AquaTechnica 4(3): 143-149(2022)
ISSN 2737-6095
DOI https://doi.org/10.33936/at.v4i3.4466
https://doi.org/10.5281/zenodo.7478219

Larval rearing and cannibalism in European hake (*Merluccius merluccius* Linnaeus, 1758) in Spain

Cría de larvas y canibalismo en la merluza europea (*Merluccius merluccius* Linnaeus, 1758) en España

Paula Iglesias, Juan José Otero, José Iglesias
Instituto Español de Oceanografía (IEO). Centro Oceanográfico de Vigo. Subida a Radio Faro nº 50, 36390 Vigo (Pontevedra). Spain.
Correspondencia: José Iglesias ***E-mail:*** *joseiglesias88@hotmail.com*

Artículo original | *Original article*

Keywords
Merluccius merluccius
Growth
Survival
Larvae rearing
Cannibalism

ABSTRACT | The European hake, *Merluccius merluccius*, is considered a promising species for diversification in aquaculture. This work presents growth data for reared European hake larvae up to two months of life, and cannibalism within the cohort was evaluated. Larvae were fed a combination of rotifer (2 ind mL-1) and Artemia nauplii (0.2 ind. mL-1) at first feeding and later were grown with dry pellet until 105 days in a 500-L capacity circular tank. Rearing temperature and salinity were 14.5ºC and 34 psu, respectively, and a daily photoperiod of 12L:12D was used. Two-month-old larvae reached a total length of 17.66 ± 1.22 mm and a dry weight of 7.30 ± 1.65 mg, with a 12% survival rate. The final total length and wet weight (105 days old) were 52 ± 4.20 mm and 9.72 ± 2.32 mg, respectively. The length and dry weight growth equations and the length-weight relationship were estimated for the first two months of life. Larval survival at two and three months after hatching was 12% and 8%, respectively. Cannibalism was first observed from 35 days after hatching onwards..

Palabras clave
Merluccius merluccius
Crecimiento
Supervivencia
Cría de larvas
Canibalismo

RESUMEN | La merluza europea, *Merluccius merluccius*, se considera una especie con gran potencial para la diversificación en la acuicultura. Este trabajo presenta datos de crecimiento de larvas de merluza europea criadas hasta los dos meses de vida, y se evaluó el canibalismo dentro de una cohorte. Las larvas se alimentaron inicialmente con una combinación de rotífero (2 ind mL-1) y nauplios de Artemia (0,2 ind mL-1) y posteriormente se alimentaron con pellets de pienso seco hasta los 105 días de vida en un tanque circular de 500 L de capacidad. La temperatura de cultivo y la salinidad fueron 14.5ºC y 34 psu, respectivamente, y se utilizó un fotoperíodo diario de 12L:12O. Las larvas de dos meses alcanzaron una longitud total de 17,66 ± 1,22 mm y un peso seco de 7,30 ± 1,65 mg, con una tasa de supervivencia del 12%. La longitud total final y el peso húmedo (105 días de edad) fueron de 52 ± 4,20 mm y 9,72 ± 2,32 mg, respectivamente. Se determinaron las ecuaciones de crecimiento en longitud y peso seco y la relación longitud-peso para los dos primeros meses de vida. La supervivencia de las larvas a los dos y tres meses después de la eclosión fue del 12% y el 8%, respectivamente. El canibalismo fue observado por primera vez a partir de los 35 días después de la eclosión.

INTRODUCTION

The European hake, *Merluccius merluccius* (Linnaeus, 1758) is considered in some countries like France, Spain, and Chile a promising species for diversification in aquaculture (Jolivet *et al*., 2012). This species is well adapted to temperate climates and has increased demand for European consumers.

Several studies from tagging and recaptured surveys have demonstrated that *M. merluccius* is a fast-growing species (de Pontual *et al*., 2006; Piñeiro *et al*., 2007). Such evidence of a high growth rate was verified by Jolivet *et al*. (2012) on growing wild-caught juveniles of 30-35 cm in captivity for 7 months within a temperature range of 9-13 ºC. The high growth rates obtained by these authors agree with observations made by Iglesias *et al*. (2010) and clearly show that hake growth potential can be considered like that of cod.

Once the optimal conditions to achieve spawning in captivity were described (Iglesias *et al*., 2010), European hake farming depends on defining larval rearing protocols as well as determining the growth rates during the first months of life.

In previous studies. we have described hake eggs and larvae characteristics (Sánchez *et al*., 2011), the larval ontogeny during yolk sac consumption (Ortiz-Delgado *et al*., 2012), the optimal prey size in larval first feeding (Iglesias *et al*., 2011) and the effect of the lipid droplet adherence on larval growth and survival (Iglesias *et al*., 2013). In this work, we describe the larval rearing technology and present for the first time, the growth in length and weight of the European hake larvae up to 105 days after hatching.

MATERIALS AND METHODS

In June 2008, wild adult hakes were caught in the Ría of Vigo (Northwest Spain) at a depth of 35 m using the methods described by Iglesias *et al.* (2010). The first spontaneous spawning took place in April 2009 (Sánchez *et al*., 2011) at the Spanish Institute of Oceanography facilities.

Naturally fertilized eggs (40000, equivalent to 267 eggs L-1) were incubated in a 150 L circular tank with an open water system and central outlet consisting of a vertical cylinder with 500 µm mesh and bottom central aeration. Water temperature throughout the embryonic development period was 14 ± 0.5ºC with a salinity of 34 ± 1.1 psu. After hatching, 16000 larvae were transferred to a 500-L capacity circular rearing tank at an initial density of 32 larvae L-1. A black walled-and-bottomed tank was used, provided with a central water outlet (PVC vertical cylinder with 500 µm mesh) and gentle aeration. Water inflow was 2 L min-1, and a light intensity of 600 lux at the water surface was supplied with a daily photoperiod of 12L:12D throughout the larval rearing process.

Following the recommendations of Iglesias *et al*. (2013) to estimate larval quality at hatching, two different types of hake larvae were sampled: those with a lipid droplet adhered to the posterior side of yolk-sac (good quality) and larvae with a lipid droplet moving freely within the yolk-sac.

Live food enriched with phytoplankton (see below) was offered from day 6 post-hatch on. Rotifer *Brachionus plicatilis* Müller, 1786 (2 ind. mL-1) and *Artemia franciscana* nauplii Kellog, 1906 (0.2 ind. mL-1), from Salt Lake City (Inve Aq. INC.), were provided at first feeding. From day 20 to 60 only *Artemia metanauplii* (0.4-1.0 ind. mL-1) were used and their size was later increased to 1.5-2.0 mm. A "green water system" was used in the first week, consisting of 150.000 cells mL-1 of *Isochrysis galbana* and 500000 cells mL-1 of *Nannochloropsis oculata* in order to achieve adequate tank turbidity and better nutritional prey conditions. From day 8 after hatching an open water system (4 h day-1) was used until the end of the experiment. A dry commercial pellet (Gemma 0.8 mm, from Skretting SA) was provided as a complement to the Artemia from day 55 on. The period of co-feeding of both diets lasted 8 days, during which Artemia was gradually decreased until being eliminated from the diet.

During the first two months, the hake larvae were individually measured (total length in mm and dry weight in mg) every ten days, using 15 individuals per sample. The dry weight of 15 larvae was recorded individually using a Mettler UM3 microbalance (0.000001g accuracy) after being kept in an oven for 24 h at 90 °C. In the post-larval stage, the TL (mm) and wet weight (g) were determined, and survival was estimated at 60, 90, and 105 days.

Specific growth rate (SGR, % day-1) was calculated according to the formula:

$$SGR = 100 * (LnWn - LnWn-1) / (tn - tn-1)$$

where Wn is the dry weight (mg) at day n,

Wn-1 is the dry weight (mg) at day n-1 and tn and tn-1,

are the days of culture at times n and n-1, respectively.

The length and dry weight growth equations for the first two months were:

$TL = a.eb.d$, where TL is total length (mm) and d is age (days)

W = a.eb.d, where W is dry weight (mg) and d is age (days)

The Length/Weight relationship for European hake larvae was calculated as:

W = a.TLb, where W is the dry weight in mg and TL the total length in mm.

Correlation indexes were determined for all length and weight equations.

r = SXY / SX * SY

where SXY is the covariance of (X,Y), SX is the variance of X and SY is the variance of Y

Cannibalism was visually monitored daily in the culture tank and once this phenomenon was observed, a four-day experiment was designed to study in detail how the processes of prey attack and ingestion were taking place. Finally, the existence of this phenomenon in hake larvae was graphically depicted. For this, at day 80 after hatching a larger specimen was removed from the culture tank and placed with 4 smaller fish from the same larval tank to monitor daily mortality.

RESULTS

Hatching took place four days after incubation at 14ºC and the hatching rate was 78%. At hatching, the group of larvae with adhered lipid droplets (good quality larvae, sensu Iglesias *et al.*, 2013) represented only 20% of the total larvae sampled, which, as we discuss later, will substantially influence the subsequent larvae survival.

The yolk sac was consumed six days after when exogenous feeding began. Larvae' total length and dry weight at hatching were 3.28 ± 0.11 mm and 0.059 ± 0.003 mg, respectively. One month later, larvae reached 6.08 ± 0.56 mm and 3.55 ± 0.22 mg and at day 58, they achieved 17.66 ± 1.22 mm and 7.30 ± 1.65 mg length and weight, respectively. The growth regression equations (n=75) were: TL = 2.9998 e 0.0292.d, r2 = 0.96, and W = 0.0244 e 0.0982.d, r2 = 0.98 (Figs. 1 and 2).

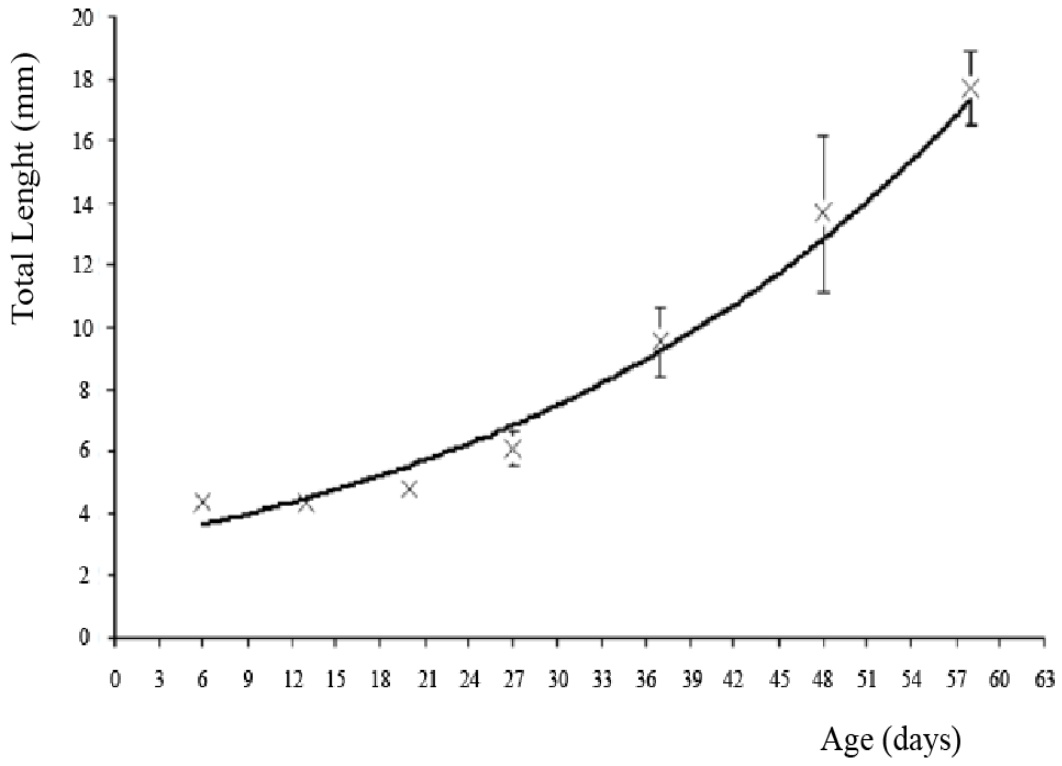


Figure 1. Growth in total length (mm) of European hake larvae reared for two months in captivity. Data are means ±SD.

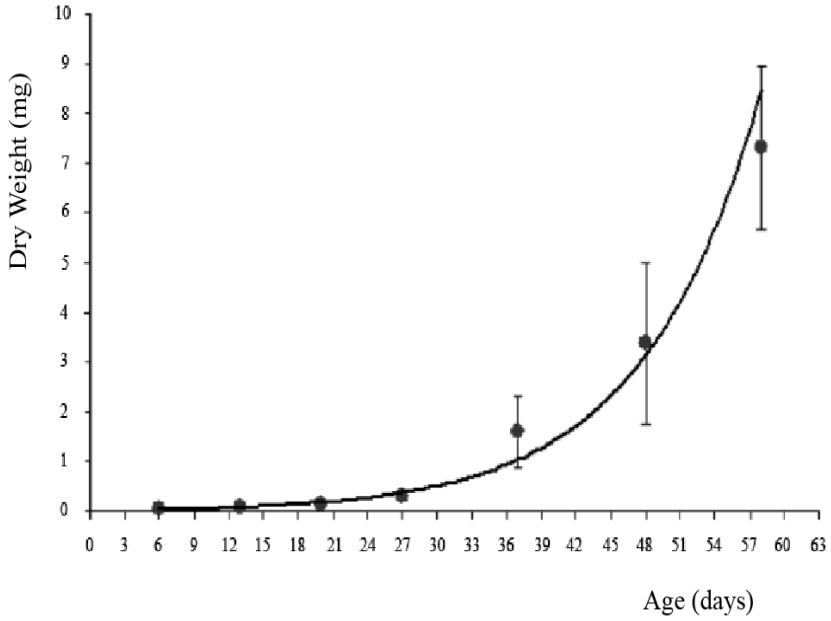


Figure 2. Growth in dry weight (mg) of European hake larvae reared for two months in captivity. Data are means ±SD.

During the first month, the specific growth rate ranged from 5.89% (the first two weeks) to a maximum of 16.15% at day 37 after hatching. However, after weaning, when live prey was gradually replaced by dry pellets, the SGR was substantially reduced until reaching 7.65% at the age of 58 days.

The equation defining the length/weight relationship for the first two months of the European hake larvae was: W = 0.0006.TL3.3085; r2 = 0.97 (Fig. 3).

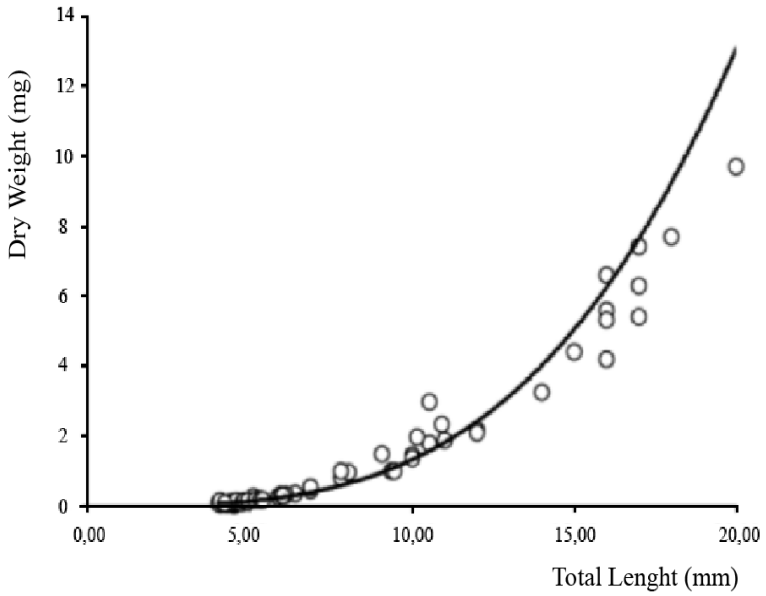
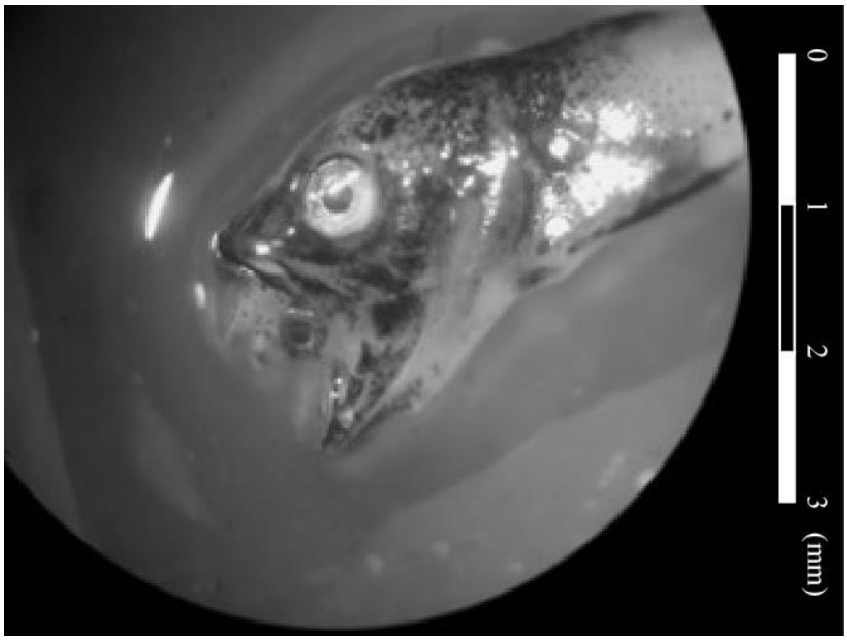


Figure 3. Length-weight relationship of European hake larvae reared from hatching to 58 day-old.

Larval survival at two and three months after hatching was 12 and 8% respectively. Cannibalism was first observed on day 35 and from this moment onwards, mortality continued to rise gradually due to the high degree of cannibalism observed, with only the most predatory individuals surviving.

This fact was confirmed in the cannibalism experiment where the largest specimen consumed the four smaller ones over a period of four days (eating one per day). It was observed that the predator first attacked the tail of its prey and then ingested the entire body of the smaller fish by mean of 3-4 successive suction movements (Photograph 1). The mean total length and wet weight of the last three survivors (105 days old), were LT = 52 ± 4.20 mm and W = 9.72± 2.32 mg, respectively.



Photograph 1: Larger hake juvenile (80 days after hatching), ingesting the entire body of a smaller specimen in the cannibalism experiment.

DISCUSSION

Hatching took place after 4 days of incubation at 14ºC and yolk-sac consumption occurred 6 days after hatching (dah) when exogenous feeding began. These data fit very well with those of Sánchez *et al.* (2011) working with this species at the same temperature in Spain and those reported by Bjelland and Skiftesvik (2006) in Norway. However, Bustos and Landaeta (2005) have indicated 9 days for the complete yolk-sac absorption in Merluccius australis (Hutton, 1872) in Chile, but those larvae were reared at a lower temperature (11.5ºC).

This work demonstrates that it is possible to rear European hake larvae for two months using rotifer in co-feeding with Artemia nauplii at first feeding. Other authors like Bjelland and Skiftesvik (2006), used rotifer and wild zooplankton nauplii as initial prey and Iglesias *et al*. (2011) report that from day 9 after hatching rearing on Artemia nauplii can be used as the only prey with similar results at first feeding.

The length and weight data reported in this work agree with previous experiments done under laboratory conditions (Bjelland and Skiftesvik, 2006; Sánchez *et al*., 2011; Iglesias *et al*., 2013). In relation to the wild population, Palomera *et al.* (2005) reported a length growth equation (Standard length = 2.092 e0.04.d) similar to the one provided in this study (TL= 2.9998 e 0.0292.d).

As has been observed in wild populations, the high specific growth rates were observed during the first month (5.89-16.15%) corresponding with the period when live food (rotifer and Artemia) is used. However,

in our study, when live prey was replaced by inert particles, SGR was reduced gradually until reaching 7.65%. Hake post-larvae did not accept easily dry pellets and high mortalities occurred during the weaning phase. Bustos and Landaeta (2005) also found an important growth rate reduction (0.08 mm L per day) working with Merluccius australis in Chile. Feeding ad-libitum on forage fish older will-caught juveniles, Jolivet *et al*. (2012) obtain specific growth rates between 0.029 and 0.299% per day. Therefore, additional research is needed on the nutritional requirements of juvenile hake to develop an optimal dry pellet.

We are currently testing a new feeding strategy using an intermediate larger prey (of about 2 cm long) between the Artemia and dry pellet stages, the mysidaceans: *Siriella armata* (Milne-Edwards, 1837) and *Leptomysis* sp., which are very well-accepted by hake postlarvae. As provisional results, we can advance that they improve the survival rate in the weaning phase, but this experience is still ongoing.

Very few comparisons can be made on hake larval survival. Our results of 12% survival at two months after hatching are the most favorable of those found in the literature. Bustos and Landaeta (2005) grow Merluccius australis larvae for up to only 45 days with no data on survival. Palomera *et al*. (2005) made a wide description of the wild larvae of *M. merluccius*, but they do not cite survival data either. Bjelland and Skiftesvik (2006) grow *M. merluccius* larvae until they reach the weaning stage, to the age of 50 days after hatching. Jolivet *et al*. (2012) give higher values of survival rates (14-65%) but these are for wild older juveniles (30-40 cm) of *M. merluccius* caught at sea and acclimatized to laboratory conditions.

Bustos *et al*. (2007) for M. australis and Iglesias *et al*. (2013) for *M. merluccius* described two different types of hake larvae at hatching: those with a lipid droplet adhered to the posterior side of yolk-sac (strong larvae, good quality) and larvae with a lipid droplet moving freely within the yolk-sac (weak larvae).

These authors referred that a high percentage of non-adhered lipid droplet larvae at hatching results in high mortality in the first fifteen days of larval rearing. Since 80% of the hatched larvae at our work had non-adhered lipid droplet, this could be one of the main reasons of the mortality observed during the first two months.

On other hand, the mortality observed after the second month may be a consequence of the need for larger prey at that age. Morote *et al*. (2011) mentioned that during larval growth, wild hake larvae increase the number of prey rather than selecting a larger prey. However, we have observed that after two months, European hake is a very voracious fish that shows cannibalism, with large specimens attacking smaller ones in the rearing tank. Cannibalism was first observed in hake larvae at day 35 and is the first mention of this phenomenon in so early larval stages. Therefore, this factor should be considered important for future research on hake larvae culture.

The main conclusions of this work were: Two-month-old larvae reached a total length of 17.66 ± 1.22 mm and a dry weight of 7.30 ± 1.65 mg, with a 12% survival rate. The total length and wet weight of the last survivors (105 days old), were 52 mm and 9.72 mg, respectively. The length and dry weight growth equations and the length-weight relationship were estimated for the first two months of life. Cannibalism was observed from day 35 onwards. Larval survival at two and three months after hatching was 12% and 8% respectively. The three main causes of mortality were: the poor larval quality at hatching, the lack of a suitable dry pellet at weaning, and the negative effect of cannibalism.

Acknowlegements

This research was funded by the CULMER project of the Spanish Institute of Oceanography. We would like to thank all technicians from the IEO aquaculture station at Vigo, for their invaluable help, without which it would not have been possible to carry out this work.

REFERENCES

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

Bustos, C.A. and M.F. Landaeta. 2005. Desarrollo de huevos y larvas tempranas de la merluza del sur, *Merluccius australis*, cultivados bajo condiciones de laboratorio. Gayana 69, 402-408.

Bustos, C.A., M.A. Landaeta, E. Bay-Schmith, R. Lewis and X. Moraga. 2007. Effects of temperature and lipid droplet adherence on mortality of hatchery-reared southern hake Merluccius australis larvae. Aquaculture 270, 535-540.

de Pontual, H., A. Groison, C. Piñeiro and M, Bertignac. 2006. Evidence of underestimation of European hake growth in the Bay of Biscay, and its relationship with bias in the agreed method of age estimation. ICES Journal of Marine Science 63, 1674-1681.

Iglesias, J., M.J. Lago, F.J. Sánchez and R. Cal, 2010. Capture, transport and acclimatization to captivity of european Hake (*Merluccius merluccius* L.). Preliminary data on feeding and growth. Aquaculture Research 41, 607-609.

Iglesias, J., M.J. Lago, J.J. Otero, F.J. Sánchez and R. Cal. 2011. Tamaño de presa optimo en la primera alimentación de las larvas de la merluza europea, *Merluccius merluccius*. In: Actas of XIII Congreso Nacional de Acuicultura. P- 017. Castelldefels. Spain. 21/1172011-23711/2011.

Iglesias, J., M.J. Lago, J.J. Otero, C. Gómez, R. Cal and F.J. Sánchez. 2013. Effect of the lipid droplet adherence on growth and survival of the European hake (*Merluccius merluccius*) larvae. Aquaculture Research. DOI:10.1111/are.12121.

Jolivet, A., H. de Pontual, M. Hervy, Y-M. Paulet and R. Fablet. 2012. Preliminary observations of survival and growth of European hake in captivity. Aquaculture Research 43, 949-954.

Morote, E., M.P. Olivar, A. Bozzano, F. Villate and I. Uriarte. 2011. Feeding selectivity in larvae of ther European hake (*Merluccius merluccius*) in relation to ontogeny and visula capabilities. Mar. Biol. 158: 1349-1361.

Ortiz-Delgado, J.B., J. Iglesias, F.J. Sánchez, R. Cal, M.J. Lago, J.J. Otero and C. Sarasquete. 2012. A morphohistological and histochemical study of hatchery-reared European hake, *Merluccius merluccius* (Linnaeus, 1758), during the lecitho-exotrophic larval phase. Scientia Marina 76(2), 259-271.

Palomera, I., M. P. Olivar and B. Morales-Nin. 2005. Larval development and growth of the European hake *Merluccius merluccius* in the northwestern Mediterranean. Scientia Marina 69(2), 251-258.

Piñeiro, C., J. Rey, H. de Pontual, R. Goñi. 2007. Tag and recapture of European hake (*Merluccius merluccius* L.) off the Northwest Iberian Peninsula: first results support fast growth hypothesis. Fisheries Research 88, 150-154.

Sánchez, F.J., J.J. Otero, R. Cal, M.J. Lago, C. Gómez and J. Iglesias. 2011. The first spontaneous spawning of European hake *Merluccius merluccius* L.: Characteristics of eggs and early larval stages. Aquaculture Research 43: 1729-1733.

Recibido: 01-03-2022
Aprobado: 02-12-2022
Versión final: 07-12--2022