Scientia Marina 78(3)
September 2014, 371-375, Barcelona (Spain)
ISSN-L: 0214-8358
doi: http://dx.doi.org/10.3989/scimar.04034.28D

Culture viability of *Sardina pilchardus* (Fish, Teleost): Preliminary results of growth in captivity up to 18 months

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Summary: Larvae of *Sardina pilchardus* were obtained in captivity from fertilized eggs captured in the wild and grown in a 10000-L tank. *Isochrysis galbana, Artemia franciscana* nauplii and live zooplankton were used as prey during the first two weeks; afterwards, the animals were fed on *Artemia* metanauplii enriched with *Isochrysis galbana*. A dry feed (Gemma 0.4 and 0.8) from Skretting S.A. (Burgos, España) was supplied from the 3rd to the 18th month. The total length reached by sardines at one year of life was 162.02±9.49 mm, corresponding to a wet weight of 36.12±10.82 g. Total length of the last survivor individual at 18 months was 182.37 mm. An 18-month experiment of sardine culture is described for the first time, and the growth data reported can help to determine its potential as a candidate for marine aquaculture.

Keywords: *Sardina pilchardus*; sardine; culture viability; larval rearing; juvenile growth; feeding behaviour; swimming behaviour.

Viabilidad del cultivo de *Sardina pilchardus* (Peces, Teleósteos): Resultados preliminares de su crecimiento en cautividad hasta los 18 meses

Resumen: Larvas de *Sardina pilchardus* fueron obtenidas en cautividad a partir de huevos fecundados capturados en el mar y cultivadas en un tanque de 10000 L. *Isochrysis galbana*, nauplios de *Artemia franciscana* y zooplancton vivo fueron utilizados como presa durante las dos primeras semanas; a partir de ahí, las sardinas fueron alimentadas con metanauplios de *Artemia* enriquecidos con la microalga *Isochrysis galbana*. Los piensos secos (Gemma 0.4 and 0.8) de la compañía Skretting S.A. (Burgos, España) fueron suministrados desde el tercer mes hasta el final de la experiencia (18 meses). La longitud media total alcanzada por las sardinas al año de vida fue de 162.02±9.49 mm, correspondiendo a un peso húmedo de 36.12±10.82 g. La longitud total a los 18 meses fue 182.37 mm. En este trabajo se describe por primera vez un experimento de larga duración (18 meses) de crecimiento de sardina cultivada y los datos aportados pueden contribuir a determinar su interés potencial como candidata para la acuicultura marina.

Palabras clave: *Sardina pilchardus*; sardina; viabilidad del cultivo; cultivo larvario; crecimiento de juveniles; comportamiento alimentario; comportamiento natatorio.

Citation/Como citar este artículo: Iglesias J., Fuentes L. 2014. Culture viability of *Sardina pilchardus* (Fish, Teleost): Preliminary results of growth in captivity up to 18 months. Sci. Mar. 78(3): 371-375. doi: http://dx.doi.org/10.3989/scimar.04034.28D

Editor: E. Mañanos.

Received: February 07, 2014. **Accepted**: June 04, 2014. **Published**: July 28, 2014.

INTRODUCTION

One of the most representative species on the Spanish coast regarding fishing and human consumption is the sardine, *Sardina pilchardus* (Walbaum, 1792). It is a pelagic species with a wide distribution extending in the north-east Atlantic from the Celtic Sea and North Sea in the north to Mauritania in the south. Populations of Madeira, the Azores and the Canary Islands are at the western limit of the distribution (Parrish et al. 1989). Sardine is also found in the Mediterranean and Black Seas. According ton the Food and Agriculture Organization (FAO), the world catch of *S. pilchardus* in 2011 was 1036708 t. Spain and Portugal have a major international fishing fleet dedicated to the capture of this species; current knowledge about the identity of

the sardine Iberian stock was studied by Riveiro et al. (2012). During the last few decades, sardine catches in the Atlantic Iberian stock (ICES, International Council for the Exploration of the Sea, subdivisions VIIIc and IXa) have shown some fluctuations, peaking in 1981 at 217000 t, and thereafter showing a general decrease. As a result of the decline of the population, the sustainability of the fishery is considered at risk and in its latest advice ICES recommended a drastic reduction in fishing effort (ICES 2013). The scarcity of the species led to a huge increase in the price of sardines in 2013, which exceeded that of hake in some periods of the year (source: www.pescadegalicia.com). This fact encouraged us to analyse the culture viability of this species.

Laboratory experiments using wild sardine specimens have dealt with acclimation to captive conditions (Marcalo et al. 2008), induction to spawning (Olmedo et al. 1990), embryonic development (Miranda et al. 1990) and larval rearing (Blaxter 1969, Miranda et al. 1992, Silva and Miranda 1992). Garrido et al. (2007, 2008) described both the feeding behaviour of sardines under culture conditions, and the diet and food intensity in the wild. However, none of these studies has focused on long-term growth in captivity.

The main objective of this paper was to study the growth of sardine, *Sardina pilchardus* under culture conditions, from hatching to adult stage, in order to determine the culture viability of the species.

MATERIALS AND METHODS

Eggs and larvae source

Larvae used in this experiment were from fertilized eggs collected from the wild with oblique trawls of a zooplankton net in the inner part of the Ria de Vigo (NW Spain) on 23 May 2010. Each trawl lasted 10 min, the depth of the water column was 9-15 m, water temperature was 17.5°C and salinity was 34.5.

A 250-μm zooplankton net of 2 m diameter was used with a final cod end of 200 μm mesh. After ruling out on board the fraction greater than 2 mm, the zooplankton retained was transported in seawater to the facilities of the Spanish Institute of Oceanography (IEO) in Vigo.

Rearing conditions

Around 40000 sardine eggs (estimated by volumetric counting) were placed in a 10000-L cylindrical tank (2.60 m diameter and 1.9 m water depth) provided with central aeration, a surface inlet and a lateral surface cylindrical outlet (60 cm high, 300 μm mesh), which was used for both incubation and larval culture. According to Russell (1976), this species has large eggs (1.30-1.90 mm diameter) with a large perivitelline space, oil globule and segmented yolk. When they reached the IEO (23 May 2010) most of these eggs (80%) were in gastrula stage, although 20% were in blastocyst and embryonic stage. After three days, almost all eggs had hatched; therefore, 26 May was considered the sardine larvae hatching date in this experiment. Hatching temperature was 19.2°C.

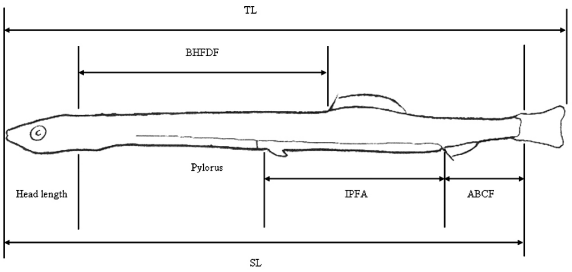

Fig. 1. – Diagram of sardine postlarva to illustrate body measurements.

Daily supply of *Isochrysis galbana* (Parke, 1949) was performed in order to maintain a "green water" system with an approximate concentration of 20×10^4 cells mL^{-1}. One to 6 million *Artemia franciscana* (Kellog, 1906) nauplii were delivered in four daily doses during the first weeks of larval culture. In addition, live wild zooplankton was added once a week. Thereafter, Artemia metanauplii enriched with *I. galbana* were added. From the third month onwards, only a dry feed, Gemma 0.4-0.8 (Skretting España S.A., Burgos) was supplied using surface feeders.

A daily partial water replacement was carried out during the first three months by opening the circuit with a water flow of 4 L min^{-1} for 4 h. Afterwards, an open water system was used. Natural photoperiod was applied (16:8 light: dark). Ambient temperature (13-20°C) was used, and the salinity range was 33.5-35.0. The oxygen, ammonium and nitrite levels were recorded daily, and pH was determined weekly.

Feeding and swimming behaviour

Observations of feeding and swimming behaviour of the sardines (shoaling and distribution in the tank) were conducted throughout the culture process.

Growth sampling

The first length sampling was performed at 23 days of age (n=5). Samplings were taken every 5 days up to day 55 of life. Subsequently, samplings were conducted every 15 days (up to day 160) and thereafter on a monthly basis until one year. After one year old, the specimens' total length (TL) and wet weight were recorded sporadically.

Distance from back of head to first dorsal fin ray, distance from anus to base of caudal fin, insertion of pelvic fin to anus, standard length and TL (Fig. 1) were recorded individually. In the first two months measurements were made under the microscope, and from day 85 onwards, a digital ichthyometer and a caliper were used.

Based on recorded TL data, a growth curve and its corresponding equation were obtained.

Sampled specimens were individually preserved in absolute alcohol. An additional study of age determination and validation based on otolith readings is being

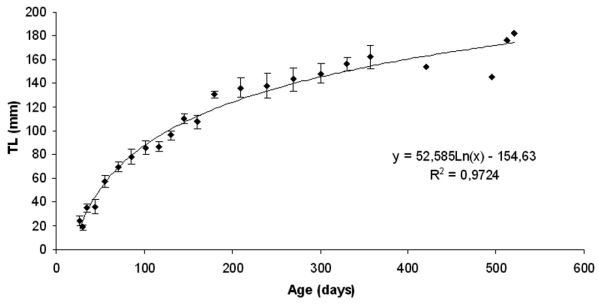

Fig. 2. – Growth in total length (TL) of *Sardina pilchardus* in captivity up to 18 month of life.

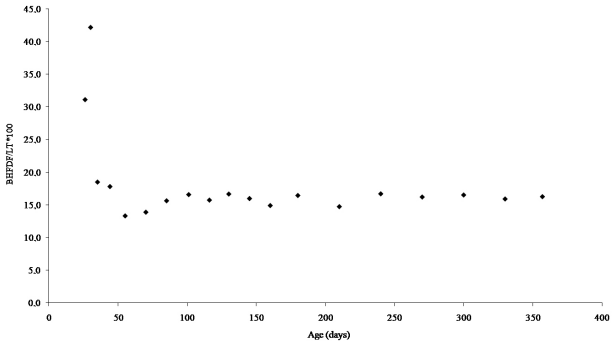

Fig. 3. – Variation with age of the relationship between back of head to first dorsal fin ray distance (BHFDF) and total length.

drawn up in collaboration with the ICES ageing group at the IEO.

To avoid interfering in the growing conditions of this 18-month experiment, survival was not determined; only qualitative estimations based on visual observations were conducted.

RESULTS

Artemia nauplii and metanauplii, wild zooplankton, and even dry feed supplied in the present study were well accepted by the sardine larvae and juveniles; this fact was inferred by the drastic reduction in the prey density (clearance) recorded daily in the culture tank. The microalgae *Isochrysis galbana* also contributed to the sardine diet but in a smaller proportion. However, mortality was also very high, being observed a reduction from four to less than one larvae per litre during the first month.

At one month old the sardines behaved erratically, swimming in small groups throughout the tank; this behaviour changed when food was provided only in a feeding area, with sardines forming a single group around it; however, this reaction was not observed when a homogeneous distribution of preys was produced by increasing the aeration intensity.

During the second and third month of life, sardines began to swim in a loose single shoal, swimming continuously upstream (against the flow). This behavioural pattern was only modified when a disturbance occurred in the tank (sampling, food supply, etc.).

From the third month and especially from one year old when sardines already fed on dry feed, they showed a very active feeding behaviour. The sampling process was particularly difficult during this period because of the sardines' elusive behaviour, which occasionally caused some individuals to jump out of the culture tank.

The highest peak in mortality was observed during the larval rearing period. Afterwards, the sardines continued to die at a lower rate until one year of life, when there were only 15 live individuals left. Thereafter, 1-2 specimens died every month until the end of the experiment. Throughout the ongrowing process, it was observed that certain individuals showed an abnormal head with a flattened nose due to collision and friction against the tank walls, which did not seem to affect their survival.

The growth in length (TL) from hatching to 18 months of life in captivity is shown in Figure 2; sardines reached a mean value of 162.02±9.49 mm at one year old and 182.37 mm at 18 months old. The growth equation during this period was:

$$y = 52.58\ Ln(x) - 154.6;\ R^2=0.972$$

Table 1 shows the evolution of the measures recorded during the experiment. It is noteworthy that the back of head to first dorsal fin ray distance varied substantially throughout the life cycle of the sardine (Fig. 3). From hatching up to the first month of life, this distance was large and represented nearly 40% of the larvae's TL, decreasing considerably until the second month to about 15-18% of the TL; thereafter, these values remained constant during the post-larvae, juvenile and adult stages.

Wet weights reached at 10, 12 and 18 months were 29.5±5.92, 36.12±10.82 and 37.37 g, respectively.

DISCUSSION

This study verified that sardine larvae and juveniles eat actively in captivity Artemia nauplii and metanauplii, wild zooplankton, and even dry feed. Previous studies based on stomach content have

Table 1. – Summary of all measurements taken during the sardine growth experiment. Age (m): age in months; TL (mm): total length; SL (mm): standard length; BHFDF (mm): back of head to first dorsal fin ray; ABCF (mm): anus to base of caudal fin; IPFA (mm): insertion of pelvic fin to anus.

Age (m)	TL (mm)	SL (mm)	BHFDF (mm)	ABCF (mm)	IPFA (mm)
1	23.80±4.27	19.84±5.54	7.40±1.08	3.96±1.04	6.93±1.44
3	78.17±6.40	63.83±6.52	12.20±1.30	15.83±2.56	16.00±1.41
6	130.40±2.97	107.00±4.69	21.40±0.50	26.80±1.64	28.00±0.55
9	143.4±9.91	124.60±8.35	23.20±3.30	29.40±3.13	30.60±3.08
12	162.02±9.49	141.18±9.95	26.30±2.10	35.83±3.54	38.55±2.22
18	182.37	155.44	27.97	41.41	43.10

shown that wild sardines have a very diverse diet. Garrido (2003) reported that stomach contents are volumetrically dominated by crustacean naupliar stages and small copepods, which fits with the data reported in our work, but also that occasionally the natural diet is numerically dominated by microplankton, especially chain-formed diatoms (99% during upwelling events). Cunha et al. (2005) also reported high numbers of phytoplankton cells, mainly diatoms and dinoflagellates, but these generally represent less than 10% of the total prey biovolume. The presence of phytoplankton (*Isochrysis galbana*) in the culture tank used in this work, which is characteristic of a green water system, also contributed to the sardine diet, though to a lesser extent.

To explain the use of both phytoplankton and zooplankton-based diet, Garrido et al. (2007) states that sardines use two feeding strategies and switch between them depending on prey size: filter-feeding when single phytoplankton cells and zooplankton <780 μm were introduced into the tank, and particulate-feeding when bigger preys were offered.

In previous culture experiments concerning sardine larval rearing, rotifer *Brachionus plicatilis* (Müller, 1786), Artemia and wild zooplankton (nauplii and juvenile copepods) were used as diet (Miranda et al. 1992, Álvarez 2002).

The behaviour observed in the first month of life, consisting of swimming erratically in small groups and suddenly concentrating in a single shoal when food is supplied, is a typical behaviour of clupeid species (Gibson and Ezzi 1990). The same occurs after the second and third month of life, when they swim continuously upstream forming a single shoal, showing a very active feeding behaviour even on dry pellets, similarly to sea bass and sea bream. Other authors have also used pellets to feed wild adult sardines captured from the sea (Garrido et al. 2007, Peleteiro et al. 2004).

Although survival estimation was not the main goal of this study (owing to the difficulty involved in estimating mortality in a tank as large as 10000 L), it is interesting to note that the highest peak in mortality was observed during the larval rearing period. Miranda et al. (1992), Silva and Miranda (1992) and Marcalo et al. (2008) also reported very low survival during the first feeding experiments on sardine larvae.

The mean growth values of 162.02±949 mm at one year old and 182.37 mm at 18 months of the present study are higher than those reported in wild individuals, whose age was estimated by Álvarez and Alemany (1997) on the basis of daily growth rings of the Galician sardine. This higher growth in captivity agrees with previous observations for other cultured species. For example, the overall growth rates obtained by Iglesias et al. (2010) for hake maintained in captivity were higher than those reported by Pontual et al. (2003) from tagged and released wild fish. However, the TL value reached at 18 months in captivity (182.37 mm), was virtually the same as the average value for the year class 1 (18.2 cm) contributed by ICES (2012) for the area corresponding to the geographical position of Galicia (IXa-N).

The wide variation observed (from 40 to 15% of the TL) throughout the life cycle in the distance from the back of head to the first dorsal fin ray is not cited in the literature for wild individuals but the biological meaning of this fact should be considered for future research.

In contrast with the length values reported in this study, the mean wet weights reached at 10, 12 and 18 months (29.5±5.92, 36.12±10.82 and 37.37 g, respectively) are lower than those quoted by ICES (2012) for the year class 1 (55 g). The reduced weight increase observed in captivity between months 12 and 18 could be attributed to the inappropriate commercial inert diet used in this study, which may not have been suitable for this species; in fact it is prepared by Skretting, S.A. as a weaning diet for marine fish larvae with a high protein requirement such as turbot. Therefore, further studies on this subject are still needed.

Considering the growth rates obtained in captivity, and the fact that commercial minimum size (11 cm TL) is achieved in approximately 6 months, it can be concluded that sardine appears to be an interesting candidate for aquaculture and therefore species cultivation is feasible. However, other aspects, such as survival estimations, inert diet improvement and culture process costs, must be analysed before a decision can be made on its viability. Nevertheless, culture experiments would be valuable for other research fields, such as age validation studies. In fact, when otoliths are analysed, this study could help to interpret growth data from field-collected individuals.

ACKNOWLEDGEMENTS

We appreciate the technical assistance of M. Calero and M.J. Lago from the IEO during the rearing process.

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