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Larval development in European hake (*Merluccius merluccius* L.) reared in a semi-intensive culture system

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Abstract

Eggs of European hake (*Merluccius merluccius* L.) were stripped from fish caught at sea. Larvae were kept under semi-intensive conditions at around 12 °C. In addition, eggs were incubated in single wells at 9.2, 12.7 and 14.5 °C, where hatching, development and survival were closely examined. During the larval stage, a total of 299 larvae were sampled to follow development and growth. In addition a small number of juveniles were sampled. Larvae hatched approximately 4 days after fertilization, and were 2.9 mm in total length (TL). At 6-day post hatching (dph), the larvae were 4.1 mm (TL), the jaw apparatus was developed, and the larvae had started to feed. Most of the growth during the early larval period is restricted to the head, and there is almost no increase in length for the first 3–4 weeks post hatching. Teeth and pelvic fins appear at 25 dph. Development of unpaired fins at approximately 30 dph marks the start of the larval–juvenile transition. Weaning to formulated feed was accomplished 50 dph, when external morphology was similar to that of adult hake.

Keywords: European hake, *Merluccius merluccius* L., larval development

Introduction

European hake (*Merluccius merluccius* L., hake) is a popular commercial species in Europe and can garner high prices (Casey & Pereiro 1995). The total landings of hake in the northeast Atlantic are about 80 000 tonnes a year, but has been as high as 175 000 tonnes (Casey & Pereiro 1995). Thus, there is a growing interest in farming this species.

Hake spawn at depths of 70–150 m (Hickling 1927; Coombs & Mitchell 1982), at water temperatures of 10–13 °C (Arbault & Boutin 1968; Coombs & Mitchell 1982; Alvarez, Motos, Uriarte & Egaña 2001). They are difficult to keep alive after capture (Hickling 1933; Belloc 1935) and, therefore, have only rarely been kept in captivity. The first known captive broodstock of hake is currently being held in tanks in the hope that they will spawn naturally (R. Salte, Norwegian University of Life Sciences, per. comm.).

Artificial fertilization of hake gametes has been reported (Raffaele 1888; Coombs & Mitchell 1982; Marrale, Alvarez & Motos 1996). In these studies, only the embryonic development and yolk sac larvae are described. Descriptions of cultured larvae beyond the start of exogeneous feeding are not available, although there are some (albeit incomplete) descriptions of wild-caught larvae and juvenile (Schmidt 1907; Schnakenbeck 1928; Arbault & Boutin 1968). Although these reports provide some information about development, the timing of events is not available. From an aquaculture perspective, it is important to know when developmental events occur (e.g. hatching, time to start feeding, growth and behaviour at different stages); this information can only be obtained from rearing experiments.

In this paper, we describe a protocol for the semi-intensive culture of hake that allowed us to describe their larval development, growth during the early life stages and the influence of temperature on development and survival.

Material and methods

Egg collection and incubation

Gametes were obtained from live hake caught at spawning grounds west of Florø, Norway (61°36′N, 05°02′E). In total, 65 hake were caught: 57 females

(size 75–83 cm), seven with ripe eggs and eight males (size 67–70 cm), three with ripe milt. Eggs were kept in ovarian fluid at approximately 10 °C before fertilization. Milt was used immediately after stripping. Mixing 150 mL eggs with about 5 mL of milt resulted in fertilization of the eggs. Approximately 3 L of seawater was added, and the gamete mixture was left undisturbed for 10–15 min. The eggs were then carefully rinsed with seawater to remove excess milt and roe fluids. Fertilized eggs were placed into plastic bags containing 3 L of seawater, and transported for 10 h. Temperature in the bags at arrival was approximately 13.5 °C. The bags with eggs were placed in incubators at a temperature of 12 °C. The bags were emptied into the incubators when the water in the bags reached the same temperature as that in the incubators (approximately 30 min).

The tanks used for egg incubation are 250 L upstream systems consisting of a cylindrical upper section and a conical lower section. Tanks were outfitted with an overflow sieve (PVC pipe covered with 250 μm mesh plankton netting) positioned just beneath the water surface. Flow was held at approximately 1 L min^{-1}, yielding a daily water renewal rate of 6. Hake eggs exhibited a strong hydrofugal nature that caused them to adhere to the water surface (as described in Porebski 1976 and in Coombs 1994). In order to prevent dehydration of eggs trapped at the surface, seawater was sprayed over the surface using a handheld vaporizer. Spraying water vapour on the surface also released dead eggs trapped at the surface–air interface and, as dead eggs are negatively buoyant, they sank and could be more easily flushed out of the system.

Larvae/juvenile rearing

Newly hatched larvae were transferred to larger systems. At this stage, the larvae were passive and buoyant, and could easily be collected. The incubators used for larvae rearing are 2800 L upstream systems consisting of a cylindrical upper section and a conical lower section. An overflow sieve was constructed of a polyethylene frame covered with 250 μm mesh plankton netting. The sieve was positioned just beneath the water surface. Flow was held at approximately 1 L min^{-1}, yielding a daily water renewal of 50%. Larvae were kept in this system until they started metamorphosis approximately 35 days post hatching (dph). From 5 dph, the larvae were offered a mixed diet of rotifers, *Brachionus plicatilis* (Muller) (cultured with bakers yeast and algae) and zooplankton nauplii, mainly *Acartia granii* (Sars). *Tetraselmis* sp. algae were added to the incubator. From 24 dph, only algae and zooplankton were offered. The concentration of prey and algae in the incubators was not measured. Approximately 1–2 million rotifers or natural zooplankton, and 12–15 L of algal cell suspension, were added daily to each of the incubation tanks. As there was no aeration in the system, algae, prey and feeding larvae all aggregated in patches in the upper section of the units.

Larvae/juveniles were transferred to flat-bottom tanks (50 L) around 35 dph to start weaning onto formulated feed. At first, a combination of live feed and formulated feed was offered, while at 50 dph they were only fed pellets (Aglonorse Larvae Feed No. 2+betaine, SSF – The Norwegian Herring Oil and Meal Industry Research Institute, Bergen, Norway). The tanks were cleaned daily.

Sampling and observation

Egg and oil globule diameters were measured (n = 100) about 24 h after fertilization. Egg samples were rinsed in distilled water for approximately 15 min and then kept frozen (− 18 °C) for 5 months. These eggs samples were then freeze dried and weighed (n = 88).

General observations of behaviour were made daily during the larval and juvenile phase. Larvae were sampled from the incubator (Table 1). Metomidate hydrochloride (1 mg L^{-1}) was used to anaesthetize the larvae before examination. After examination, larvae were preserved in Karnovsky fixative. Total length and body height were measured for all larvae. Yolk length and oil globule diameter were measured for as long as they were present. Eye diameter and length of the lower jaw were measured from 6 dph using

Table 1 Sampled material of reared European hake larvae (*Merluccius merluccius* L.)

	Days post hatch						
	0	4	6	8	11	17	25
Total number of sampled larvae	96	83	23	25	9	34	29
Yolk length	96	83	23				
Oil globule diameter	96	83	23				
Total length	96	83	23	24	9	34	29
Body height	96	83	23	25	9	34	29
Length of lower jaw			17	13	7	15	11
Diameter of eye	8	6	12	17	9	18	11
Dry weight	20	20	20	20	9	20	10

Values in the columns are the number of larvae used at for the different measurements.

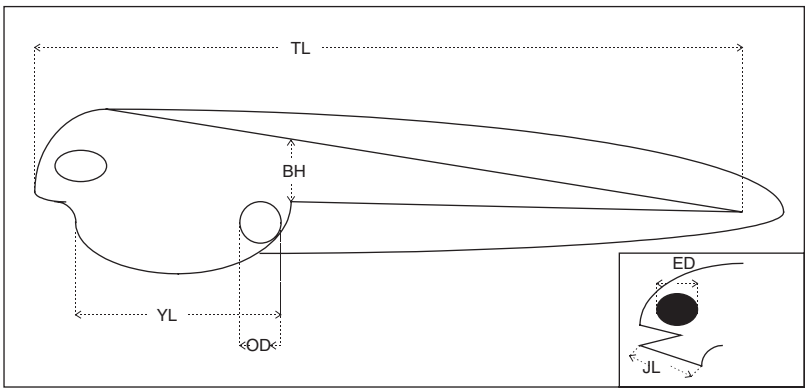

Figure 1 Simplified drawing of larvae showing how the different measurements were taken. TL, total length from snout to end of notochord; BH, body height measured vertically at the anus; YL, maximum length of the yolk; OD, diameter of the oil globule. The caption in the lower right corner shows the head of an older larva and the measurements taken on preserved larvae. JL, length of the lower jaw from the most anterior part until the angle of the jaw; ED, eye diameter measured parallel to the forehead.

larvae that had been preserved in fixative for approximately 7 months (Fig. 1). Gut content and development of the eyes, gut and jaws were noted. For dry weight measurements, preserved larvae were rinsed in distilled water and then dried for 24 h at 60 °C. All morphometric measurements were made using a binocular microscope outfitted with a micrometer eyepiece. Photos of eggs and larvae were taken using a binocular microscope fitted with a digital camera.

When larvae were about to enter the juvenile stage, the number of individuals was too low to sustain regular sampling. However, some juveniles were measured for length, and photographed, in order to obtain an indication of their growth and development.

Single-well experiment

In addition to the large-scale trial, a small-scale experiment was carried out to more closely monitor events such as hatching, survival of yolk sac larvae and morphological development at different temperatures. The single-well incubation experiment was conducted at three temperatures: 9.2, 12.7 and 14.5 °C. Each well was filled with 11 mL of autoclaved water at a salinity of 25 g L^{-1}, and one egg (24 h post fertilization) was added to each well – ca. 100 eggs per temperature. The egg was gently submerged using a pair of fine forceps. The 9.2 °C group was held in a refrigerator with a glass door, and were thereby exposed to some light, while the two others were incubated in the dark. The wells were taken out of the refrigerator at noon every day and observed under a binocular microscope for hatching and mortality. Aspects of morphological development, such as corporeal pigmentation, fins, eye pigment and depletion of yolk and oil drop, were described. Hatching per cent was calculated as the number of larvae hatched vs. the number of incubated eggs. Survival is expressed as the percentage of larvae still alive at total yolk depletion.

Results

Egg characteristics

Hake eggs are spherical with a smooth outer surface (Fig. 2). The yolk is homogeneous and transparent, with a pale yellowish colouration. A single yellow oil globule is present. Mean egg diameter was 1.07 ± 0.01 mm, the oil globule measured 0.29 ± 0.00 mm, while the dry weight was 59 ± 6 µg.

Single-well experiment

All larvae hatched within 2 days at all temperatures (about 90% of them hatched the first day). The majority of larvae hatched at around 60 d °C (day-degrees = number of days × temperature in °C) after fertilization. The highest hatching per cent occurred at 12.7 °C = 61%. The best survival to yolk depletion was at 14.5 °C = 55%. Complete results of this experiment are presented in Table 2.

Larval rearing

Results regarding start feeding were obtained from the rearing experiment conducted at a temperature of approximately 12 °C. After hatching, larvae re-

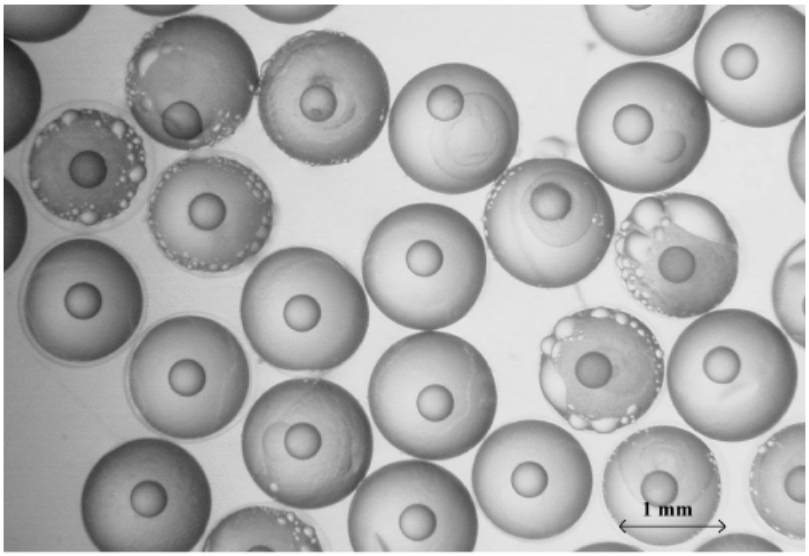

Figure 2 Eggs of European hake (*Merluccius merluccius* L.).

Table 2 Hatching and survival to yolk depletion at different temperatures for reared hake (*Merluccius merluccius* L.) larvae

Temperature (°C)	*n*	Hatching (dpf–df °C)	Hatching (%)	Yolk depletion (dph–dh °C)	Survival (%)
9.2	114	7–64	30	7–64	26
12.7	104	5–64	61	6–76	41
14.5	91	4–58	46	5–73	55

n is the initial number of eggs. Time to hatch was defined as the day on which 50% of the eggs had hatched. Hatching % is calculated on the basis of *n*. Survival is the percentage of hatched larvae that survived until yolk was depleted.
dpf, days post fertilization; dph, days post hatching; df °C, day-degrees (dpf × incubation temperature); dh °C, day-degrees (dph × incubation temperature).

mained inactive with their head down in the upper section of the incubator for the first 2 days, and then became more evenly distributed in the water column. At 4 dph larvae were still inactive, but short periods of swimming were observed in association with sampling (escape reactions). At start feeding (6 dph), there was again a concentration of larvae near the surface. Larvae also tended to stay near the sides of the incubator.

The yolk was depleted before the oil globule; the latter lasted for several days after the onset of feeding. First feeding was observed at 6 dph when approximately 30% of the yolk and 70% of the oil globule remained. Total depletion was observed at 8 and 11 dph (~ 100 and 130 d °C) for yolk sac and oil globule respectively. At 6 dph, five of 23 larvae had algae in the gut. At 8 dph, 10 of 25 larvae had algae in the gut, while five of 25 had eaten rotifers or other zooplankton prey. On later sampling days, more than 90% of the sampled larvae had prey in their guts. The number and size of prey items found in the gut were not measured. The concentration of prey in the incubation tanks was not evaluated in detail, but was maintained at *ad libitum* levels throughout the experiments. Algae and zooplankton were not uniformly distributed in the tanks, but tended to patch in certain areas near the surface. Feeding larvae were observed in these patches, but often stayed in areas where the concentration of prey was visibly less dense.

Juvenile

Larvae/juveniles (35 dph) were transferred from the large incubators (2800 L) to flat-bottomed smaller tanks (50 or 500 L) for weaning. The juveniles (50 dph) did not respond to any of the formulated feeds except the one with betaine added. The feeding response to this feed was spontaneous. Juveniles would only eat sinking pellets, and did not touch feed on the bottom or at the surface. At this stage,

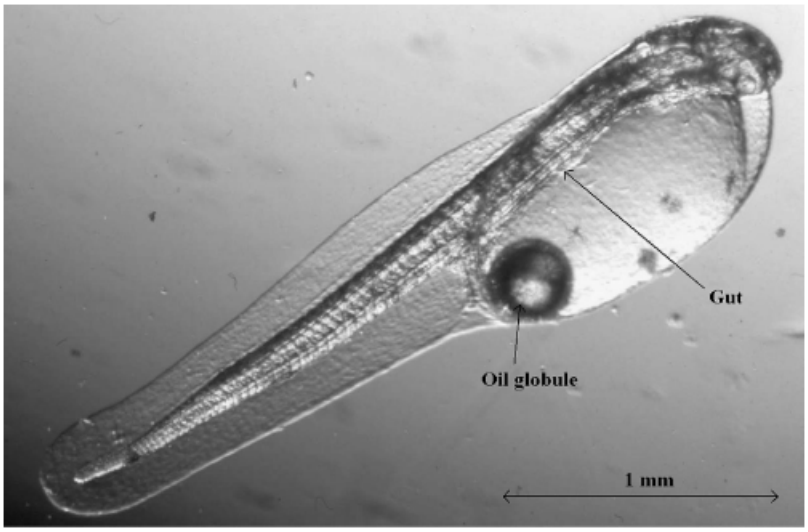

Figure 3 European hake larva (*Merluccius merluccius* L.), 0 dph (0 d °C). Total length 3.0 mm. Eyes are not pigmented. Fins are not present, and the body is surrounded by a wide primordial finfold. The mouth is not present, and no intestine is visible. The pigmentation pattern consists of scattered pigment cells around the yolk, oil globule, head, trunk and three areas at the posterior part of the body. Black pigment is dominant, but yellow pigment is also present. The yolk is pale transparent yellow, and the single oil globule is located in the posterior part of the yolk sac.

juveniles were inactive most of the time and stayed at the bottom of the tank. Cannibalism was observed after transferring the juveniles from the large incubators.

Development

The following description is based on larvae reared in large incubators (∼ 12 °C), and daily observations of embryos and larvae in the single-well experiment (9.2, 12.7 and 14.5 °C). As larvae were raised at different temperatures, only d °C were used to age the larvae in this case. Descriptions of larvae on each sampling day are presented in Figs 3–9.

Eye

The first eye rudiments are seen as 'bumps' on the head of the embryo 20 d °C before hatching, and the lenses are visible 10 d °C before hatching. At hatching, the eyes are semi-circular and pressed against the yolk sac. Pigmentation begins in the posterior part of the eye at 30 d °C post hatching, and proceeds from a subjective greyish, brown-grey until they become black at 90 d °C.

Digestive system

A straight intestine is first observed 30 d °C after hatching. It starts to bend at 60 d °C, and at 70 d °C the gut has curled into a loop. At 90 d °C a part of the gut is extended into a larger sac. Peristaltic movements are first observed around 100 d °C.

Mouth and jaws

The mouth had not yet formed at hatching. From 50 d °C, the mouth starts to form, and at 90 d °C the jaws are developed and movable. Thereafter, snapping was observed regularly in the single-well system, and most larvae sampled from the ongrowing tanks had captured prey. Teeth were first observed in larvae aged 300 d °C (25 dph).

Fins

The pectoral fins were not present at hatching. At 30 d °C small fin buds were present. As the fins grew, they became pigmented, and around 90 d °C the pectoral fins were well developed and functional (i.e. they move). The pelvic fins appeared much later. The first rudiments were observed as dark pigmented areas around 300 d °C (25 dph), but only larvae that had reached a length of more than 5 mm had pelvic fins at this age. The pelvic fins grew to a large size, and juveniles seemed to use them to maintain station on the bottom. Unpaired fins (dorsal, caudal and anal fins) began to appear around 300 d °C (25 dph); this marks the start of the transition to the juvenile period.

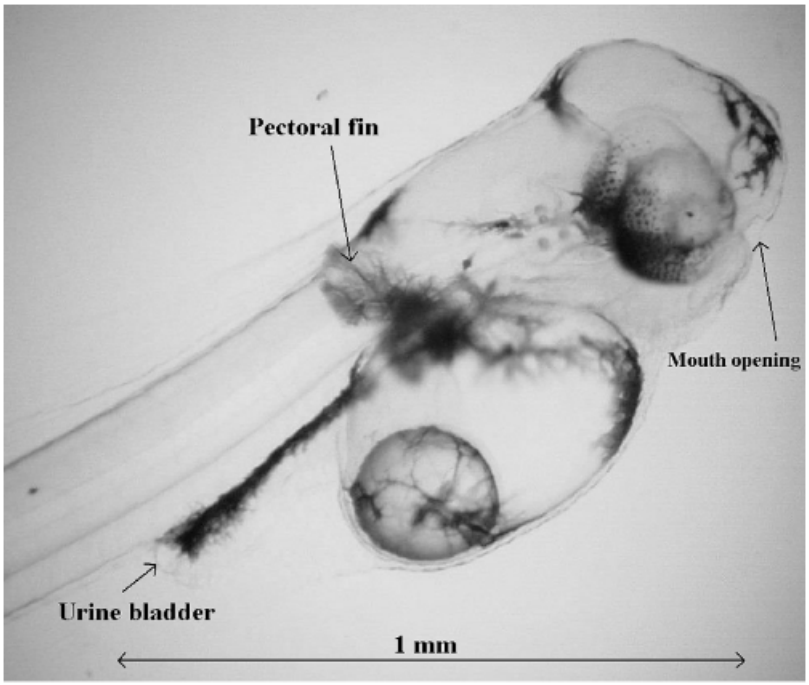

Figure 4 European hake larva (*Merluccius merluccius* L.), 4 dph (~ 50 d °C). Total length 4 mm. The posterior section of the eyes is slightly pigmented. Pectoral fins are present and pigmented. The pigmentation pattern is as earlier, but more pronounced. Jaws are not present, but 8% of the examined larvae had open mouths. The urine bladder is visible near the anus. The yolk sac is reduced to about 50% of its initial length, while the size of the oil globule is reduced by about 15%.

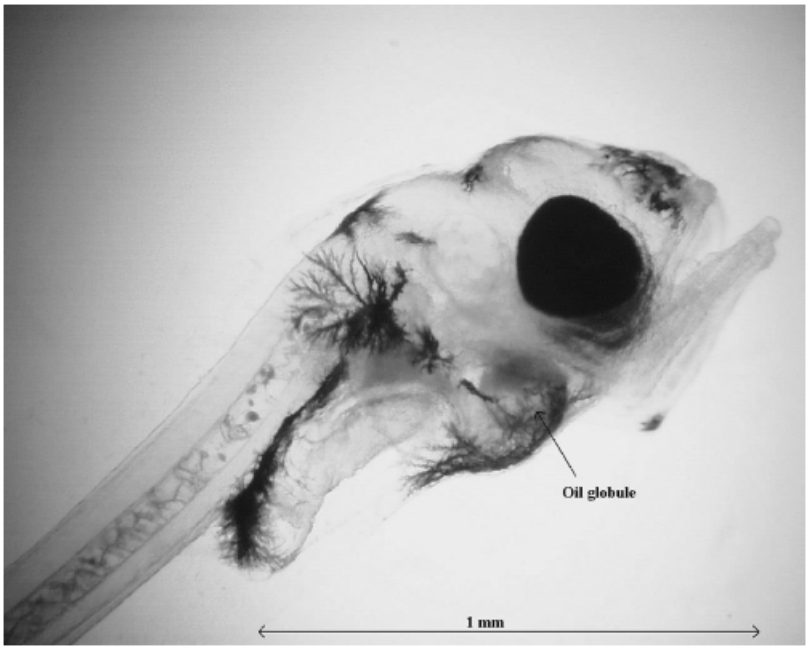

Figure 5 European hake larva (*Merluccius merluccius* L.), 8 dph (~ 100 d °C). Total length 4 mm. The eyes are fully pigmented. The gut is clearly present. Jaws are functionally Although present, swimbladder and prey items in the gut are not present in the specimen photographed.

Swimbladder

A gas filled swimbladder is first seen in larvae aged 100 d °C (8 dph), when it was present in 16% of the larvae. At 11 dph it was present in 50% of the larvae. In older larvae, pigmentation made it difficult to clearly establish the presence of a swimbladder.

Growth

The standard length of larvae increased by more than a millimetre for the first few days, while there was no growth in length from 4 to 11 dph (no significant difference between sampled larvae from these ages: *t*-test, $P < 0.05$). From 11 dph, the length began increasing again. Body height of larvae increased un-

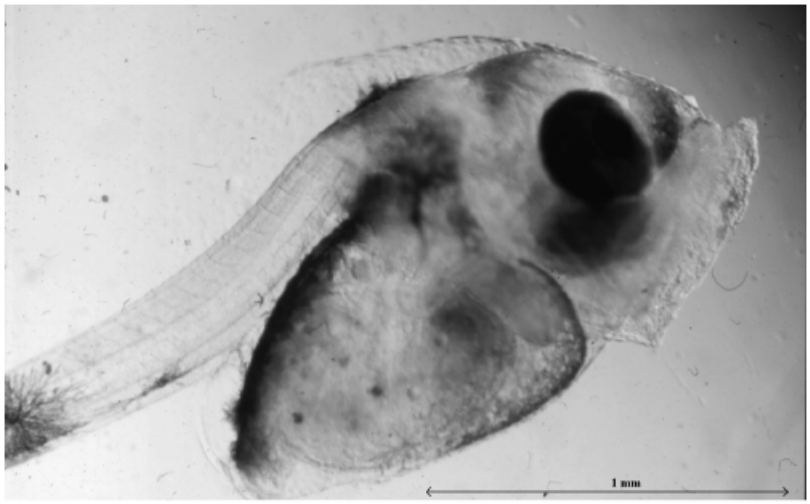

Figure 6 European hake larvae (*Merluccius merluccius* L.), 11 dph (~ 130 d °C). Total length is 4.1 mm. Gut/stomach is filled with prey.

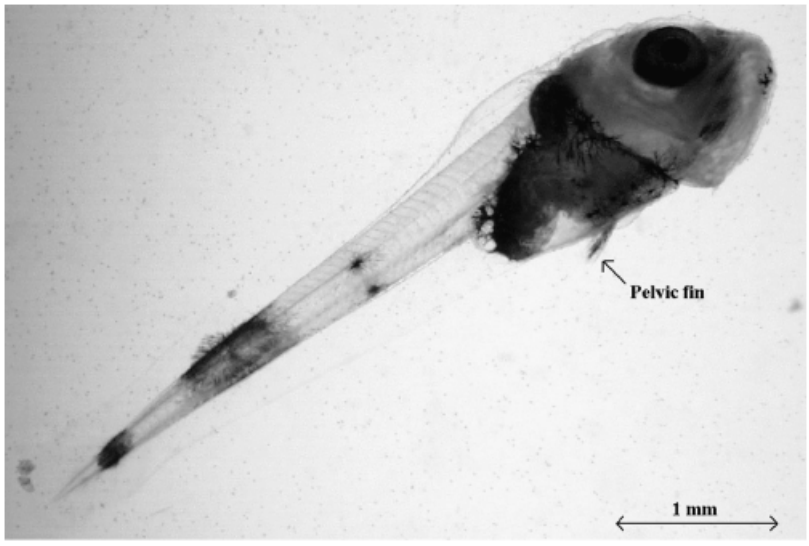

Figure 7 European hake larva (*Merluccius merluccius* L.), 25 dph (~ 300 d °C). Total length is 5 mm. At this age, all larvae have a swim bladder. Blood circulation is visible in the gill filaments and in the heart. All larvae have developed teeth, and the largest ones also have a small barbell. Larvae more than 5 mm in length have pelvic fins. The primordial finfold is still present in the caudal part of the body, but is absent around the anterior half.

til 6 dph, but there was no difference in height between larvae aged 6, 8 and 11 dph (*t*-test, $P < 0.05$). From 11 dph and onwards the body height increased again. Diameter of the eye more than doubled from 0 to 8 dph, while growth thereafter was slower (Fig. 10). The jaw was not present before 6 dph, but grew rapidly during the period 6–11 dph. Larval dry weight increased slightly from 0 to 6 dph, but there was no significant increase between 6 and 11 dph. After 11 dph, the larvae again gained weight, and by 25 dph it had doubled compared with newly hatched larvae. After 25 dph, regular sampling was not possible (not enough material remained), but a few juveniles were measured for length until 250 dph, and further growth is illustrated in Fig. 10.

Discussion

For hake to be commercially farmed it is essential to be able to keep broodstock from which to collect naturally spawned and fertilized eggs. We made several attempts to catch live hake for this purpose, without success. Species from this genus are especially sensitive to handling-related damage (Hickling 1933; Belloc 1935). Further, only 4.8% of hake females have hydrated oocytes on any given day during the spawning season (Murua, Motos & Lucio 1998). Hickling (1930) also mentions the low rate of ripe females caught during cruises (26 of 1932 mature females – 1.3%). Given the low numbers of females captured on our collection cruises, the probability of obtaining

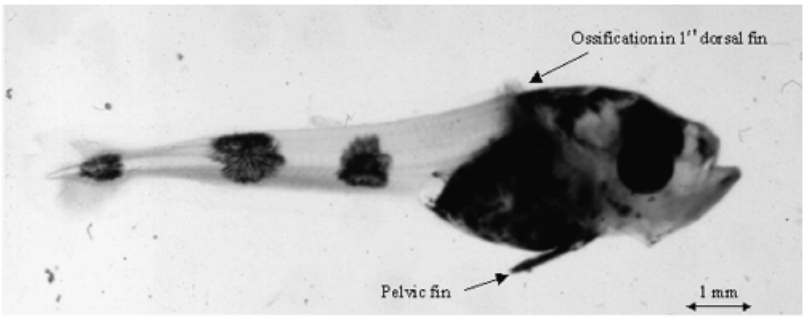

Figure 8 European hake larva/juvenile (*Merluccius merluccius* L.), 33 dph (~ 400 d °C). Total length is 10 mm. The body is deep, and the head is large, while the posterior part is still quite slender. The unpaired fins have started to form, and ossification is seen in the first dorsal fin. The pelvic fins reach halfway to the anus. Both pectoral and pelvic fins are pigmented. The abdomen is heavily pigmented, and other patterns are as previously described; with three clear areas along the posterior part of the body.

ovulated eggs was relatively low. Nonetheless, 12% of the females collected had ripe eggs. The low catch of males was probably due to the mesh size in the gillnets we used, as males mature at a younger age and are normally smaller than females (Hickling 1930). A stock of wild-caught Southern hake (*Merluccius australis* (Hutton)) has been maintained in net pens in Chile and there has also been some success with broodstock (Anon. 1999, 2004; Carvajal 2003). In an experiment with silver hake (*Merluccius bilinearis* (Mitchill)), wild caught broodstock spawned in captivity (Buckley, Smigielski, Halavik, Burns & Laurence 1993). A major effort was recently put into capturing live hake using rod and reel, resulting in a healthy broodstock (R. Salte, Norwegian University of Life Sciences, per. comm.). Therefore, the possibilities for hake are improving.

In the discussion that follows, the results from our trials with hake will be compared with analogous information collected during the development of intensive culture systems for cod.

The procedure used to transfer newly hatched larvae from egg incubators to larger tanks for startfeeding was chosen based on the limited experience we had with this species. In the future, it would be useful to test other systems, such as those used for cod, halibut (*Hippoglossus hippoglossus* L.) and haddock – 1500 L flat-bottomed tanks with aeration and a central outlet pipe.

The diameter of eggs and oil globule reported in this study are consistent with previous findings (0.94–1.11 and 0.25–0.4 mm respectively) for artificially fertilized hake eggs (Raffaele 1888; Coombs & Mitchell 1982; Marrale *et al.* 1996) as well as for eggs collected in ichthyoplankton surveys (Ehrenbaum-Helgoland 1905–1909; Arbault & Boutin 1968). The adhesive and hydrofugal nature (see Zaitsev 1971) of hake eggs, which causes them to be trapped by surface tension, has been described for various *Merluccius* species (Porebski 1976; Coombs 1994). The phenomenon is not of a spontaneous adherence character, as there has to be an external force (like air bubbles) forcing the eggs up towards the surface. Contact with the air causes the eggs to 'adhere' to the surface. Eggs at the surface eventually die of dehydration. Spraying the incubator surface with seawater vapour appeared to reduce egg dehydration. This problem probably never occurs under natural conditions; hake spawn at approximately 70–150 m depths (Hickling 1927; Coombs & Mitchell 1982), and the positive buoyancy causes the eggs to rise towards the surface layers. In ichthyoplankton surveys, hake eggs were found from 150 to 0 m (Coombs & Mitchell 1982; Alvarez *et al.* 2001). The method used for cod – aeration to keep eggs in suspension (Thompson & Riley 1981; Kjørsvik, Pittman & Pavlov 2004) – does not work with hake eggs, as they become even more hydrofugal when exposed to air.

Development of the embryo is similar to that described for most marine fish larvae (Coombs & Mitchell 1982; Marrale *et al.* 1996). Pigmentation appears early in development; the first sign of pigment appeared just after closure of the blastopore. Just before hatching, the embryo reached all the way round the inside of the egg, and its nose and tail touched. Larvae hatched approximately 50 d °C (~ 4 days) after fertilization when kept in the incubators, while the larvae in the single-well system needed an additional 10 d °C before hatching. These latter values is comparable with that reported by Coombs and Mitchell

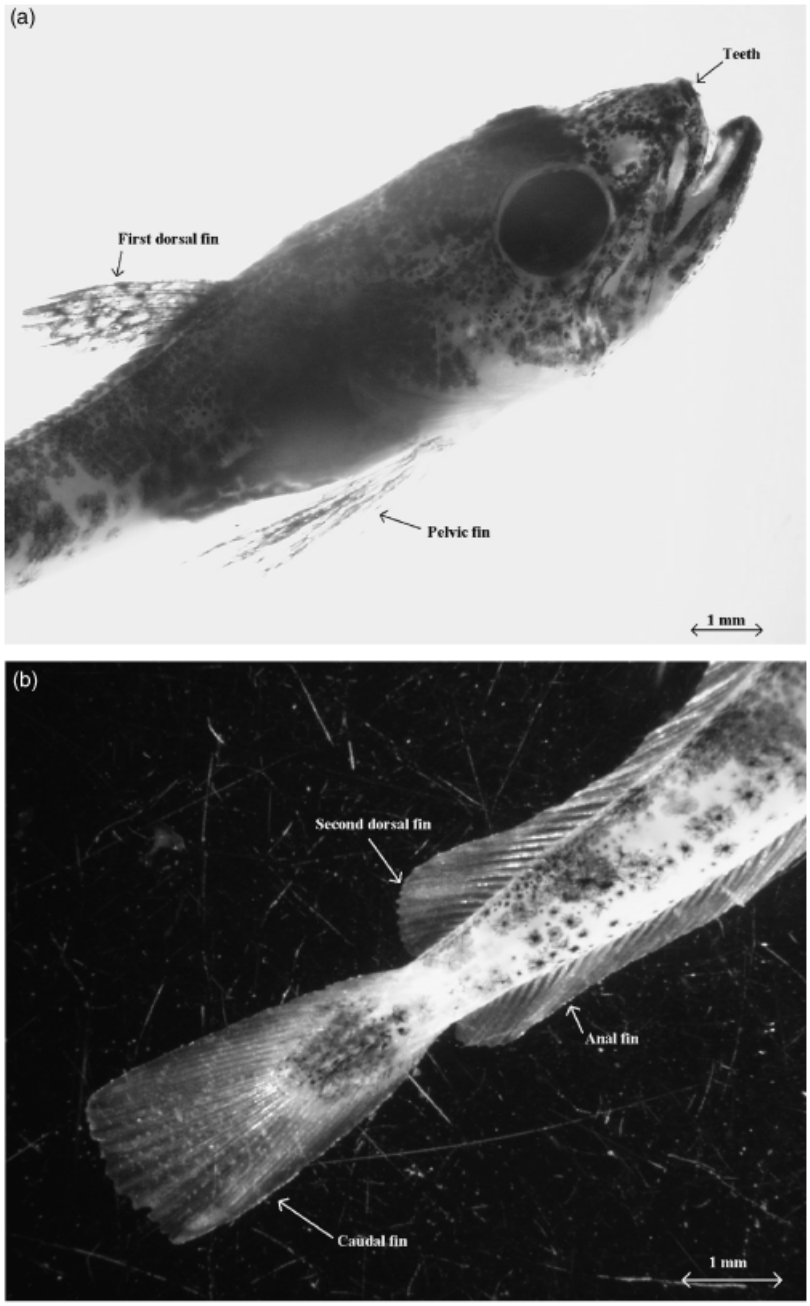

Figure 9 European hake juvenile (*Merluccius merluccius* L.), 59 dph (~ 700 d °C). Total length is 23.5 mm. (a) Anterior part. (b) Posterior part. Transformation to the juvenile form is completed. The body is slender, and the large head is dorso-ventraly flattened. The pigmentation pattern on the posterior part with three pigment bands is not as dominant as earlier. Pelvic fins reach beyond the anus.

(1982), who observed hatching at 60 d °C at 13 °C while in Marrale *et al.* (1996) time to hatch was a bit shorter (between 38 and 50 d °C at a temperature varying between 13 and 17 °C). Compared with cod, which hatch about 100 d °C after fertilization (Russell 1976), the hake have a short egg period. A high proportion of the larvae hatched synchronously (within a day), while the remaining hatched on the following day. In an intensive rearing context, this is a very positive feature, since day 0 (day of hatch) is easy to estimate and the developmental state of all larvae is similar at start feeding. The highest hatching percent was achieved at 12.7 °C (~ 60%). Coombs and Mitchell (1982) reported much higher embryonic mortality, and had best results at 8.1 °C, with 32% hatching. Temperature in the sea during the spawning period of hake is between 10 and 13 °C (Arbault & Boutin 1968; Coombs & Mitchell 1982; Alvarez *et al.* 2001).

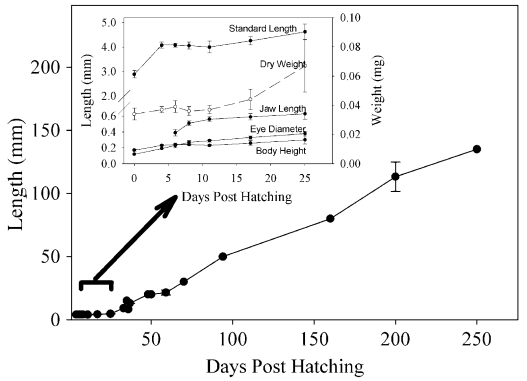

Figure 10 Growth of European hake larvae and juveniles (*Merluccius merluccius* L.). The figure insert shows total length, body height, jaw length and eye diameter on the left axis (filled circles) and dry weight on the right axis (open circle). The larger figure presents length growth of larvae trough juveniles.

Several authors have described larvae and juveniles caught in the wild (size range 3–31 mm length) (Schmidt 1907; Schnakenbeck 1928; Arbault & Boutin 1968). The development of larvae in our experiment followed the pattern described for wild-caught larvae. Like most larvae that hatch from pelagic eggs, newly hatched hake are poorly developed (Kendall, Ahlstrom & Moser 1984). Newly hatched larvae were 2.89 ± 0.15 mm (TL), which is consistent with previous findings (Ehrenbaum-Helgoland 1905–1909; D'Ancona 1956), but differs greatly from the data of Marrale *et al.* (1996) who reported length at hatch to be 1.63–1.74 mm. Newly hatched yolk sac larvae are pigmented in a manner typical of all species in the genus *Merluccius* (Coombs & Mitchell 1982), with three bands of dark pigment along the posterior part of the body. The yolk and oil globule are also pigmented from hatching.

Hake larvae have two kinds of energy reserves, yolk and oil globule. During the yolk sac phase, the yolk is depleted first while the oil globule lasts for several days longer (remnants remain visible at 11 dph).

First feeding was observed at 6 dph (∼ 70 d °C), when 30% of the yolk and most of the oil globule remained, but at the beginning only algae were present in the gut. According to van der Meeren (1991) the 'green gut' is a result of drinking activity. No other prey was found until 8 dph (∼ 100 d °C), when the yolk was depleted and only the oil globule remained. Compared with cod, which initiate feeding 5 dph at 5 °C (25 d °C) and have totally depleted yolk at 8 dph at 5 °C (40 d °C) (Ellertsen, Moksness, Solemdal, Strømme, Tilseth, Westgård & Øiestad 1980), hake initiate feeding very late and the energy reserves last longer. Functional jaws are present in hake larvae at 6 dph (70 d °C); later than the 25 d °C found for cod (Ellertsen *et al.* 1980). Jaw length as measured in this study does not provide any indication of how large prey the larvae are able to consume, but the relative growth of the jaw through development should be indicative of changes in diet. Compared with other species, larvae of North pacific hake (*Merluccius productus* (Ayres)) have big mouths and consume a large size range of prey (Hunter 1980). In our experiment, rotifers and zooplankton nauplii were offered as first feed. Considering the development of jaw size, an earlier shift to larger zooplankton as food would have been preferable. Teeth, which will further increase success in prey capture, were first seen in larvae at 4.5 mm length (25 dph), which was also noted by Schnakenbeck (1928) for wild caught larvae.

Development of the digestive system follows the same pattern as that exhibited by cod, with a straight gut turning into a loop before the mid-gut expands to form a 'stomach' (Kjørsvik *et al.* 2004).

Development of fins is related to changes in mobility and, thereby, activity level (Hunt von Herbing, Boutilier, Miyake & Hall 1996). Pectoral fins are not present at hatch, but soon start to form and are functional at first feeding (6 dph). Pelvic fins start to appear when larvae are about 5 mm (TL); this is also noted in field-caught larvae (Schmidt 1907; Palomera, Olivar, & Morales-Nin 2005). Unpaired fins appear around 30 dph at a length of 10 mm and mark the start of the transition to the juvenile period. In the field, a length of 10–12 mm is the size at which other fins start to appear (Arbault & Boutin 1968; Schmidt 1907; Palomera *et al.* 2005). The presence of large pelvic fins in juvenile hake is related to their demersal behaviour, which starts at this stage; juveniles remain rather passively on the bottom of the tank. In the Mediterranean, a hake study based on otolith growth suggests that the demersal phase begins at approximately 40 dph at a length of approximately 140 mm (Arneri & Morales-Nin 2000).

The presence of a swimbladder is important in larval development, as it controls buoyancy and makes swimming activity and prey capture more energy efficient. As for cod (Ellertsen *et al.* 1980; Hunt von Herbing *et al.* 1996), filling of the swimbladder in hake larvae occurred at the transition to exogenous feeding. A gas-filled swimbladder was first seen in larvae 8 dph, but only in 16% of the larvae sampled. Even

though this increased to 50% at 11 dph, it is likely that swimbladder inflation was inhibited by the oily water surface in the incubator (Planas & Cunha 1999). Such lack of swimbladder inflation is associated with high mortalities caused by buoyancy problems and ineffective foraging (Thompson & Riley 1981).

Fully developed juveniles have a slender body with a dorso-ventrally flattened head. During the transition to the juvenile form, large differences in inter-individual size develop. Cannibalism was observed, but not before transferring from the large incubators to the weaning tanks. Even though we did not have any direct observation of cannibalism in the large incubators, a marked decrease in the number of larvae was noted. Combined with the increasing difference in inter-individual size, it is very likely that cannibalism also occurred here. For most marine species, cannibalism is a problem in aquaculture (Hecht & Pienaar 1993). As is the case for all species that are cannibalistic, the only solution in an intensive rearing context is to offer the right kind of food in high enough amounts, and to undertake extensive grading.

The presence of an attractant seemed to be important when the juveniles were weaned to formulated feed, as the juveniles immediately started to eat when the betaine feed was offered. The reason why hake should be more 'picky' than e.g. cod, haddock and halibut (which all are weaned without this attractant added to the feed) is unknown and requires further investigation. The Dover sole, *Solea solea* L. also requires betaine as an attractant in the feed for proper feed uptake (Mackie & Mitchell 1982).

The early growth of hake is characterized by rapid development of the head and trunk region, while the rest of the larvae does not seem to grow much until later. During the first 4 days after hatching, length and body height increased, while further growth through 25 dph was mostly expressed in an enlargement of the head (as seen in photos and indicated as growth of the eye and jaw). When the mouth is large enough to catch many types of prey, growth in length and height continues, while growth of the eye and jaw slows down. This growth pattern, with early development of head and trunk, and slow initial growth in length, was also noted by Schmidt (1907), Schnakenbeck (1928) and Palomera *et al.* (2005) for wild-caught larvae. It is also known from other species in the family, e.g. North Pacific hake (Ahlstrom & Counts 1955; Bailey 1982) and Silver hake (Buckley *et al.* 1993) and other species such as Atlantic horse mackerel, *Trachurus trachurus* L. (Russell 1976) and Pacific bonito, *Sarda chiliensis* (*Cuvier*) (McFarlane, Cripe & Thompson 2000).

The growth of juvenile and adult hake is well studied. Otolith or length frequency distribution analyses suggest a growth rate of 0.9–1.15 cm a month (Hickling 1933; García-Rodríguez & Esteban 1995; Morales-Nin & Aldebert 1997). In tagging experiments, the growth rate reported was 1.0–1.4 cm a month (Belloc 1935; Fritz 1959; de Pontual, Bertignac, Battaglia, Bavouzet, Moguedet & Groison 2003). This study produced the first direct measure of growth of hake larvae; juveniles reached a length of 12 cm after 200 days, which represents a monthly increase of about 1.8 cm. Otolith microstructure of reared and wild hake larvae were comparable (Morales-Nin, Bjelland & Moksness 2005), indicating that the cultured fish in this experiment were growing reasonably well.

The development of hake for aquaculture is still at an early stage. This study provides some basic information about egg incubation, hatching, start feeding and weaning. All areas require more research, and with a healthy broodstock now in captivity, the chance of success has increased.

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References

Ahlstrom E.H. & Counts R.C. (1955) Eggs and larvae of the Pacific hake, *Merluccius productus*. *Fishery Bulletin* **56**, 295–329.

Alvarez P., Motos L., Uriarte A. & Egaña J. (2001) Spatial and temporal distribution of European hake, *Merluccius merluccius* (L.), eggs and larvae in relation to hydrographical conditions in the Bay of Biscay. *Fisheries Research* **50**, 111–128.

Anon. (1999) Hazaña en los mares del sur. *Aquanoticias* **50**, 6–13.

Anon. (2004) Primer desove de merluzas producidas en laboratorio. *Revista Aqua* **91**, 1.

Arbault S. & Boutin N. (1968) Ichthyoplancton. Oeufs et larves de poissons teleosteens dans le Golfe de Gascogne en 1964. *Revue des Travaux de l'Institut des Peches Maritimes* **32**, 412–476.

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

Bailey K.M. (1982) The early life history of the Pacific hake, *Merluccius productus*. *Fishery Bulletin* **80**, 589–598.

Belloc G. (1935) Étude monographique du merlu. *Merlucius merluccius* L. *Revue des Travaux de l'Office des Pêches maritimes* **8**, 145–202.

Buckley L.J., Smigielski A.S., Halavik T.A., Burns B.R. & Laurence G.C. (1993) Growth and survival of the larvae of three temperate marine fish species at discrete prey densities. II. Cod (*Gadus morhua*), winter flounder (*Pseudopleuronectes americanus*), and silver hake (*Merluccius bilinearis*). In: *Physiological and Biochemical Aspects of Fish Development* (ed. by B.T. Walter & H.J. Fyhn), pp. 183–195. University of Bergen, Bergen, Norway.

Carvajal P. (2003) Hake farming in Chile. *IntraFish* **2**, 25.

Casey J. & Pereiro J. (1995) European hake (*M. merluccius*) in the North-east Atlantic. In: *Hake: Biology, Fisheries and Markets* (ed. by J. Alheit & T.J. Pitcher), pp. 125–147. Chapman & Hall, London, UK.

Coombs S.H. (1994) Identification of eggs of hake, *Merluccius merluccius*. *Journal of the Marine Biological Association of the United Kingdom* **74**, 449–450.

Coombs S.H. & 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. *Journal du Conseil. International Council for the Exploration of the Sea* **40**, 119–126.

D'Ancona U. (1956) Uova, larve e stadî giovanili di Teleostei. Gadidae. *Fauna Flora Golfo Napoli* **38**, 178–255.

de 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.

Ehrenbaum-Helgoland E. (1905–1909) *Merlucius merluccius L.* In: *Eier Und Larvaen Von Fischen. Nordisches Plankton* (ed. by E. Ehrenbaum-Helgoland), pp. 260–264. Verlag von Lipsius & Tischer, Kiel und Leipzieg, Germany.

Ellertsen B., Moksness E., Solemdal P., Strømme T., Tilseth S., Westgård T. & Øiestad V. (1980) Some biological aspects of cod larvae (*Gadus morhua* L.). *Fiskeridirektoratets skrifter : Serie Havundersøkelser* **17**, 29–47.

Fritz R.L. (1959) Hake tagging in Europe and the United States, 1931–1958. *Journal du conseil* **24**, 480–485.

García-Rodríguez M. & Esteban A. (1995) Algunos aspectos sobre la biologia y pesca de la merluza mediterranea *Merluccius merluccius* (Linnaeus, 1758) en la Bahia de Santa Pola (sureste de la peninsula Iberica). *Boletin del Instituto Espanol de Oceanografia* **11**, 3–25.

Hecht T. & Pienaar A.G. (1993) A review of cannibalism and its implications in fish larviculture. *Journal of the World Aquaculture Society* **24**, 246–261.

Hickling C.F. (1927) The natural history of the hake. Part 1 – periodic changes in the fishery. *Fishery Investigations – Series 2* **10**, 1–33.

Hickling C.F. (1930) The natural history of the hake. Part 3 – seasonal changes in the condition of the hake. *Fishery Investigations – Series 2* **12**, 1–60.

Hickling C.F. (1933) The natural history of the hake. Part 4 – age-determination and the growth-rate. *Fishery Investigations – Series 2* **13**, 1–85.

Hunt von Herbing I., Boutilier R.G., Miyake T. & Hall B.K. (1996) Effects of temperature on morphological landmarks critical to growth and survival in larval Atlantic cod (*Gadus morhua*). *Marine Biology* **124**, 593–606.

Hunter J.R. (1980) The feeding behavior and ecology of marine fish larvae. In: *Fish Behavior and its use in the Capture and Culture of Fishes. ICLARM Conference Proceedings* 5 (ed. by J.E. Bardach, J.J. Magnuson, R.C. May & J.M. Reinhart), pp. 287–330. International Centre for Living Aquatic Resource Management, Manila, Philippines.

Kendall A.W., Ahlstrom E.H. & Moser H.G. (1984) Early life history stages of fishes and their characters. In: *Ontogeny and Systematics of Fishes: Proceedings of an International Symposium Dedicated to the Memory of Elbert Halvor Ahlstrom* (ed. by H.G. Moser, W.J. Richards, D.M. Cohen, M.P. Fahay, A.W. Kendall & S.L. Richardson), pp. 11–22. Special Publication Number 1, American Society of Ichthyologists and Herpetologists, Allen Press, Lawrence, Kansas.

Kjørsvik E., Pittman K. & Pavlov D. (2004) From fertilisation to the end of metamorphosis – functional development. In: *Culture of Cold-Water Marine Fish* (ed. by E. Moksness, E. Kjørsvik & Y. Olsen), pp. 204–278. Blackwell Publishing, Oxford, UK.

Mackie A.M. & Mitchell A.I. (1982) Further studies on the chemical control of feeding behaviour in the Dover sole, *Solea solea*. *Comparative Biochemistry and Physiology* **73A**, 89–93.

Marrale D., Alvarez P. & Motos L. (1996) Development and identification of European hake, *Merluccius merluccius* L., embryonic and yolk-sac larval stages. *Ozeanografika* **1**, 5–26.

McFarlane M.B., Cripe D.J. & Thompson S.H. (2000) Larval growth and development of cultured Pacific bonito. *Journal of Fish Biology* **57**, 134–144.

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

Morales-Nin B., Bjelland R.M. & Moksness E. (2005) Otolith microstructure of a hatchery reared European hake (*Merluccius merluccius*). *Fisheries Research* **74**, 300–305.

Murua H., Motos L. & Lucio P. (1998) Reproductive modality and batch fecundity of the European hake (*Merluccius merluccius* L.) in the Bay of Biscay. *California Cooperative Oceanic Fisheries Investigations Reports* **39**, 196–203.

Palomera I., Olivar M.P. & Morales-Nin B. (2005) Larval development and growth of the European hake *Merluccius merluccius* in the northwestern Mediterranean. *Scientia Marina* **69**, 251–258.

Planas M. & Cunha I. (1999) Larviculture of marine fish: problems and perspectives. *Aquaculture* **177**, 171–190.

Porebski J. (1976) Application of the surface adhesion test to identify the eggs of the hake *Merluccius* spp. *Collection of Scientific Papers – International Commision for Southeast Atlantic Fisheries* **3**, 102–106.

Raffaele F. (1888) Le uova galleggianti e le larve dei Teleostei nel golfo di Napoli. *Mittheilungen aus der Zoologischen Station zu Neapel* **8**, 1–85.

Russell F.S. (1976) *The Eggs and Planktonic Stages of British Marine Fishes*. Academic Press, London, UK, 524p.

Schmidt J. (1907) On the post-larval development of the hake (*Merluccius vulgaris* Flem.). *Meddelelser fra Kommissionen for Havundersøgelser. Serie: fiskeri – bind* **2**, 3–19.

Schnakenbeck W. (1928) Beitrag zur Kenntnis der Entwicklung einiger Meeresfische I. *Berichte der Deutschen Wissenschaftlichen Kommission für Meeresforschung* **4**, 119–229.

Thompson B.M. & Riley J.D. (1981) Egg and larval development studies in the North Sea cod (*Gadus morhua* L.). *Rapports et Procès-verbaux des Réunions Conseil international pour l'Exploration de la Mer* **178**, 553–559.

van der Meeren T. (1991) Algae as 1st food for cod larvae, *Gadus morhua* L. – filter feeding or ingestion by accident. *Journal of Fish Biology* **39**, 225–237.

Zaitsev Y.P. (1971) *Marine Neustonology, 205*. Israel Program for Scientific Translations, Jerusalem, Israel.

