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Solea senegalensis
(Kaup, 1858) larvae
using a co-feeding
regime in Cantabria
(northern Spain)

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ISSN: 0212-1565

Núm. 186

Págs. 14

Madrid, España 2007



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El Instituto Español de Oceanografía agradece la colaboración de los evaluadores anónimos en la revisión crítica de esta publicación (*The Instituto Español de Oceanografía thanks its anonymous referees for their critical revision of this publication*).

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First results of rearing
Solea senegalensis (Kaup, 1858)
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Received March 2005. Accepted March 2007
Contributing Editor: Juana Rosa Cejas Pulido



Edita (Published by): INSTITUTO ESPAÑOL DE OCEANOGRAFÍA
Avda. de Brasil, 31 · E-28020 Madrid, España

ISSN: 0212-1565

Núm. 186

Págs. 14

Madrid, España 2007

FIRST RESULTS OF REARING *Solea senegalensis* (KAUP, 1858) LARVAE USING A CO-FEEDING REGIME IN CANTABRIA (NORTHERN SPAIN)

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ABSTRACT

The present paper describes the first trials in Cantabria (northern Spain) on the larval growth and survival of *Solea senegalensis* (Kaup, 1858), involving co-feeding with live and inert feed, which could condition larvae to better accept a commercial diet. The larval growth rate, in terms of length and dry weight, was similar up to day 70. The average partial growth rates during different time intervals break down as 14.53 % from days 0-22; 7.4 % from days 22-43; and 4.76 % from days 43-70, with the first 22 days of growth clearly being the most important period. Some significant differences in length were found on days 26, 33, 39 and 43. The average survival rate was high (81 %) at day 70. Weaning trial began on day 80, when the larvae had reached a wet weight of approximately 100 mg. Previously, the larvae had been redistributed into two kinds of tanks: elongated S tanks (450 l, 3 m²), and circular LE tanks (430 l, 1.54 m²), stocked at a density of approximately 1 000-1 500 larvae/m². At the end of weaning, significant differences were observed in length and wet weight between larvae weaned in the two kinds of tanks. In sum, these first results of breeding *S. senegalensis* in Cantabria, obtained at ambient temperature in a geographical setting different from the species's native range, indicate that *S. senegalensis* has good prospects as an alternative species for the region's aquaculture operations, since it is well accepted by consumers and enjoys a high market value.

Keywords: *Solea senegalensis*, larvae, growth, survival, co-feeding.

RESUMEN

Primeros resultados del cultivo larvario de *Solea senegalensis* (Kaup, 1858) en Cantabria (norte de España)

Se describen las primeras experiencias sobre el crecimiento y la supervivencia en larvas de *Solea senegalensis* (Kaup, 1858) en Cantabria (norte de España) utilizando un régimen que combina alimento vivo e inerte (coalimentación) que podría condicionar a las larvas a aceptar mejor una dieta comercial. La tasa de crecimiento en longitud y peso seco de las larvas fue similar desde el inicio del cultivo hasta el día 70. Las tasas parciales medias de crecimiento fueron 14,53 % en los días 0-22, 7,4 % en los días 22-43 y 4,76 % en los días 43-70, con mejor tasa de crecimiento durante los primeros 22 días. Se encontraron algunas diferencias significativas en longitud los días 26, 33, 39, 43 y 70. La tasa de supervivencia media a día 70 fue alta (81 %). La experiencia de destete comenzó el día 80, cuando el peso húmedo de cada larva era aproximadamente 100 mg. Previamente, las larvas fueron distribuidas en dos tipos de tanques: tanques alargados S, de 3 m² de planta y 450 l de capacidad, y tanques circulares LE, de 1,54 m² y 430 l, a una densidad de aproximada de 1 000-1 500 larvas/m². Al final del destete se encontraron diferencias significativas en longitud y peso entre las larvas destetadas en los dos tipos de tanques. En conclusión, estos primeros resultados del cultivo de *S. senegalensis* en Cantabria, realizado a temperatura ambiente y en una zona geográfica diferente a la natural de esta especie, indican las buenas perspectivas que ofrece *S. senegalensis* como especie alternativa en la acuicultura de esta zona, ya que es bien aceptada por los consumidores y tiene un valor comercial elevado.

Palabras clave: Lenguado senegalés, larva, crecimiento, supervivencia, coalimentación.

* Received March 2005. Accepted March 2007.

Contributing Editor: Juana Rosa Cejas Pulido.

1. INTRODUCTION

The commercial production of marine fish species in Spain focuses principally on three species: seabream, seabass, and turbot, whose aquaculture has reached an optimal level of development. Indeed, due to high production, local markets are becoming saturated, and therefore investigation of new species' aquaculture potential has become a key strategy for incorporating highly valued species into the market, such as the Senegalese sole *Solea senegalensis* (Kaup, 1858), one well adapted to temperate climates and which is extensively cultivated along the southern coast shared by Spain and Portugal (Drake, Arias and Rodríguez, 1984; Dinis, 1992; Dinis, Reis and Arrobas, 1996).

S. senegalensis has been under study for more than 20 years (Rodríguez, 1984; Dinis, 1986), although slowly and discontinuously, which is why the species has yet to reach its optimal level of industrial production. Even though its potential market value is high, limited information is available regarding its biology, nutritional requirements, and techniques for intensive rearing of *S. senegalensis* larvae (Vázquez et al., 1984; Dinis and Reis, 1995; Marín-Magán, Anguis and Cañavate, 1995; Mourente and Vázquez, 1996). Consequently, mortality in the transition from live to inert feed in sole larvae has been the major limitation on sole farming in this region. However, due to the economic interests involved, research has recently intensified regarding these aspects of the species's aquaculture (Dinis et al., 1999; Martínez et al., 1999; Ribeiro, Sarasquete and Dinis, 1999; Ribeiro et al., 1999; Yúfera et al., 1999; Parra and Yúfera, 2001; Fernández-Díaz et al., 2001; Howell et al., 2003; Imsland et al., 2003), mostly aimed at reducing its dependence on live feed, and searching for the ideal diet or weaning method (Cañavate and Fernández-Díaz, 1999; Dinis et al., 2000; Yúfera, Pascual and Fernández, 1999; Yúfera et al., 2001; Chereguini and Díez, 2003; Morais et al., 2004). The use of a combined live-inert diet during the larval phase could contribute not only to improving the larvae's nutrition, but also ease weaning by increasing acceptance of commercial feed and reducing dependence on live feed (Rosenlund, Stoss and Talbot, 1997; Ben Khemis et al., 2003).

Although *S. senegalensis* is abundant along the southern Iberian Peninsula, where most of these studies have been carried out, its distribution covers almost the entire coastline of Portugal and Spain, as far north as the Bay of Biscay, and therefore its aquaculture can be considered viable in most of these areas. In the Cantabrian Sea, and Iberian Atlantic generally, there is a growing need for an alternative species to the turbot *Scophthalmus maximus* (L., 1758), whose farming technology is already well developed, as well as the seabream *Pagellus bogaraveo* (Brünnich, 1768), so that there is high interest in embarking on large-scale sole aquaculture. Therefore, it is very important for us to learn the major zootechnical characteristics of those regions where sole farming has not been carried out to date.

The aim of the present project was to carry out the first experiments in Cantabria (northern Spain) on the larval growth and survival of *S. senegalensis*, in order to introduce this species as an alternative for the local aquaculture industry, given its high market value and popularity with consumers. These experiments have been conducted involving co-feeding with live and inert feed, which could better condition the larvae to accept a commercial diet.

2. MATERIALS AND METHODS

This study was carried out in the El Bocal Aquaculture Centre at the Instituto Español de Oceanografía (IEO) in the Cantabrian regional capital of Santander. Larval culture began with fertilized eggs from the Instituto de Formación Agraria y Pesquera de Andalucía (IFAPA) Centro El Toruño in Cadiz (southern Spain). Once hatched, the larvae were distributed into three circular 280 l polyester tanks with conical bottoms, at an initial density of 50 larvae/l, with aeration and water replacement systems at environmental temperature (16.1 ± 2 °C), running the water through 1 µm filters at a rate of 35 l/h. Once the larvae had completely absorbed their vitellin sacs and opened their mouths, the co-feeding phase using live and inert feed began. Before feeding, the water replacement system was turned off, and the alga *Nannochloropsis occulata* was introduced into the tanks at a density of 300×10^3 cells per ml. During feeding, the tanks were lighted constantly at 2000 lux on the surface until metamorphosis, at which point the lighting was changed to 16 h of light per 8 h of darkness until weaning. Continuous water inflow was maintained at a daily 20% exchange until metamorphosis, and increased gradually every six days to achieve a 100% exchange thereafter.

The live-inert feeding regime was carried out according to CIFPA protocol, as shown in table I. The rotifers *Brachionus plicatilis* (O. F. Müller, 1786) were previously enriched with *Isochrysis galbana* (Parke, 1949), and the *Artemia nauplii* were enriched with DHA Super Selco (INVE). The size of the dry feed was

$$\begin{aligned} M1 \text{ (OYC)} &= 100-150 \mu\text{m}; M2 \text{ (OYC)} = 150-350 \mu\text{m}; \\ M3 \text{ (OYC)} &= 350-650 \mu\text{m}; NRD 5/8 \text{ (INVE)} = 500-800 \mu\text{m} \end{aligned}$$

The rotifers were given once a day, and *Artemia nauplii* twice daily. The inert feed was distributed in four feedings per day, with an automatic feeding system.

In order to compare growth in terms of length and dry weight, we took samples of larvae from the three tanks on different days of the larval culture period to measure individually the total length of larvae, using a profile project Nikon V-12B. Dry weight was determined on pooled samples with several larvae, which were rinsed with distilled water and dried in a 60 °C oven after 24 h, using a Mettler MT5 microscale. Moreover, when the larvae reached a larger size their wet weight was also recorded on different days, eliminated excess water with filter paper.

After the 22nd day of culture, the larvae began to reach benthic size, and due to the high larval density in tanks 2, 4, and 6, half of the larvae in each was placed into a series of similar tanks until weaning. Mortalities were removed daily and survival rate was calculated.

When the larvae reached a wet weight of 100 mg, weaning experiment began, on the 80th day of culture. Previously, the larvae had been redistributed into two kinds of tanks: four elongated S tanks (450 l, 3 m²), and four circular LE tanks (430 l, 1.54 m²), stocked at a density of approximately 1 000-1 500 larvae/m². For another week, both Artemia and inert feeds were still given, after which the larvae received only inert feed. At first, the type of commercial feed used was NRD 5/8 (0.5-0.8 mm particles, from INVE), which was gradually replaced with Perla Plus 0 (0.9-1.4 mm particles, from TROUW) as the larvae grew in size. For comparison of growth in terms of length and weight, all postlarvae from both tank types were measured and weighed at the end of weaning. Mortality was also recorded.

2.1 Statistical analysis

The length data of larvae from the three tanks on different culture days were compared with an analysis of variance, and in those cases where a Kolmogorov-Smirnov test indicated that the data were not distributed normally ($p = 0.000$), nonparametric analysis was applied, using a Kruskal-Wallis one-way anova (SPSS program).

To determine whether there were differences in the rate of larval growth among the three larvae groups, larval growth (length and dry weight) was adjusted with the exponential equations

$$\begin{aligned} L_t &= L_0 e^{gt} \\ W_t &= W_0 e^{gt} \end{aligned}$$

where g is the growth rate.

Logarithms were used to convert these expressions into regression lines, which were analyzed with Student's t-Test, comparing slopes and elevations (Zar, 1984). The difference was considered statistically significant at $p < 0.05$.

Once the larvae had been weaned, the length and wet weight data obtained in both tank types were also compared with an analysis of variance, and in those cases where a Kolmogorov-Smirnov test indicated that the data were not distributed normally ($p = 0.000$), nonparametric analysis was applied, using a Kruskal-Wallis one-way anova (SPSS program).

3. RESULTS AND DISCUSSION

As shown in figure 1, no differences in length were found on days 0, 8, 14, 18 and 22 ($p = 0.3918$, $p = 0.2665$, $p = 0.1487$ and $p = 0.1518$, respectively). However, some significant differences were found on

days 26, 33, 39 and 43 ($p = 0.0297$, $p = 0.0172$, $p = 0.0043$, $p = 0.0022$, respectively). On days 50, 56 and 62 ($p = 0.5233$, $p = 0.2222$ and $p = 0.1142$, respectively), no differences were found, either. The mean dried weight of pooled larvae on different days of culture are shown in figure 2.

The average wet weights of the larvae from the three tanks on different days are shown in figure 3. At 70 days, differences in length were found but not in wet weight ($p = 0.0478$ and $p = 0.0888$, respectively).

The survival and partial growth rates in dry weight at different time intervals are shown in table II, where we see a mean survival rate of 81% on day 70, and the highest growth rate during the first 22 days. Larval growth rates dropped after metamorphosis in all three tanks, as observed in other studies on this species (Cañavate and Fernández-Díaz, 1999; Parra and Yúfera, 2001). However, these growth rates were somewhat lower than those reported by Cañavate and Fernández-Díaz (1999) and Fernández-Díaz et al. (2001) for the same time intervals at a temperature of 19–21 °C; this can be attributed to the fact that our study was conducted at the lower local ambient temperature (16.1 ± 2 °C), confirming once again the impact of temperature on this species's growth. In any case, the survival rate was similar.

Comparing the slopes of the regression lines for larval growth in terms of length and of dry weight until day 70, the growth rates were similar, as shown in figures 4 and 5.

Larval size showed high variability at day 70, as reported by other co-feeding studies (e.g. Rosenlund, Stoss and Talbot, 1997) involving various species, and which had been previously observed in the sole *Solea solea* (L., 1758), a morphologically similar species difficult to distinguish from *S. senegalensis*. As suggested by Howell (1997), such variability could be due to a high degree of interaction among individuals due to the feeding method used in those studies, which consisted of frequently giving small amounts of inert feed.

When weaning was over, significant differences in length and wet weight were found between the set of larvae weaned in one of the LE and the other ($p = 0.0000$ and $p = 0.0000$, respectively). However, those larvae weaned in the S tanks showed no such differences ($p = 0.4678$ and $p = 0.1010$). When all of the weaned larvae were compared according to the type of tank used, significant differences were found: larvae in the S tanks presented lower growth in terms of length and weight ($\bar{L}_s = 4.28 \pm 0.75$ cm and $\bar{W}_s = 0.97 \pm 0.56$ g), whereas larvae from the LE tanks were larger ($\bar{L}_s = 5.81 \pm 0.75$ cm and $\bar{W}_s = 2.93 \pm 1.66$ g), as shown in figures 6 and 7. As to the survival rate, it was double in the S tanks than in the LE tanks (Chereguini and Díez, 2003), although the values obtained in both were lower than those reported by other authors (Dinis, 1992; Cañavate and Fernández-Díaz, 1999).

In conclusion, these first results of larval culture of *S. senegalensis*, at the ambient temperature of a geographical area where it had not been previously farmed industrially, indicate its

aquaculture potential in this zone as an alternative species, one with a high potential market value. However, our results could be improved by working with higher temperatures of 19-21 °C, and by experimenting with new commercial feed during the weaning phase in order to improve the survival rate.

4. ACKNOWLEDGEMENTS

This study was supported by Spain's national development plan for sole farming, Desarrollo de los principales aspectos para el cultivo integral del lenguado (*Solea senegalensis*), financed by the national aquaculture advisory committee Junta Asesora de Cultivos Marinos (Jacumar), in conjunction with regional centers around Spain, of the IFAPA (Cadiz), IEO (Santander and Vigo), IRTA (Catalonia) and the Centro de Investigación y Desarrollo Agroalimentario (Murcia).

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Table I. Co-feeding sequence used during the larval rearing of *S. senegalensis*. The alga used was *Nannochloropsis occulata*. The dry feed sizes were: M1 = 100-150 μm ; M2 = 150-350 μm ; M3(OYC) = 350-650 μm ; NRD 5/8 (INVE) = 500-800 μm

Co-feeding regime (days)	Algae ($\times 10^6$ cells ml^{-1})	Rotifers (indiv ml^{-1})	<i>Artemia</i> (nauplii ml^{-1})	Dry feed
Tanks (2, 4, 6)				
3-9	0.3	20	-	M1
7-14	-	-	8	M1
15-22	-	-	12	M2
23-43	-	-	7	M2
44-70	-	-	7	M3; NRD 5/8

Table II. Partial growth rates in dry weight during different time intervals, and survival rates as measured on different days

Day	Growth rates (%)			Survival (%)			
	0-22	22-43	43-70	27	43	70	85
T2	13.23	7.25	6.5	100	100	94	57
T4	15.95	6.81	4.96	100	100	53	44
T6	14.42	8.12	2.83	100	100	96	94
Mean \pm SD	14.53 \pm 1.36	7.4 \pm 0.67	4.76 \pm 1.84	100	100	81	65

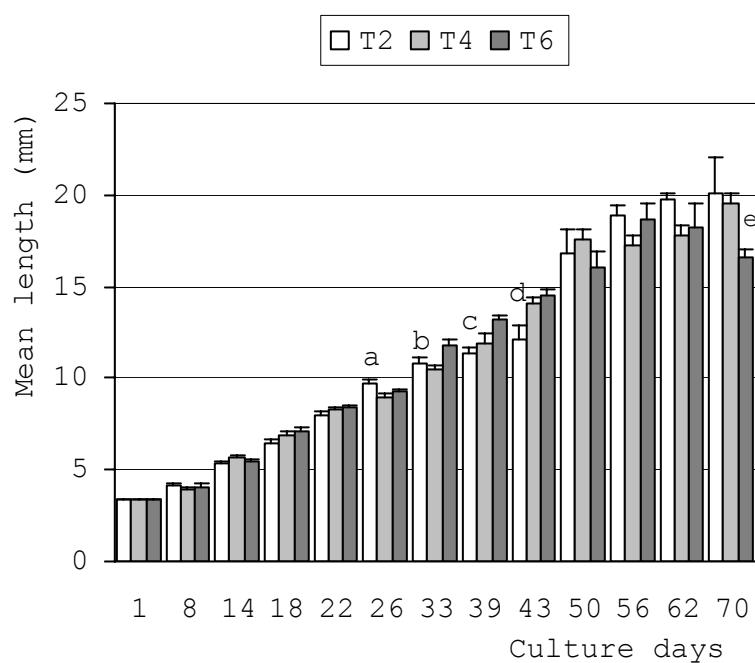


Figure 1. Mean and standard error of length of *S. senegalensis* larvae (mm) raised in three tanks, at different culture days. Each bar represents a different tank: white bars represent T2, dotted bars T4, and striped bars T6. Means with letters are significantly different

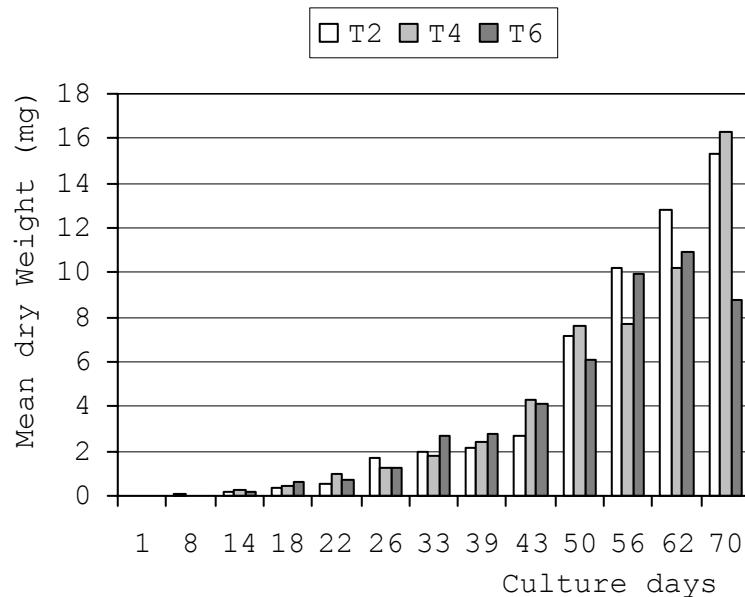


Figure 2. Mean dry weight of pooled *S. senegalensis* larvae (mg) raised in three tanks, at different culture days. Each bar represents a different tank: white bars represent T2, dotted bars T4, and striped bars T6

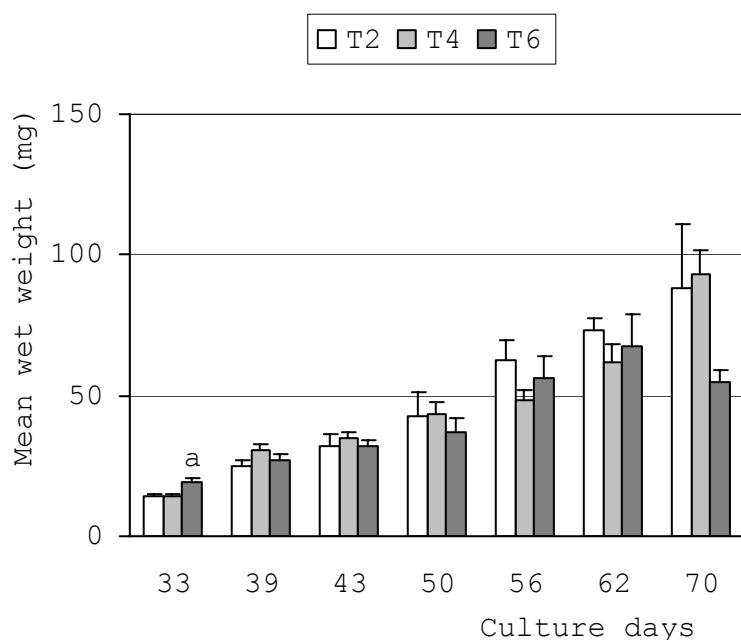


Figure 3. Mean and standard error of wet weight of *S. senegalensis* larvae (mg) raised in three tanks, at different culture days. Each bar represents a different tank: white bars represent T2, dotted bars T4, and striped bars T6. Means with letters are significantly different

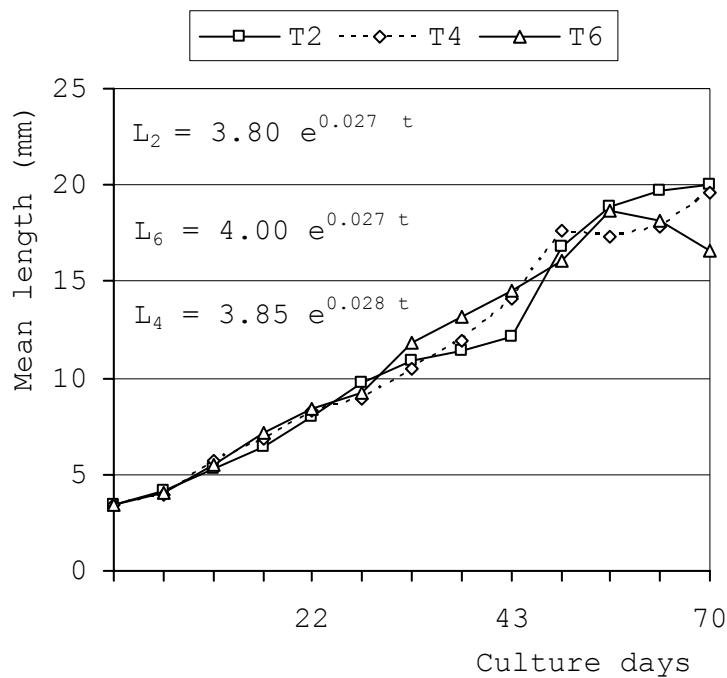


Figure 4. Larval growth, by length (mm), of *S. senegalensis* larvae raised in three tanks. Each line represents a different tank: the continuous line with squares represents tank 2, the line of dashes represents tank 4, and the continuous line with triangles represents tank 6

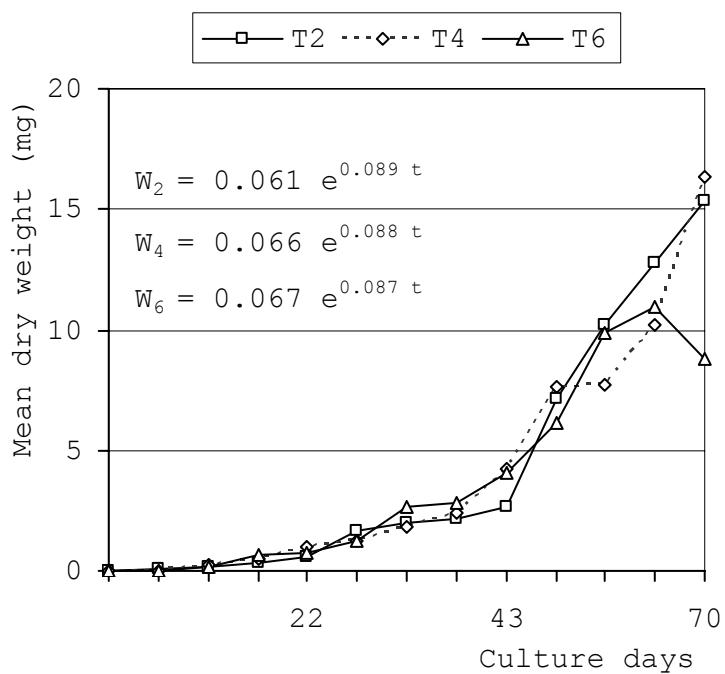


Figure 5. Larval growth, in dry weight (mg), of *S. senegalensis* larvae raised in three tanks. Each line represents a different tank: the continuous line with squares represents tank 2, the line of dashes represents tank 4, and the continuous line with triangles represents tank 6

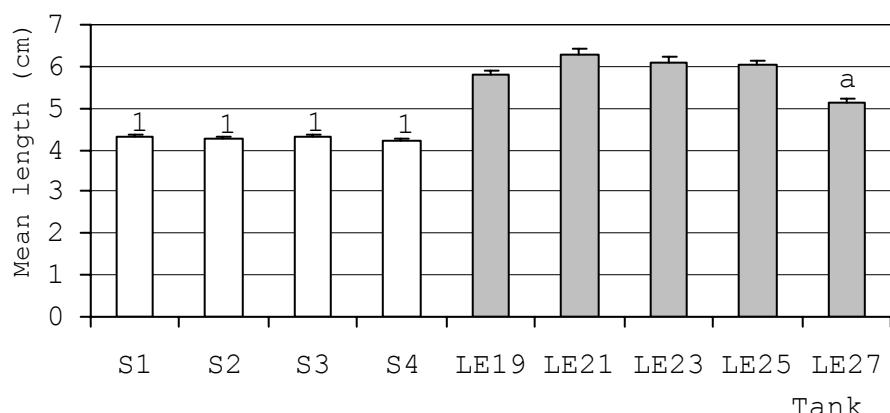


Figure 6. Mean and standard error of length of postlarvae (cm) weaned in two different tank types, S and LE. Each bar represents a different tank type: white bars represent the S tank, and dotted bars the LE tank. Means with letters are significantly different for the same kind of tank, and those with numbers are significantly different between the two types

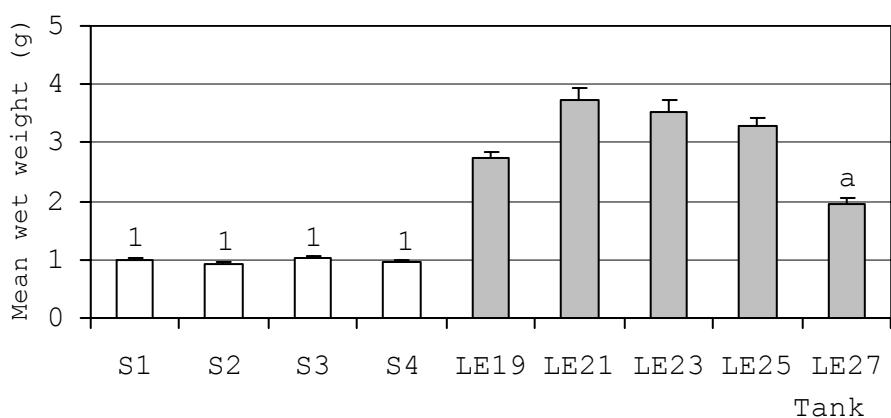


Figure 7. Mean and standard error of wet weight of postlarvae (g) weaned in the two different tank types, S and LE. Each bar represents a different tank type: white bars represent the S tank, and dotted bars the LE tank. Means with letters are significantly different for the same kind of tank, and those with numbers are significantly different between the two types

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GUÍA PARA LOS AUTORES

Idiomas

Se aceptarán originales en español o inglés, indistintamente.

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Los originales se presentarán mecanografiados en DIN-A4, a un espacio, dejando 2 cm de margen derecho, 2,5 cm de margen izquierdo, 3 cm de margen superior y 2,5 cm de margen inferior. El tipo de letra será Courier New 11.

El trabajo, cuando su naturaleza lo permita, se articulará en: RESUMEN; ABSTRACT; 1. INTRODUCCIÓN; 2. MATERIAL Y MÉTODOS; 3. RESULTADOS; 4. DISCUSIÓN; 5. AGRADECIMIENTOS y 6. BIBLIOGRAFÍA.

La numeración de los apartados comenzará en la introducción (1. INTRODUCCIÓN) y los títulos de los mismos se escribirán en mayúsculas, negrita y sin subrayar.

La paginación irá en el centro del margen inferior, empezando por la página 4 que será donde comience la introducción.

Entre apartados se dejarán tres líneas y entre un título y el principio de su texto correspondiente, dos.

En los puntos y aparte se dejará una línea y se comenzará a escribir, igual que el resto del texto, al principio del renglón.

Los subapartados se numerarán con dos, tres, cuatro,... cifras, dependiendo de su categoría y siguiendo su orden lógico correspondiente. Todos se mecanografiarán en minúsculas y sólo el primero en cursiva. Ej.:

4. DISCUSIÓN

4.1 Condiciones físicas del medio

4.1.1 Forma del recipiente

4.1.2 Aireación

4.2 Rendimientos

4.3 Aplicaciones

Los símbolos y signos químicos, físicos o matemáticos se escribirán siempre ateniéndose a las normas internacionales vigentes: SI (Sistema Internacional de Unidades), ISO (International Standard Organization) y UNE (Una Norma Española). Dichos símbolos, por tanto, se escribirán siempre sin punto y permanecerán invariables en plural. Las normas ISO y UNE servirán siempre de referencia en la elaboración de originales.

En español las mayúsculas también se acentuarán siguiendo las normas correctas de ortografía.

Para facilitar la lectura de los números de muchas cifras, éstas pueden separarse en grupos apropiados, preferentemente de tres cifras, a contar desde el signo decimal en uno y otro sentidos; los grupos deben ir separados por un pequeño espacio, pero nunca por un punto u otro signo.

El signo decimal es una coma en la parte baja de la línea. En los textos escritos en inglés puede utilizarse también un punto, siempre en la parte baja de la línea.

Los números que indiquen años tampoco llevarán punto pero, al contrario que en el caso anterior, en su lugar no se dejará ningún espacio. Por ejemplo: la forma correcta de escribir año mil novecientos noventa y nueve es 1999.

El nombre vulgar de las especies, cuando se citen por primera vez (en los títulos en español y en inglés, en el resumen, en el abstract y en el resto del texto), debe ir seguido de su nombre científico y éste, a ser posible, del nombre del autor que la describió y del año. En las veces posteriores en que aparezca el nombre de la especie no se volverá a citar ni autor ni año.

Irán en cursiva los nombres de géneros y especies, así como los nombres de revistas y simposios y los títulos de libros.

No se aceptarán llamadas a pie de página.

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Las cabeceras de las distintas páginas se presentarán como se indica en los ejemplos que aparecen a continuación. Irán dentro del margen superior de la página a 1,5 cm del borde superior, ocuparán el ancho de la mancha de texto (16,5 cm) y el tipo de letra será Times New Roman 8.

- Cabecera de la página 3:

Inf. Téc. Inst. Esp. Oceanogr. 167. 1997: 44 pp.

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- Cabecera de las páginas pares:

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J. P. Rubín et al.

- Cabecera del resto de las páginas impares:

El ictioplancton, el mesozooplancton y la hidrología

Inf. Téc. Inst. Esp. Oceanogr. 167. 1997: 44 pp.

- Cabecera de página de sumario, cuando el trabajo lo lleve:

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El título del trabajo en las cabeceras será un título abreviado.

Página de título, resumen y abstract

En esta primera página de texto (página 3 del trabajo) figurará el título del trabajo (en mayúsculas y negrita) y los nombres de los autores (en negrita y minúsculas), con su institución, dirección postal (calle, ciudad, país) y los correos electrónicos de todos los autores (en fina y minúsculas). A continuación resumen y abstract, que no excederán de 125 palabras cada uno, darán a conocer los objetivos del trabajo así como los procedimientos seguidos y los resultados y datos más significativos obtenidos. Ambos epígrafes se mecanografiarán en mayúsculas negrita, en el centro de la página y sin numeración. Al principio del abstract se incluirá, en negrita y minúsculas, el título del trabajo en inglés. Al final de cada apartado figurarán hasta un máximo de ocho palabras clave, no incluidas en el título y por orden de importancia, representativas del trabajo. Los epígrafes palabras clave y keywords irán también en minúsculas y negrita.

A pie de página, como llamada del título y en cursiva, figurarán las fechas de recepción y aceptación del trabajo así como la coordinación científica editorial.

El tipo de letra a emplear en resumen, abstract y direcciones será Courier New 9, y en las fechas de recepción y aceptación y en la coordinación científica editorial Courier New 8.

Introducción

La introducción no excederá de 500 palabras, indicará brevemente los objetivos del estudio y proporcionará suficiente cantidad de información como para aclarar el planteamiento del trabajo y la hipótesis que se pretende comprobar.

Material y métodos

Este apartado será lo más conciso posible pero deberá proporcionar toda la información necesaria para permitir a cualquier investigador especializado evaluar la metodología empleada.

Resultados

El apartado de resultados será lo más claro posible y se ceñirá a los resultados de la investigación esenciales para establecer los principales puntos del trabajo.

Discusión

Se incluirá una breve discusión sobre la validez de los resultados observados relacionándolos con los de otros trabajos publicados sobre el mismo asunto así como un informe sobre el significado del trabajo. Se desaconseja discusiones extensas sobre la literatura existente.

Bibliografía

La bibliografía se limitará a los trabajos citados en el texto y sólo figurarán en ella los trabajos publicados o "en prensa". Esta última información deberá indicarse, en lugar del año, entre paréntesis. Las referencias en el texto a los autores se harán citando el apellido del autor (en minúsculas) y a continuación, entre paréntesis, el año de la publicación, o bien poniendo entre paréntesis el(los) autor(es) y el año, separados por una coma. Las observaciones no publicadas, las comunicaciones personales o los trabajos en preparación o en evaluación se citarán exclusivamente en el texto, sustituyendo el año de publicación por "observación no publicada", "manuscrito" ("MS") o "inédito"; "comunicación personal" ("com. pers."); "en preparación" o por "en evaluación", respectivamente. Cuando la publicación sea de más de tres autores sólo se citará el primero de ellos y a continuación la abreviatura *et al.* En la bibliografía, sin embargo, aparecerán los nombres de todos los autores, separados por comas. Las referencias bibliográficas figurarán por orden alfabético y, para un mismo autor, por orden cronológico. Los nombres de las revistas se escribirán preferentemente sin abbreviar. Si se prefiere utilizar las abreviaturas, éstas se ajustarán siempre a lo indicado en el *Periodical Title Abbreviations*. 8.^a edición. Gale Research Inc. Detroit; Londres. 1992. Si esto no es posible se escribirán sin abbreviar.

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Ejemplos de citas bibliográficas:

- De una revista:

Guiry, M. D. 1974. A preliminary consideration of the taxonomic position of *Palmaria palmata* (Linnaeus) Stackhouse = *Rhodymenia palmata* (Linnaeus) Greville. *J. Mar. Biol. Ass. (UK)* 54: 509-529.

- De un libro:

Sinderman, C. J. 1970. *Principal diseases of marine fish and shellfish*. Academic Press. Londres; Nueva York: 870 pp.

- De un artículo de un libro que forma parte de una serie:

Fraga, F. y R. Prego. 1989. Condiciones hidrográficas previas a la purga de mar. En: *Las purgas de mar como fenómeno natural. Las mareas rojas* (Cuadernos da Área de Ciencias Mariñas). F. Fraga y F. G. Figueiras (eds.) 4: 21-44. Ediciós do Castro. Seminario de Estudos Galegos. Sada (A Coruña), España.

- De un artículo de un simposio:

Figueiras, F. G. y F. Fraga. 1990. Vertical nutrient transport during proliferation of *Gymnodinium catenatum* Graham in Ría de Vigo, Northwest Spain. En: *Toxic Marine Phytoplankton. Proceedings of the Fourth International Conference on Toxic Marine Phytoplankton* (26-30 de junio, 1989. Lund, Suecia). E. Graneli et al. (eds.): 144-148. Elsevier. Nueva York.

Los autores serán responsables de que todas las citas bibliográficas estén completas y de la exactitud de las mismas.

Tablas, figuras, láminas, mapas y fotografías o diapositivas

Todas las ilustraciones (figuras, láminas, mapas y fotografías o diapositivas) deben ser originales y se prepararán en archivos electrónicos independientes del texto. Sólo se incluirán aquéllas que muestren datos esenciales; nunca deberá producirse duplicidad de datos por la presentación de los mismos en texto, tablas e ilustraciones.

Las ilustraciones se presentarán con el tamaño definitivo de impresión, leyendas incluidas, teniendo en cuenta que la superficie que queda impresa es de 16,5 cm x 24 cm. Si se quiere incluir más de una por página se presentará, igual que el resto del trabajo, la página ya maquetada.

El grosor de las líneas y el tamaño de letras y otros símbolos serán adecuados para que sean visibles y claros. Los símbolos menores en ningún caso serán inferiores a 1,5 mm.

En la elaboración de tablas y en los rótulos de figuras se utilizará el mismo tipo de letra que en el texto.

Los rótulos irán siempre en minúscula y sin negrita.

Todas las ilustraciones serán en blanco y negro y se procurará que no sean apaisadas.

Las figuras se delinearán cerradas, es decir, con los correspondientes ejes de abscisas y ordenadas unidos entre sí por sus paralelas. El nombre de cada variable se escribirá a lo largo de su eje, coincidiendo el final con el extremo del mismo.

Las tablas, en cambio, no llevarán nunca líneas verticales.

Las tablas se numerarán con números romanos: tabla I., etc., y la leyenda irá como cabecera; las ilustraciones (figuras, láminas, mapas y fotografías o diapositivas) se numerarán con números arábigos, todas se denominarán figuras: figura 1., etc., y llevarán la leyenda en la parte inferior.

Las leyendas irán centradas en la página, llevarán sangría francesa -comenzando la segunda línea y siguientes debajo del principio del texto de la leyenda- y ocuparán el ancho de la mancha de texto (16,5 cm). El tipo de letra será Courier New 9.

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Las imágenes en formato electrónico se escanearán siempre en escala de grises -lo que facilita enormemente su manejo- al tamaño de publicación o algo mayor y con una resolución entre 300 y 600 ppp. Si se envían sin incrustar dentro de otra aplicación (por ej. Power Point) se guardarán como .tif o .eps.

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Los originales serán revisados criticamente por, al menos, dos evaluadores.

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Informes Técnicos. Instituto Español de Oceanografía features original articles on the applications and development of working techniques and partial research findings, or final analyses of findings by study groups.

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Use the following format whenever possible: ABSTRACT; RESUMEN (translation into Spanish of the Abstract); 1. INTRODUCTION; 2. MATERIAL AND METHODS; 3. RESULTS; 4. DISCUSSION; 5. ACKNOWLEDGEMENTS and 6. REFERENCES.

Begin enumeration of these sections with the Introduction (**1. INTRODUCTION**), writing their titles in boldface capitals, without underlining.

Centre page numbers on the bottom margin, beginning on page 4, which is where the Introduction begins.

Leave three lines between sections. Leave two lines between a title and the beginning of its corresponding text.

Leave one line between paragraphs. Do not indent the first line of a paragraph.

Number subsections with two or more figures, depending on their category and following a logical order. Use lowercase type for all subsections, putting only the first in italics, e.g.:

4. DISCUSSION

4.1 Physical conditions of the medium

4.1.1 Form of recipient

4.1.2 Aeration

4.2 Yield

4.3 Applications

Chemical, physical or mathematical signs and symbols should follow standard international usage: SI (*Système International d'Unités*), ISO (International Standard Organisation) and UNE (*Una Norma Española*). Therefore, these symbols should always be written without periods, and will remain unmodified when plural. Always refer to the ISO and UNE norms when preparing texts for publication.

In Spanish, accent capital letters, following correct spelling norms.

To simplify the reading of long numbers, they may be separated into appropriate groups, preferably with three places, counting from the decimal point in one or the other direction; these groups should be separated by a space, but never by a comma or other sign.

The decimal sign is a comma on the line. Texts in English may also use a point, on the line.

Numbers indicating years should follow this format: 1999 (for nineteen ninety-nine).

The first citation of the vernacular name of a species in the Spanish and English titles, the abstract, the resumen, and the body of the text should be followed by its scientific name, and then, whenever possible, by the name of the author who described it, and the year. Omit the author and the year in subsequent citations.

Italicise genus and species names, as well as the titles of journals, symposia, and books.

Footnotes will not be accepted.

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Page headings should follow the format below, with the heading placed 1.5 cm below the top margin, running across the whole width of the printed page (16.5 cm), and using a Times New Roman 8 font.

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Inf. Téc. Inst. Esp. Oceanogr. 167. 1997: 44 pp.

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ISSN: 0212-1565

- Even-numbered page headings:

Inf. Téc. Inst. Esp. Oceanogr. 167. 1997: 44 pp.

J. P. Rubin et al.

- Other odd-numbered page headings:

Ictyoplankton, mesozooplankton and hydrography

Inf. Téc. Inst. Esp. Oceanogr. 167. 1997: 44 pp.

- Summary page heading, when used:

Inf. Téc. Inst. Esp. Oceanogr. 167. Madrid, 1997: 44 pp.

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Use an abbreviated title on the page headings.

Title, abstract and resumen page

On the first page of text (page 3 of the paper), put the paper's title (in boldface uppercase letters), names of the authors (in boldface lowercase letters), and institution, mailing address (street, city, country) and e-mail address (in regular type lowercase letters). Next, the abstract, in English and Spanish versions (resumen, in Spanish), no more than 125 words each, setting out the paper's objectives, as well as the procedures followed and the most relevant data and findings obtained. The headings abstract and resumen should be centred and unnumbered, in boldface uppercase letters. Include the title of the paper in Spanish at the beginning of the Spanish abstract, in boldface lowercase letters. At the end of each one, list a maximum of eight key words, not included in the title and in order of importance, indicative of the paper's contents, with the headings keywords and *palabras clave* in boldface lowercase letters.

At the bottom of the page, with a reference mark to the title and in italics, include the date of reception and acceptance of the paper, as well as its Contributing Editor.

Use a Courier New 9 font for the abstract, resumen and addresses, and a Courier New 8 font for the reception and acceptance dates and Contributing Editor.

Introduction

The introduction should not exceed 500 words, briefly indicating the study's objectives and providing sufficient information to clarify the paper's basic focus and the hypothesis being tested.

Materials and Methods

Make this section as concise as possible, while giving all the information necessary to enable any specialist to evaluate the methodology used.

Results

This section should be as clear as possible, and limited to findings essential for establishing the paper's main points.

Discussion

Include a brief discussion regarding the validity of the results observed in relation to those of other published papers on the same topic, as well as a report on the paper's significance. Extensive discussion of the literature is discouraged.

Bibliography

Limit bibliographies to those works cited in the text which have been published or are "in press". If a paper is in press, this phrase should replace the year at the end of the bibliographic reference, in parentheses. For references in the text, cite the author's surname (capitalising the first letter only), followed by the year of publication in parentheses; when the entire reference is enclosed in parentheses, the surname(s) of the author(s) should be followed by a comma and the year. Cite unpublished observations, personal communiqués or works in preparation or under evaluation in the text only; rather than the year of publication, they should be followed by: "unpublished observation", "manuscript" ("MS") or "unpublished", "personal communiqué" ("pers. comm."), "in preparation" or "under evaluation" or "submitted". When the publication has more than three authors, cite only the first, followed by *et al.* In the bibliography, however, all authors' names should appear, separated by commas. Alphabetise bibliographic references; references by the same author should be put in chronological order. The names of journals should, preferably, not be abbreviated. Journal abbreviations should follow those indicated in *Periodical Title Abbreviations*. Eighth Edition. Gale Research Inc. Detroit; London. 1992. If this is not possible, they should be written without abbreviation.

Use a Courier New 9 font, with a three-space hanging indentation for each bibliographic reference.

Examples of bibliographic references:

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Guiry, M. D. 1974. A preliminary consideration of the taxonomic position of *Palmaria palmata* (Linnaeus) Stackhouse = *Rhodymenia palmata* (Linnaeus) Greville. *J. Mar. Biol. Ass. (UK)* 54: 509-529.

- Of a book:

Sinderman, C. J. 1970. *Principal diseases of marine fish and shellfish*. Academic Press. London; New York: 870 pp.

- Of an article from a book which forms part of a series:

Fraga, F. and R. Prego. 1989. Condiciones hidrográficas previas a la purga de mar. In: *Las purgas de mar como fenómeno natural. Las mareas rojas* (Cuadernos da Área de Ciencias Mariñas). F. Fraga and F. G. Figueiras (eds.) 4: 21-44. Edicións do Castro. Seminario de Estudios Galegos. Sada (A Coruña), Spain.

- Of an article from a symposium:

Figueiras, F. G. and F. Fraga. 1990. Vertical nutrient transport during proliferation of *Gymnodinium catenatum* (Graham) in Ría de Vigo, Northwest Spain. In: *Toxic Marine Phytoplankton Proceedings of the Fourth International Conference on Toxic Marine Phytoplankton* (June 26-30, 1989. Lund, Sweden). E. Granelli et al. (eds.): 144-148. Elsevier. New York.

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