Aquaculture 477 (2017) 80–89

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aquaculture

# A workflow management system for early feeding of the European hake

Manuel Nande[a,b], Montse Pérez[a], Damián Costas[c], Pablo Presa[b,c,*]

[a] Grupo de Acuicultura Marina, Centro Oceanográfico de Vigo, Instituto Español de Oceanografía, Subida a Radio Faro 50, 36200 Vigo, Spain
[b] Department of Biochemistry, Genetics and Immunology, Faculty of Biology, Spain
[c] ECIMAT Marine Station of Toralla, University of Vigo, Illa de Toralla s/n, 36331 Vigo, Spain

ARTICLE INFO

*Keywords:*
Early feeding
European hake
Larval growth
Live prey
*Merluccius merluccius*
Adaptive diets

ABSTRACT

Diversification of marine species has emerged as a priority in the aquaculture agenda of many countries due to its large industrial potential and as an alternative to overharvested fisheries. Aquaculture diversification entails new challenges during early life stages of candidate species such as survival bottlenecks or body malformations, many of them due to uncoupling between classic diets and early nutritional requirements. Monospecific diets are common in fish aquaculture, e.g. beginning with a rotifer-based diet, followed by a mixed diet of rotifer and artemia nauplii and ending with artemia nauplii and metanauplii until weaning. Despite some success was reported using such protocol in early hake feeding the massive mortality observed as approaching 25 dph makes optimization of early feeding and larval management a current challenge for the domestication of this species. The main goal of this study was to design and test a workflow management system for early feeding of the European hake as a candidate species. The null hypothesis tested was that optimization of rearing settings had no effect on early growth and survival up to 30 dph as compared to classic culture protocols using commercial prey. Absence of prey in 6 dph hake larvae stomachs indicates that their external feeding at 14 °C begins just after that age. Early feeding preference depends on prey size (< 500 μm before 9 dph) as well as on pigmentation and behavior e.g. those with poor escape reactivity such as *A. franciscana* Nauplii. Significant feeding specialization on wild zooplankton such as *P. intermedius* and *T. longicornis* occurred after 9 dph (Chesson selectivity index = 0.11). Feeding activity was maximal in darkness (D) and medium light intensity (600 lx, MLI) as compared to the lethal light intensity of 1700 lx (HLI). Rotifer-based diets entailed low larvae growth and hake culture unviability after 15 dph but inclusion of wild zooplankton in early diets doubled growth of 30 dph larvae regarding artemia-based diets. The adaptive prey-size diet designed (MiACop) by combining stages of copepods (nauplii, copepodite and adult), rotifer and commercial nauplii of artemia was five-fold superior to the artemia/zooplankton diet all along the first 30 dph larvae culture. The massive cannibalism observed from 25 dph on was related to the absence of an adequate prey size such as that of mysids and euphausiids in combination with semi-dry feed to trigger weaning. Current workflow design for early feeding of the European hake can be helpful to assuring a larger proportion of juveniles entering the weaning phase.

*Statement of relevance:* First feeding of the European hake.

## 1. Introduction

Diversification of marine species has emerged as a priority in the aquaculture agenda of many countries because of its high economic potential and as an alimentary alternative to exhausted fisheries (e.g. Asche and Tveterås, 2004). One candidate species is the European hake (*Merluccius merluccius*) which is naturally distributed from Iceland to Mauritania including the Mediterranean Sea (Inada, 1981). That species occupies temperate habitats up to 15 °C, a depth range 30–1000 m and exhibits year-round batch-spawning (Hunter et al., 1992; Mehault et al., 2010), e.g. the main spawning peaks are reported between February and April in the Bay of Biscay (Pérez and Pereiro, 1985; Álvarez et al., 2004). Spawning is performed deep in rocky bottoms of the continental shelf and eggs ascend to the surface at 50–100 m depths where 3–4 mm larvae hatch after 3–4 days post-fecundation and begin feeding on nauplii, copepods and copepodites (Palomera et al., 2005; Morote et al., 2011). Larvae of ~4 cm descend to 250 m depths and aggregate in nursery areas of the Atlantic North, i.e. the Celtic sea and the Bay of Biscay, until they reach ~20 cm (Fariña and Abaunza, 1991; Sánchez and Gil, 2000; Lloris et al., 2003; Álvarez et al., 2004). In that stage, juveniles feed on larger prey such as euphausiids, mysids and fish larvae (Murua and Michael, 2010). Size at first maturity varies with latitude

* Corresponding author at: ECIMAT Marine Station of Toralla, University of Vigo, Illa de Toralla s/n, E-36331 Vigo, Spain
*E-mail addresses:* mnande@uvigo.es (M. Nande), montse.perez@vi.ieo.es (M. Pérez), dcostas@ecimat.org (D. Costas), pressa@uvigo.es (P. Presa).

http://dx.doi.org/10.1016/j.aquaculture.2017.05.001
Received 24 February 2017; Received in revised form 1 May 2017; Accepted 3 May 2017
Available online 04 May 2017
0044-8486/ 

being 42 cm in North Atlantic Europe and 37 cm in South Eastern Europe (ICES, 2014) and maturation brings about a more benthonic and sedentary life in rocky bottoms of the continental shelf (Sánchez and Gil, 2000). Benthopelagic demersal adults feed on sardine (*Sardina pilchardus* Walb), blue whiting (*Micromesistius poutassou*), mackerel (*Scomber scombrus*) and horse mackerel (*Trachurus trachurus*) and usually perform feeding emersions at night (Cohen et al., 1990; Alheit and Pitcher, 1995; Velasco and Olaso, 1998; Cabral and Murta, 2002; Lloris et al., 2003).

The European hake is one of the most valuable commercial species from the Atlantic Northeast, i.e. FAO Area 27 (Casey and Pereiro, 1995), e.g. landings from both Atlantic stocks accounted for 105,890 t in 2015 (ICES, 2016a,b). The questioned sustainability of its fisheries after decades of exploitation (e.g. Pita et al., 2016), the sanitary and commercial impact of the widespread *Anisakis* infestation and the consolidated market value of hake, prompted some EU countries attempting its domestication (e.g. Bjelland, 2001). The first domestication attempts consisted on protocols designed upon life-cycle properties as well as on previous assays of tagging and recapture (de Pontual et al., 2003; Piñeiro et al., 2007). Initial promising rearing beginnings were followed by serious difficulties to maintaining, spawning and harvesting this species (Bjelland and Skiftesvik, 2006; Jolivet et al., 2009; Jolivet et al., 2012; Treasurer and Atack, 2013). For instance, a successful rearing protocol used hake larvae stripped from wild adults and fed in semi-intensive conditions using a mixture of rotifer and *Acartia* sp. followed by weaning of a small number of juveniles from 35 dph on (Bjelland and Skiftesvik, 2006). The first spontaneous spawning in captivity was achieved in 2009 at the aquaculture facilities of IEO (CO-Vigo) after two years of adult acclimatization (Sánchez et al., 2012) following an improved protocol to found a hake broodstock (Iglesias et al., 2010; Jolivet et al., 2012). In the 2010 spawning season, a steady spawning activity made affordable the description of the European hake digestive and visual ontology during the endo-exotrophic phase of fertilized eggs (Ortiz-Delgado et al., 2012; Sánchez et al., 2012). Current challenges focus on the optimization of broodstock management and improving early egg and larvae development, e.g. testing the influence of the lipid droplet on egg viability (Iglesias et al., 2014) as well as on the fine tuning of early feeding and larval management (Costas et al., 2014a,b; Nande et al., 2014).

Despite the advancement on hake aquaculture, the European hake is a top predator which first feeding preferences are still unclear. The use of different prey types in early feeding is transversal in aquaculture as beginning with a rotifer-based diet, followed by a mixed diet of rotifer and artemia nauplii and ending with artemia nauplii and metanauplii until weaning. Although rearing success was seldom reported in hake larvae grownup to 19 dph using 2 individuals/mL rotifer (Iglesias et al., 2010), the high mortality, body malformations and defects of pigmentation observed in hake and other candidate fish species such as cod (Karlsen et al., 2015), tuna (Yúfera et al., 2014) and halibut (Shields et al., 1999) are thought to be caused by nutritional deficiencies of monospecific diets used in early feeding. Implementation of a multispecies diet from the beginning of the endo-exotrophic feeding stage has improved the performance of early stages in aquaculture (e.g. Drillet et al., 2011). Despite the technical and economic difficulties to produce copepods, they entail a better nutritional efficiency than traditional live prey for early fish stages and their industrial escalation is a priority in many aquaculture facilities (Rasdi and Qin, 2016). Nonetheless, once a potential live prey has been identified, several collateral factors such as tank volume, prey density or optimal light regime for prey hunting, altogether conform a combinatory challenge determining the viability of its use in aquaculture.

The advancement pursued in this study on early feeding of hake relied on the following goals: a) identifying the type and size of prey preferred by hake larvae upon hatching, b) establishing the optimal prey density that maximizes larvae feeding, c) assessing the influence of light intensity on early larvae feeding activity, d) assessing differences in growth among larvae batches fed zooplankton as nutritional supplement, and e) evaluating the growth efficiency of an adaptive feeding protocol designed for up to 30 dph larvae. The working hypothesis was that optimization of various, abiotic culturing settings (e.g. light regime), prey preference (species, size, density) and multispecies feeding using wild zooplankton (adaptive diets) had no effect on the early growth and survival of hake larvae up to 30 dph as compared to classic culture protocols using commercial copepods and artemia.

## 2. Materials and methods

### 2.1. Biological material

The methodologies employed for adult hake capture and transportation from Ría de Vigo as well as for their indoors acclimatization in darkness were those described by Iglesias et al. (2011). The embryonic development lasted 4 days at 14 °C and incubation of spontaneous spawning was carried out in 150 L troncoconical tanks with gentle aeration and seawater through-flow according to Iglesias et al. (2011) and Sánchez et al. (2012). Hake larvae used in current experiments hatched from eggs laid by the hake broodstock of the Spanish Institute of Oceanography (CO-Vigo) in 2013 and 2014.

### 2.2. Prey preference test using cultured zooplankton

In order to determine prey-type and the prey-size preferred by hake larvae at first feeding using commercial species of zooplankton, six opaque 1 L plastic buckets were filled with 36‰ seawater at 14 ± 0.5 °C, previously filtered (0.5 μm) and UV-sterilized. Buckets were equipped with gentle aeration (≈ 1 bubble/s), constant cool-daylight and a 75 lx lamp on top of each tank. Six types of monospecific diets were implemented from commercial zooplankton, i.e. *Acartia tonsa*, *Artemia franciscana* and *Brachionus plicatilis* (Table 1). Density of each prey was settled according to the recommended ranges for early feeding of marine fishes using live prey (Tucker, 2012). Consumption of the six prey was assayed by supplying 30, 8 dph hake larvae to each diet-specific bucket ($n$ = 180 larvae in total). Stomach content of $n$ = 10 larvae from each bucket were analyzed every 4 h, 18 h and 26 h from the beginning of the experiment. All larvae from each treatment were gently titrated through a 300 μm sieve, transferred to

**Table 1**
Morphometric characteristics of commercial zooplankton used in tests of prey-type and prey-size preference by 8 dph hake larvae. Species names with distinct superscript (a, b) differed significantly among each other in body length.

| | | | | | B | |
|---|---|---|---|---|---|---|
| | *Brachionus plicatilis*[a] rotifer | *Acartia tonsa* Nauplii[a] | *Artemia franciscana* A0 Nauplii[a] | *Acartia tonsa* copepodite[a] | *Artemia franciscana* A1 metanauplii[b] | *Acartia tonsa* copepod[b] |
| Density (ind/ml) | 5 | 5 | 2 | 2 | 2 | 1 |
| Sieve (μm) | 80 | 100 | 150 | 150 | 180 | 400 |
| Length ± SD (μm) | 219.43 ± 60.13 | 346.09 ± 68.40 | 438.83 ± 56.55 | 451.75 ± 45.50 | 658.55 ± 48.56 | 862.71 ± 67.45 |
| Width ± SD (μm) | 156.89 ± 23.34 | 131.20 ± 19.25 | 180.23 ± 36.56 | 152.07 ± 33.23 | 210.34 ± 39.26 | 284.83 ± 49.54 |

a Petri dish, anesthetized in 1 drop of 96 °C ethanol per 2 mL of water and examined in a binocular (Nikon SMZ1500). Consumption of prey-diets was globally compared using one-way ANOVA analysis from the statistical package STATISTICA 10.0©. The Tukey test was applied in pairwise signification tests between prey diets. Consumption preference among monospecific diets or among prey-size categories (Table 1) was tested with ANOVA for a nominal alpha = 0.05. The food importance index (*F*) was also computed (Ticina et al., 2000 and references therein) to estimate the ratio of the number of stomachs that contained a prey of a relative size versus the total number of stomachs containing prey. The *F*-index was independently calculated for each prey according to Segers et al. (2007) using the formula:

$$F(\%) = \frac{ns}{N_s} x\ 100$$

where *ns* is the number of stomachs that contained a specific prey and $N_S$ the total number of prey-contained stomachs.

### 2.3. Prey preference test using wild zooplankton

This experiment tested the preferred prey of wild zooplankton during early feeding of hake larvae. Wild zooplankton was collected from Ría de Vigo in September 2013 using a bongo sleeve of 200–500 μm mesh and kept in 500 L tanks with gentle aeration and 50:30:20 phytoplankton supplement of *Rhodomonas lens*, *Thalassiosira weissflogii* and *Isochrysis galbana*, respectively. Zooplankton species were photographed using a binocular Leica MZ8® and total length and width were measured as excluding appendixes (Schmitt, 1986) using the Leica Application Suite V4 software. The experiment used larvae from 6 dph to10 dph and was performed on a daily basis re-initiation, i.e. each day 100 starving hake larvae were placed in a 5 L bucket and its replica, containing and admixture of wild zooplankton at 0.2 individuals/mL as the density required to prevent passive/random feeding and maintained under gentle aeration and darkness. The zooplankton diet was composed of *P. intermedius*, *T. longicornis*, *A. clausii*, *Pseudocalanus* sp., *Centropages* sp., *Decapod* zoeae, *Cirripedia nauplii*, *Siphonophoreae*, *Nyctiphanes couchii*, *Trochophore*, and *Brachyura* (Megalopa) (Table 2). On a daily basis, 10 larvae were sampled from each bucket and its replicate at 3 h, 5 h and 24 h, and their stomach content was examined with a Leica binocular MZ5.Prey selectivity was determined using the alpha index (α*i*) (Chesson, 1978) for each type of prey from stomachs, as

$$\alpha i = \frac{\frac{ri}{pi}}{\sum_{i=1}^{m} ri/pi}$$

where *m* is the number of prey types ($m = 11$), *ri* is the proportion of prey type *I* consumed and *pi* is the proportion of prey type *I* available. A value of $\alpha i = 1/m$ ($1/m = 0.091$) indicates no selective prey choice, $\alpha i > 1/m$ indicates positive prey selection and $\alpha i < 1/m$ suggests negative prey selection (Chesson, 1978).

### 2.4. Optimal prey density test

This experiment was conducted to test the optimal prey density in culture using *A. franciscana* as the preferred cultured prey in previous experiments (see Table 1 and Fig. 1).Fifteen small glass-tanks filled with 1 L filtered (0.5 μm) seawater, UV-sterilized, 36‰ salinity and 14 ± 0.5 °C, were equipped with soft aeration, constant cool-daylight and a 75 lx lamp on top of each tank. Fresh living *A. franciscana* nauplii (A0) were prepared at three densities (individuals/mL of culture), i.e. 0.1 individuals/mL, 1 individuals/mL and 2 individuals/mL, and replicated five times each in 15 culture tanks. All prey diets were set to density ≤ 2 individuals/mL to prevent random predator-prey encounters which could trigger passive feeding. Each tank was seeded with 30, 8 dph hake larvae kept in starvation (450 larvae in total) and cultures were allowed to proceed for 20 h. All larvae were individually removed from buckets using a Pasteur pipette and their stomach content was examined in a binocular Nikon SMZ1500 after completion of the experiment. Consumption of *A. franciscana* under the three experimental densities assayed was compared with one-way ANOVA analysis using the statistical package STATISTICA 10.0©. When global ANOVA was significant for alpha = 0.05 pairwise tests between density treatments were performed with the Tukey test.

### 2.5. Light intensity test on feeding and survival

These experiments aimed testing feeding ability under different lighting regimes using *A. franciscana* prey (Fig. 1) at density 0.2 individuals/mL to foster active prey hunting. Such density was suboptimal in previous experiments but it prevents passive hunting and allows testing which light regime is more suitable for chasing scarcely distributed prey. Experiments were performed using starved hake larvae of 6–10 dph maintained in 150 L troncoconical tanks. On a daily basis, 100 larvae were seeded in three plastic buckets and their replicates, containing 5 L of 0.5 μm UV-filtered seawater at 14 ± 0.5 °C, 36‰ salinity, smooth aeration and *A. franciscana* nauplii (AF0) at 0.2 individuals/mL. Twenty-four hour experiments were performed at three light intensities per day of life as starting at 6 dph and ending at 10 dph, i.e. 1700 lx (HLI, high light intensity), 600 lx (MLI, medium light intensity) and darkness (D). Ten larvae were collected from each bucket every 3 h, 5 h and 24 h and their stomach content examined under a Leica-MZ5 lens. The percentage of feeding larvae was calculated as a proportion between the No. of occupied stomachs and the total No. of larvae per sampling. Prey consumption among light intensities was compared per day and per hour using a Factorial ANOVA analysis from the statistical program STATISTICA 10.0©. Prey consumption within category of light intensity was

**Table 2**
Consumption of wild zooplankton delivered at 0.2 individuals/mL in first-feeding tests of 6–10 dph hake larvae.

| | Body lenght ± SD (μm) | Body width ± SD (μm) | Species % ± SD in diet | Species % in 9 dph stomachs[a] |
|---|---|---|---|---|
| *Nauplii* (Cirripedia)[b] | 642 ± 87 | 414 ± 43 | 11 ± 3 | 0 |
| *Podon intermedius* | 697 ± 56 | 330 ± 23 | 7 ± 2 | 20 |
| *Acartia clausi* | 1451 ± 67 | 294 ± 34 | 24 ± 13 | 0 |
| *Temora longicornis* | 1657 ± 98 | 434 ± 84 | 20 ± 7 | 20 |
| *Brachyura* (Megalopa)[b] | 1729 ± 117 | 1982 ± 94 | 5 ± 2 | 0 |
| *Centropages* sp. | 1950 ± 32 | 586 ± 26 | 5 ± 3 | 0 |
| Decapod zoeae[b] | 2135 ± 122 | 489 ± 21 | 6 ± 2 | 0 |
| *Siphonophoreae*[b] | 4406 ± 455 | 1533 ± 211 | 13 ± 4 | 0 |
| *Nyctiphanes couchii* | 5194 ± 344 | 864 ± 238 | 1 ± 1 | 0 |
| *Pseudocalanus* | 1589 ± 27 | 498 ± 15 | 5 ± 2 | 0 |
| *Trochophore* | 488 ± 56 | 298 ± 31 | 3 ± 3 | 0 |

[a] No stomach content was observed in larvae of 6–8 dph and massive mortality was observed in 10 dph starving larvae.
[b] A finer taxonomic classification of early stages could not be achieved in this taxon.

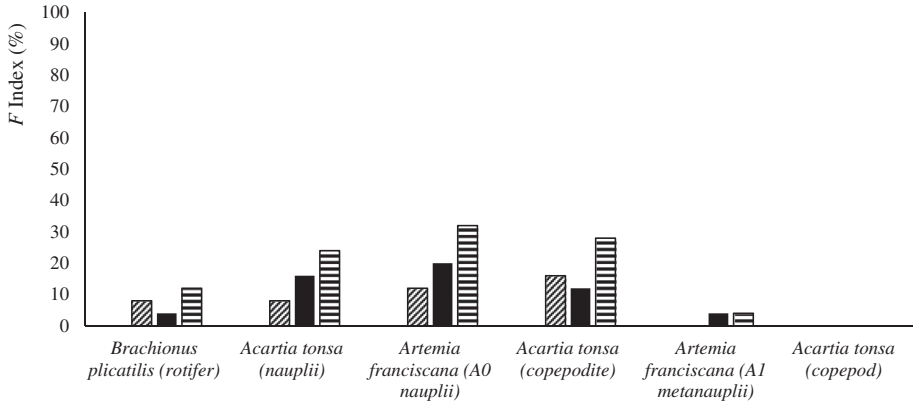


**Fig. 1.** Food importance Index (*F*) inferred from the stomach content of hake larvae after 4 h (open bar), 18 h (oblique bar), 26 h (closed bar) of cohabitation between 8 dph larvae and six prey-types. The accumulated *F*-index is the percentage of feeding larvae (horizontal bar-frame). The six zooplanktonic prey assayed were cultured at 14 °C and delivered at a fixed density (see Table 1).

compared per hour and per day with a one-way ANOVA analysis. When the global comparison test was significant for a nominal threshold alpha = 0.05, average feeding values were compared between pairs of treatments using the Tukey test.

### 2.6. Larvae rearing protocol up to 30 dph larvae

The aim of these experiments was to study the evolution of weight and length of hake larvae up to 30 dph under different rearing conditions and combined prey diets. The rearing protocol applied was designed from preliminary experiments where optimal culture conditions were tested in 1000 L tank volume. Collected hake eggs were incubated in five 150 L tanks at 14 °C with gentle aeration. Hatching occurred 4 days later and 3 dph larvae were added to 1000 L culture tanks at density 25 ± 5 i/L. A medium light intensity of 600 lx (MLI) with photoperiodic regime 16 L:8D was assured on the tank surface to favor microalgae stability, i.e. green water. Wild zooplankton was collected once a week (Table 2) and kept alive in 500 L tanks supplemented on a daily basis with microalgae *I. galbana* and *R. lens* from their exponential culture phase. Prey cultures were grown in 500–1000 L tanks under attenuated ambient light (400 lx), 36‰ salinity, 15.0 ± 1.0 °C and mild aeration in an open circuit, and daily enriched with *I. galbana* and *R. lens* at 100,000 cells/mL and 150,000 cells/mL, respectively. *Acartia clausii* and *Temora longicornis* were isolated and cultured in parallel to larvae. The commercial crustacean *A. franciscana* and the rotifer were cultured in parallel to larvae and hatched according to the manufacturer's protocol and their cultures were enriched with *I. galbana* (150,000 cells/mL) grown at 25 °C. Prey size and prey density of 0.2 individuals/mL were kept constant during the experiment by increasing the frequency of fresh prey inoculations into rearing tanks (common procedure applied also to 30 dph adaptive rearing experiments, see Section 2.7). Despite better results were observed at 2 individuals/mL the enforcement of a low prey density was required to prevent a progressive prey size increase in a 18 h culture, i.e. when prey transits from size group A (507.67 ± 65.69 μm)to size group B (609.69 ± 87.84 μm) (see Table 1) and would limit larvae hunting success.

### 2.7. Adaptive diets

This experiment aimed to describe the evolution of weight and length of hake larvae up to 30 dph using adaptive larvae-prey sizes as advancing in time (e.g. Fig. 1). Culture conditions were those used in previous experiments except prey size which was increased in parallel to larvae growth. Commercial species of copepods (e.g. *A. tonsa*) were reared in 500 L and 1000 L tanks and adult copepods were daily fed microalgae *R. lens* and *I. galbana* at 250,000 cells/mL and 150,000 cells/mL, respectively. Adaptive diets (MiACop) were prepared using combinations of commercial copepods at different stages (*A. tonsa* nauplii, copepodite and adult) and *A. franciscana* (nauplii and metanauplii). When cultures achieved 100 nauplii/mL nauplii were separated from adults using two superimposed sieves (150 μm and 40 μm), transferred to a new tank, adjusted at 5 individuals/mL and fed microalgae in inverse proportion to that used for adults, i.e. 250,000 cells/mL of *I. galbana* and 150,000 cells/mL of *R. lens*. This procedure allowed choosing different prey sizes and tailoring their concentration in growing hake larvae cultures. Hatching of commercial *A. franciscana* followed the manufacturer's protocol and its nauplii were used to feed larvae from 11 dph to 18 dph. From that age, larvae were fed *A. franciscana* metanauplii enriched for 24 h with *I. galbana* (150,000 cells/mL) at 25 °C.

### 2.8. Growth measurement and statistical analyses

Fifteen larvae were sampled from each tank at 3 dph, 15 dph and 30 dph except rotifer/zooplankton feeding assays (R/Z) which viability did not succeed beyond 15 dph. Larvae were weighted (dry weight, DW) using an ultra-precision scale UM3 Mettler (0.000001 g) and measured (total length, TL) using a binocular microscope (Leica MZ5® and the Leica Application Suite V4 software). Distributions of length and weight were analyzed using the Kolmogorov-Smirnov test to check their fitting to a normal distribution. Data from non-divergent replicates was grouped in a single class within experiment and statistical differences between diets were compared using one-way ANOVA test. The instantaneous growth of hake larvae fed different diets was computed using the formula:

$$Gw = (\ln Wt - \ln Ws)/t$$

where the *Gw* is the instantaneous growth rate, *Wt* is the larvae dry weight at the end of the experiment and *Ws* is the larvae dry weight at the beginning of the experiment. All data series were checked for normal distribution using the one-sample Kolmogorov–Smirnoff test as well as for homogeneity of variances using the Levene's test (Zar, 1999). When necessary, arcsin transformation of data was performed (Zar, 1999). Diet treatments (artemia/zooplankton vs. artemia) were compared with one-way ANOVA using the statistical package STATISTICA 10.0© (Zar, 1999). DW and TL of larvae fed rotifer/zooplankton and rotifer were not included in the analyses since both cultures did not survive beyond 15 dph. Pairwise comparisons of mean DW and TL were performed with the Tukey test against a nominal alpha = 0.05.

## 3. Results

### 3.1. Prey preference using commercial zooplankton

No differences in prey consumption were observed among species smaller than 500 μm (ANOVA, $F = 0.33$, $p = 0.74$). Significant differences in prey consumption were observed between zooplankton species < 500 μm (group A, Table 1) and those larger than 500 μm (group B, Table 1) (Tukey HSD test, $p = 0.009$) (Fig. 1). An increment of stomach content was observed from 4 h to18 h in all prey of size group A (*B. plicatilis, A. tonsa*, *A. franciscana*), but not in prey from size group B (*A. franciscana* A1 metanauplii and *A. tonsa* copepod) (Table 1).Larvae feeding decreased from 18 h to 26 h in cultures based on the rotifer *B. plicatilis* or on *A. tonsa* copepodite (group A) (Fig. 1). The large copepod *A. tonsa* (group B) was only found in 10% of stomachs in the 26 h test.

### 3.2. Prey preference using wild zooplankton

No stomach content was observed in larvae from 6 dph to 8 dph and massive mortality was observed in 10 dph starving larvae. Wild cladocerans (*P. intermedius*, averaging L × W, 697 × 330 μm) and copepods (*T. longicornis*, L × W, 1657 × 434 μm) appeared first in 20% of 9 dph larvae stomachs after 3 h of predator–prey cohabitation (Table 2).Value of Chesson's selectivity index for those two consumed zooplanktonic prey was 0.11.

### 3.3. Prey density

No differences in larvae mortality were observed among the three prey densities assayed (One-way ANOVA, $F = 0.745$, $p = 0.49$) (Fig. 2). Prey density tests using *A. franciscana* as preferred live prey (Subsection *2.2*, Fig. 1) showed significant feeding differences among the three densities assayed (Factorial ANOVA, $F = 5.77$, $p = 0.017$).Feeding at prey density 1 individual/mL (34.23% of larvae) did not differ from prey density 0.1 individuals/mL (Tukey HSD test, $p = 0.187$) or from prey density 2 individuals/mL (Tukey HSD test, $p = 0.3212$). A significantly less feeding was observed between prey density 0.1 individuals/mL (12.77% of larvae) and prey density 2 individuals/mL (51.47% of larvae) (Tukey HSD test, $p = 0.0139$) (Fig. 2).

### 3.4. Influence of light on larvae feeding and survival

The factorial adjusted model (culture time, starving time, light intensity and prey consumption) explained 93.84% of events ($R^2$ Multiple, Factorial ANOVA, $F = 3.11$, $p = 0.038$) and an identical statistical outcome was obtained using the adjusted model that explained 63.72% of cases. No stomach content was observed in 6 dph larvae and the number of feeding larvae on artemia delivered at 0.2 individuals/mL was significantly higher in days 8 dph and 9 dph as compared to 7 dph or 10 dph larvae, under the three light intensities assayed (Factorial ANOVA, $F = 14.54$, $p = 0.0005$) (Fig. 3).

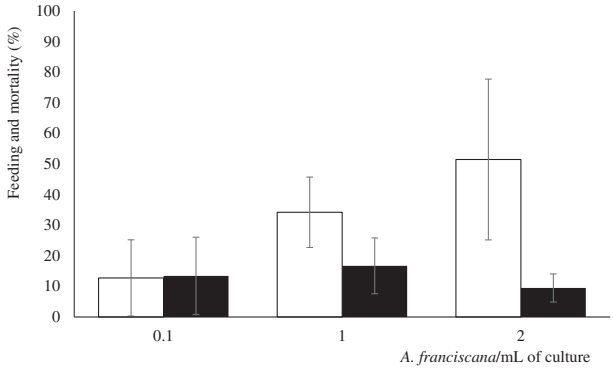


**Fig. 2.** Percentage of 8 dph hake larvae feeding after a 20 h culture at 14 °C to test three prey densities of *A. franciscana* (individuals/mL, Abscissa). The percentage of feeding larvae (open bars) as calculated upon their stomach content and the percentage of mortality (closed bars) are nuanced by their standard deviation (Ordinate).

Larvae mortality in darkness averaged 14.34 ± 3.45% across days and reached up to 91.23 ± 5.65% after 24 h under high light intensity (HLI = 1700 lx). Significant differences in stomach content were observed among light intensities from day 7 dph to day 9 dph (Factorial ANOVA, $F = 11.34$, $p = 0.0004$). The highest consumption was observed under darkness (Factorial ANOVA, $F = 17.71$, $p = 0.0008$).The percentage of feeding larvae increased with time of predator-prey cohabitation and showed a similar pattern both, in darkness and low light intensity (600 lx) from 6 dph to 10 dph. At 10 dph no feeding differences were observed between medium light intensity (MLI = 600 lx) and darkness (Fig. 3).

The percentage of larvae feeding under HLI = 1700 lx increased from 3 h to 5 h but decreased dramatically at 24 h (One-way ANOVA $F = 43.18$, $p = 0.0016$) in days 7 dph, 8 dph and 9 dph (Tukey HSD tests, $p = 0.03$, $p = 0.01$ and $p = 0.0003$, respectively) (Fig. 3). Percent of larvae feeding under MLI = 600 lx showed significant consumption differences between 3 h and 5 h of cohabitation (One-way ANOVA $F = 17.83$, $p = 0.00019$) in days 7 dph and 8 dph ((Tukey HSD test, $p = 0.00016$, $p = 0.0014$, respectively) and showed a maximum at 24 h. Percent of larvae feeding under darkness showed significant consumption differences after 3 h and 24 h of cohabitation (one-way ANOVA $F = 16.89$, $p = 0.00001$) in days 7 dph, 8 dph and 9 dph (Tukey HSD test, $p = 0.00016$, $p = 0.0014$, $p = 0.021$, respectively) as well as between 3 h and 5 h in days 8 dph and 9 dph (Tukey HSD test, $p = 0.00016$, $p = 0.0014$, respectively). Maximum feeding in darkness was observed at 24 h except in 10 dph larvae (Fig. 3).

### 3.5. Larvae rearing assays up to 30 dph

Since no differences were observed from 3 dph to 30 dph in dry weight DW (One-way ANOVA, $F = 1.89$, $p = 0.14$) of larvae fed the same prey in different 1000 L tanks were grouped per prey for statistical analyses. Larvae fed rotifer or rotifer/zooplankton showed a DW at 15 dph of 0.051 ± 0.005 mg and 0.082 ± 0.004 mg respectively and both cultures died afterwards. The number of prey found in larvae fed artemia/zooplankton increased with age, i.e. 100% of 8 dph filled stomachs contained artemia, 15 dph stomachs contained 79% artemia, 13% cladocera and 8% copepods, and 30 dph stomachs contained76% artemia, 18% copepods and 6% cladocera (Table 3). Dry weight of larvae fed artemia/zooplankton was 0.120 ± 0.001 at 15 dph and 0.551 ± 0.171 at 30 dph (Fig. 4) and its instantaneous growth was 6.09% and 10.13% (per day DW% gain), respectively. Dry weight of larvae fed artemia was 0.094 ± 0.095 at 15 dph and 0.247 ± 0.061 at 30 dph (Fig. 4) and its instantaneous growth was 4.18% and 6.45%, respectively. Significant dry weight differences were observed between 30 dph larvae cultures fed artemia/zooplankton and those fed artemia (one-way ANOVA, $F = 14.15$, $p = 0.0003$) (Fig. 4).

The instantaneous growth of larvae fed a mixture diet (MiACop) based on copepods, *A. franciscana* and different stages of *A. tonsa* at 15 dph and 30 dph was 12.23% and 14.8%, respectively (Fig. 4). Significant dry weight differences were observed at 15 dph and 30 dph between larvae fed the progressive MiACop diet (DW = 0.288 ± 0.186 and 2.636 ± 0.941, respectively) and both, those fed only artemia (One-way ANOVA, $F = 18.29$, $p = 0.0002$) and those fed the artemia/zooplankton diet (One-way ANOVA, $F = 57.78$, $p = 0.0001$) (Fig. 4). Cannibalism began to be observed in culture tanks after 25 dph.

## 4. Discussion

Although knowledge of nutritional needs is critical to optimize a

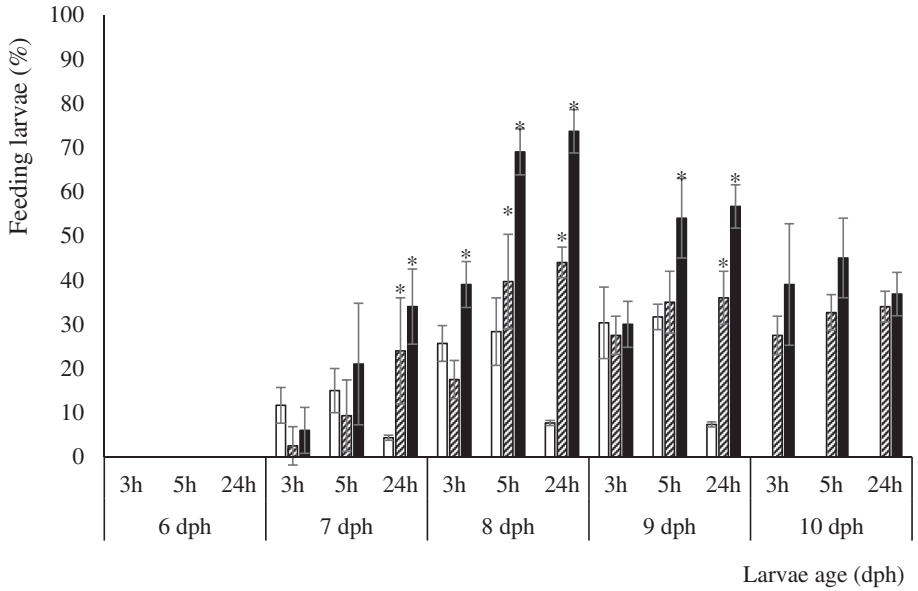


**Fig. 3.** Percentage of hake larvae feeding from 6 dph (mouth opening) to 10 dph as observed after 3 h, 5 h and 24 h of larvae-prey cohabitation at 14 °C using *A. franciscana* A0 at 0.2 individuals/mL density under three light intensities: 1700 lx (HLI, High Light Intensity, open bar), 600 lx (MLI, Medium Light Intensity, oblique bar), and darkness (D, closed bar). Factorial ANOVA was performed on three variables (hours, days and light intensity). Significant differences among light intensities per day are indicated by an asterisk (*$p < 0.05$). Vertical bars indicate the standard deviation of the variable.

feeding protocol for early stages of any fish (e.g. Roberts et al., 2014) early feeding of hake larvae is still unresolved from the perspective of aquaculture. From an ontogenetic insight of hake larvae reared at 14 °C, the apparition of the mature digestive system from 6 dph, i.e. open mouth and anus, mouth size, and gut morphology (Palomera et al., 2005; Ortiz-Delgado et al., 2012) is determinant for larvae viability (Morote et al., 2011) and coincides with the acceleration of lipid metabolism from the oil drop as energy input during the endo-exogenous feeding transition (Ortiz-Delgado et al., 2012; Iglesias et al., 2014). Also, the endogenous dependent maturation of the retinal tissue gives signs of visual capacity around 6dph (Bozzano and Catalán, 2002) what allows hake to initiate external feeding activity. From a nutritional insight, the experiments designed herein aimed calibrating some key parameters determining first feeding in hake, such as prey-type, prey-size, prey-predator encountering probability (prey density) and prey visibility (light intensity and prey pigmentation) in order to work out a workflow management system for early feeding of this species.

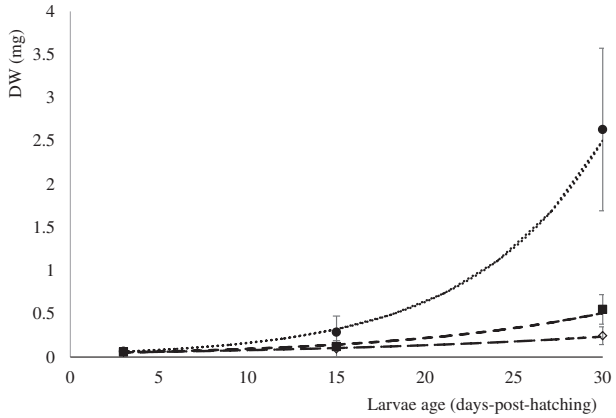


**Fig. 4.** Evolution of dry weight (DW) up to 30 dph of hake larvae fed three live prey diets: *A. franciscana* (0.2 individuals/mL, open diamond, y(A) = $0.0457e^{0.0547x}$, $R^2 = 0.9865$); *A. franciscana* (0.1 individuals/mL)/wild zooplankton (0.1 individuals/mL) (closed square, y(A/Z) = $0.0408e^{0.0841x}$, $R^2 = 0.9817$) and the adaptive larvae-prey size diet MiACop (*A. franciscana*, 0.1 individuals/mL)/(*A. tonsa* copepod size adapted to the larval-size, 0.1 individuals/mL) (closed circle, y(L-size/P-size) = $0.0408e^{0.0841x}$, $R^2 = 0.9817$).

### 4.1. Prey preference using commercial zooplankton

The higher preference that 8 dph hake larvae showed for prey of similar size-range, e.g. *Artemia franciscana* and copepodites of *Acartia tonsa* together with the increment of stomach content at 18 h for all cultured zooplankton of similar size assayed at different densities, except adults of *A. tonsa* copepod and *A. franciscana* metanauplii suggest that hake larvae was unable to catch prey larger than 500 μm before 9 dph. That result is congruent with previous assays on 8 dph hake larvae using common live prey in aquaculture, i.e. rotifer (*Brachionus plicatilis*) and brine shrimp nauplii (*A. franciscana*) provided their size range was well below 500 μm (Iglesias et al., 2011). Such prey preference in culture agrees with that found in stomach contents of wild

**Table 3**
Average number of artemia/zooplankton prey in 8 to 30 dph hake larvae stomachs and percentage of feeding larvae containing those prey.

| Species | 8 dph | | 15 dph | | 30 dph | |
|---|---|---|---|---|---|---|
| | Average prey No. in filled stomachs | Percentage of feeding larvae | Average prey No. in filled stomachs | Percentage of feeding larvae | Average prey No. in filled stomachs | Percentage of feeding larvae |
| *A. franciscana* Nauplii | 1.00 ± 0.00 | 100 | 6.34 ± 0.57 | 79.17 ± 7.21 | 24.34 ± 1.52 | 76.05 ± 0.91 |
| *Podon intermedius* (Cladocera) | 0 | 0 | 1.20 ± 0.60 | 12.55 ± 7.25 | 1.80 ± 0.80 | 6.26 ± 0.39 |
| *Temora longicornis* (copepod) | 0 | 0 | 0.67 ± 0.34 | 8.34 ± 7.22 | 5.67 ± 0.58 | 17.69 ± 1.04 |

hake larvae which is skewed towards copepods nauplii (100–450 μm in length) as well as with the size-range of prey found in larvae stomachs of other hake species (Sumida and Moser, 1980). Those results suggest that there is a relationship between the mouth-size of hake larvae and their preference for prey of similar size and also with the reported positive relationship between stomach volume and prey volume (Cass-Calay, 2003). Previous studies on prey preference in fishes ranged from those with clear size-dependent relationships to those in which species altered their niche breadth as they grow up. The latter model is not supported after Pearre (1986) and Shirota (1970) who suggested that larval fish showed predictable size distributions of ingested prey (Pepin and Penney, 1997). At first sight current data may suggest that prey preference of 8 dph hake larvae is a size-related choice (i.e. prey length < 500 μm) better than a species-related choice. However the patent higher preference for *A. franciscana* nauplii which exhibits a lower mobility and a poor escape reactivity (less efficient swimmer) than other prey such as copepod nauplii of similar size (Gauld, 1959) suggests that not only prey size but also prey pigmentation and behavior have an influence on prey preference (Checkley, 1982; Peterson and Ausubel, 1984; Garrison and Link, 2000).

### 4.2. Prey preference using wild zooplankton

Absence of stomach content from 6 dph to 8 dph in hake larvae exposed to wild zooplankton was likely due to a prey-size well above 500 μm. Presence of cladocerans (*P. intermedius*, averaging L × W, 697 μm × 330 μm) and copepods (e.g. *T. longicornis*, averaging L × W, 1657 × 434 μm) in 20% of 9 dph larvae stomachs indicates that they are competent for predating on larger prey as compared to 6–8 dph larvae. This size selectivity has also been observed in early feeding experiments of other species such as sprat larvae (*Sprattus sprattus*) which stomachs contained nauplii of *Acartia* spp. and nauplii of *Temora longicornis*, whereas larger larvae consumed up to 80% *Acartia* spp. copepodites and adults as well as cladocera (Dickmann et al., 2007). While it has been shown that the higher variety of prey is consumed during the initial learning process in cohabitation (Croy and Hughes, 1991) a prey specialization seems to occur afterwards since the Chesson selectivity index showed an α-value of 0.11 for both types of wild zooplanktonic prey (*P. intermedius* and *T. longicornis*) which suggests a positive selection of 9 dph hake for those species. Similarly, positive prey selection of wild European hake larvae from the Mediterranean has been reported on the calanoide copepod *Clausocalanus* spp. among several available prey as inferred from a Chesson's selectivity index of 0.25 (Morote et al., 2011).

The absence of significant differences in body length and width between consumed and non-consumed prey of wild zooplankton in early hake larvae feeding implies that despite body size is a practical selection criteria for hake larvae, also pigmentation (prey detection), swimming behavior (prey responsiveness) (Gago et al., 2010) and prey nutrition value (Fox et al., 1999), are playing factors to be evaluated in studies of prey preference. The survival capacity of a copepod against a predator depends on its ability to avoid attacks, e.g. the poor escape responsiveness of copepods such as *Pseudocalanus* species (Viitasalo et al., 2001; Petrik et al., 2009) and *Paracalanus* species (McLaren and Avendaño, 1995) make them more vulnerable to predation than other species. Additionally, larval swimming skills also influence predator–-prey encounters and the good swimming capability of *M. merluccius* larvae provided its early development of a strong caudal peduncle (Palomera et al., 2005) surely assists this species in early predation activities, e.g. the swimming characteristics of *Clausocalanus*, which is rapid but aimless, make it an easy prey for the rapid *M. merluccius* larvae (Mazzocchi and Paffenhöfer, 1999).

### 4.3. Prey density

Prey density influences larval fish feeding rates, activity, growth rate, evacuation time, etc. (Lubzens et al., 1989). A high copepod density has a positive influence on larvae intake in several fishes (Frimpong and Lochmann, 2005) as has been observed herein using a prey density of 2 individuals/mL (51.47% larvae) and was likely motivated by a higher encounter probability. However, a high density of big prey is not beneficial in hake culture because their slow digestion counteracts with the rapid growth of the remaining prey which size overcomes the optimal consuming size (Sumida and Moser, 1980; Morote et al., 2011). Also, the concentration of microalgae in larval rearing tanks is lower than in supplementary enriched cultures for prey, what causes the decay of some of the prey essential nutrients such as DHA y EPA decay, e.g. *A. franciscana* nauplii starved at 12 °C exhibited loss rates per day$^{-1}$ up to 51% of DHA, 15% of EPA, 30% of other *ny3* fatty acids and 11% of total lipid content (Evjemo et al., 2001). For this reason, the increase of intake frequency of low-density fresh-made optimal-sized prey is preferable. Therefore, the minimum prey density assayed with acceptable intakes, growth and survival of hake larvae lies between the 0.2 and 1.0 individuals/mL as has also been observed in Atlantic cod larvae *Gadus morhua* (Buckley, 1979).

### 4.4. Light intensity

Irrespective of the light intensity applied, the absence of wild zooplankton in 6 dph stomachs indicates that their external feeding at 14 °C begins just after that age (Palomera et al., 2005; Ortiz-Delgado et al., 2012). A consequence of larvae rearing at high light intensity (HLI, 1700 lx) was its massive mortality (90%) as compared to darkness (15%). Optimal light ranges in early feeding are not common to all fishes and some species develop well at very low light intensities in hatchery, e.g. *Sparus aurata* at 50 and 150 lx, or in the absence of light, e.g. *Clupea harengus* (Boeuf and Le Bail, 1999), while other species are able to grow at high light intensities such as the Atlantic cod reared at 2400 lx without any effect on larvae survival (Puvanendran and Brown, 2002). In hake, little is known on the influence of circadian rhythms caused by variation of photoperiod in the wild as opposed to the constant lighting settings assayed in hatchery. Early stages of wild hake are believed to feed in low light transmittance areas due to the depth of the water column and the sea surface turbidity (Mas-Riera, 1991). The higher hake feeding activity in green water, either in dark and medium light intensity (MLI, 600 lx,) as compared to HLI is congruent with the feeding pattern observed in wild *M. merluccius* larvae which dwelling depth is under moderate light intensity never exceeding 500 lx (Morote et al., 2011). Such feeding preference under MLI has also been observed in other fishes such as the north pacific hake (*M. productus*) (Sumida and Moser, 1980), the Atlantic cod (*Gadus morhua*) (Bainbridge and McKay, 1968), the redfish (*Sebastes marinus*), the Pacific sardine (*Sardinops sagax*) and flatfishes (Sumida and Moser, 1980), the meagre *Argyrosomus regius* (Vallés and Estévez, 2013), the gilt-head bream *Sparus aurata* (Tandler and Mason, 1984) and the silver seabream *Pagrus auratus* (Fielder et al., 2002).

Hake larvae begun chasing live prey at 7 dph, i.e. during the *endo*-exogenous feeding transition, under the three light intensities assayed and well before the digestive and visual systems were fully physiologically functional around 9–10 dph (Ortiz-Delgado et al., 2012). The poorer feeding activity at 10 dph regarding previous days can be explained as a byproduct of the experimental design where 10 dph is a too-late developmental stage for a starving larvae to begin feeding on a re-initiation basis, due to its weakness and lack of chasing experience. Current data agree with knowledge on the visual system of this species where Amacrine and Ganglion cell layers are only detectable after 9 dph of the lecithotrofic development. Those structures control sensitivity in scotopic vision through connections with rods and cone bipolar cells, determining a better adaptation to light oscillations (Ortiz-Delgado et al., 2012). Morphology of the visual system changes during the life-time of fishes to adapt to environmental conditions and some hakes experiencing transition from semi-pelagic to demersal life

(*M. capensis* and *M. paradoxus*, *M. merluccius*) gain sensitivity and visual acuity associated with sea depth (Mas-Riera, 1991). The higher prey intake of 10 dph larvae at MLI, inverting previous days trend of a higher feeding in darkness, suggests that maturation of the visual system allows larvae detecting prey under a progressively higher variety of lighting conditions (Morote et al., 2011) and their higher sensitivity and visual acuity at 9–10 dph makes HLI lethal after a 24 h exposure.

### 4.5. Larval rearing up to 30 dph

The lowest growth observed in larvae fed rotifer-based diets (rotifer and rotifer/zooplankton) and its unviability after 15 dph are facts congruent with the moderate early rotifer consumption and its progressive decrease afterwards. The increment in the number and size of prey found in larvae fed artemia/zooplankton with age (Table 1 and Table 3) suggests that both, the low size of the rotifer and its low nutritional value, are not appropriate for the fast growing hake larvae. Individual rearing of wild zooplankton species allowed to work out combinations of *A. franciscana* nauplii as the most consumed prey in early days, with selected species of zooplankton from different stages of development (Cladocera, cirrípeda, copepods, etc) and rotifers. The significantly lower DW and instantaneous growth of 30 dph larvae fed artemia as compared to artemia/zooplankton indicates that inclusion of wild zooplankton in early feeding doubles larvae growth regarding those fed only artemia. Supplementing with zooplankton enhances the quality of diets by offering larvae a wider food choice and a variety of essential amino acids which determine a higher growth rate (e.g. Katan et al., 2016). The use of wild zooplankton prey to supplement diets based on traditional cultures represents an improvement in growth, survival and overall development of larvae and reduces skeletal malformations in semi-intensive rearing assays, as observed in Atlantic cod (Busch et al., 2010), halibut (*Reinhardtius hippoglossoides*), seabream (*Pagellus bogaraveo*), seabass (*Dicentrarchus labrax*) (van der Meeren and Naas, 1997), greater amberjack (*Seriola dumerili*) (Papandroulakis et al., 2005) and in the dusky grouper *Epinephelus marginatus* (Russo et al., 2009).

### 4.6. Adaptive diets and cannibalism

Current assays on preferred prey sizes showed that those larger or smaller than the optimal size for each developmental stage of larvae had a much lower consumption (Fig. 1). Noteworthy, larvae consumed larger prey advancing in time so its digestion time increased and the frequency of prey ingestion of decreased (e.g. Sumida and Moser, 1980). Therefore, current prey density was set at 0.2 individuals/mL in order to supply fresh-made size-adapted diets in four daily intakes, thus preventing prey growth overtaking its optimal size. We show that such progressive prey-size diet approach (MiACop) which comprised a combination of different stages of copepods (nauplii, copepodite and adult) rotifer and commercial nauplii of artemia was 5 fold more efficient than artemia/zooplankton along the 30 dph hake larvae culture. That result is explained by the adaptation of prey size to the nutritional needs of larvae and is in agreement with previous assays achieved up to 24 dph in hake (Bjelland and Skiftesvik, 2006) or in halibut, seabass, and cod (Shields et al., 1999; Rajkumar, 2006; Hansen, 2011).

The massive cannibalism observed after 25 dph in rearing tanks had also been observed in previous non-intensive culture assays in hake using copepod nauplii and rotifer with very little survival after 30 dph in spite of achieving a successful weaning from 35 dph using formulated feed up to 50 dph (Bjelland and Skiftesvik, 2006). Cannibalism is a natural phenomenon which has been systematically observed in the wild, where density, spatial distribution patterns and total length ($L_T$) structure of hake recruits were identified as the main variables triggering it (e.g. Preciado et al., 2015). Causes of cannibalism in rearing conditions are likely related to the absence of an adequate live prey size. The fast growth rate of highly voracious 25 dph hake larvae triggers uncoupling between larvae size and the traditional species supplied in diets. This hypothesis gains strength since stomachs of wild juveniles older than 30 dph of *M. productus* (Cass-Calay, 2003) and *M. merluccius* (Morote et al., 2011) contain copepods but also larger prey such as euphausiids. Indeed, parallel assays have minimized cannibalism in the European hake using a combination of mysidacea, euphausiids and a semi-moist feed (Sánchez et al., 2012) achieving a three-year hake culture (Damián Costas, unpublished data).

### 4.7. Conclusion

The current null hypothesis established that optimization of abiotic culturing settings (light regime, tank volume, temperature) and live prey diets (species, size, density) would have no effect on early growth and survival of hake larvae cultured up to 30 dph as compared to classic culture protocols using commercial artemia, rotifer and copepods. We refute that null hypothesis since the advancement achieved on live prey management has allowed to a) calibrating the type and size of preferred prey from hatching to 30 dph, b) establishing the optimal prey density at 0.2 individuals/mL, c) setting the optimal light intensity ≤ 600 lx which maximizes phytoplankton production/maintenance in green cultures and attenuates a harmful direct lighting, d) calibrating a cultivation protocol for 30 dph larvae using the settings worked out along the study and a progressive adaptive diet using different stages of copepods, rotifer and commercial nauplii of artemia (Fig. 5). The current workflow design for early feeding of the European hake can be helpful to assuring a larger proportion of juveniles entering the weaning phase and therefore to foster the aquaculture of this species.

## Acknowledgements

The authors acknowledge the financial support through actions HAKEUP and HAKEU + from the AEOE-ECIMAT strategic consortium funded by the Competitive Research Units' Consolidation Program and co-funded by the European Regional Development Fund (ERDF) (Operative Program Galicia 2007–2013, Consellería de Cultura, Educación e Ordenación Universitaria of Xunta de Galicia, RN#AOE_2008-080). Support was also lent by Ministerio de Economía y Competitividad through project LETSHAKE (AGL2013 -

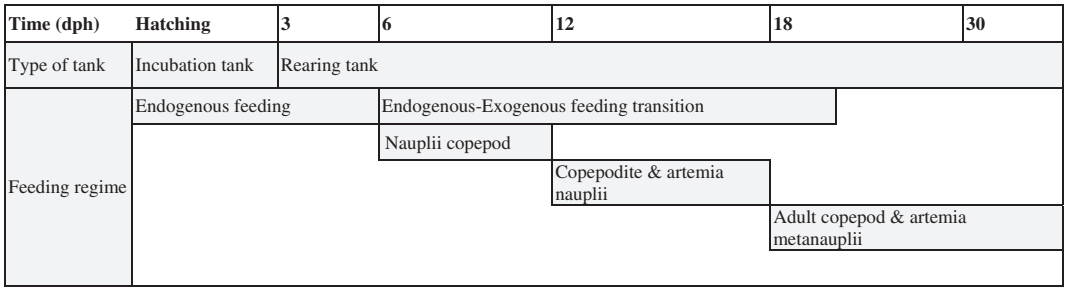


**Fig. 5.** Adaptive feeding workflow from hatching to 30 dph hake larvae using an adaptive prey-size strategy.

48468-R) co-funded by FEDER. The authors acknowledge C. Gómez-Ceruelo and M. J. Lago for their technical assistance.

## References


Alheit, J., Pitcher, T.J., 1995. Hake: Biology, Fisheries and Markets. Fish and Fisheries Series No. 15. Chapman & Hall, London, pp. 487.

Álvarez, P., Fives, J., Motos, L., Santos, M., 2004. Distribution and abundance of European hake *Merluccius merluccius* (L.), eggs and larvae in the north East Atlantic waters in 1995 and 1998 in relation to hydrographic conditions. J. Plankton Res. 26 (7), 811–826.

Asche, F., Tveterås, S., 2004. On the relationship between aquaculture and reduction fisheries. J. Agric. Econ. 55 (2), 245–265.

Bainbridge, V., McKay, B.J., 1968. The feeding of cod and redfish larvae. Spec. Publs. Int. Commn. Northw. Atl. Fish. 7, 187–217.

Bjelland, R.M., 2001. European hake, *Merluccius merluccius* (L. 1758). In: A new Candidate for Aquaculture? Rearing Techniques, Larval Development and Startfeeding. Fisheries and Marine Biology, University of Bergen, Norway Ms. Thesis. Dept.

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

Boeuf, G., Le Bail, P.Y., 1999. Does light have an influence on fish growth? Aquaculture 177 (1), 129–152.

Bozzano, A., Catalán, I., 2002. Ontogenetic changes in the retinal topography of the European hake, *Merluccius merluccius*: implications for feeding and depth distribution. Mar. Biol. 141 (3), 549–559.

Buckley, L.J., 1979. Relationships between RNA-DNA ratio, prey density, and growth rate in Atlantic cod (*Gadus morhua*) larvae. J. Fish. Board Can. 36 (12), 1497–1502.

Busch, K.E.T., Falk-Petersen, I.B., Peruzzi, S., Rist, N.A., Hamre, K., 2010. Natural zooplankton as larval feed in intensive rearing systems for juvenile production of Atlantic cod (*Gadus morhua* L.). Aquacult. Res. 41 (12), 1727–1740.

Cabral, H.N., Murta, A.G., 2002. The diet of blue whiting, hake, horse mackerel and mackerel off Portugal. J. Appl. Ichthyol. 18 (1), 14–23.

Casey, J., Pereiro, J., 1995. European hake (*M. merluccius*) in the North-east Atlantic. In: Hake. Springer Netherlands, pp. 125–147.

Cass-Calay, S.L., 2003. The feeding ecology of larval Pacific hake (*Merluccius productus*) in the California current region: an updated approach using a combined OPC/MOCNESS to estimate prey biovolume. Fish. Oceanogr. 12 (1), 34–48.

Checkley Jr., D.M., 1982. Selective feeding by Atlantic herring (*Clupea harengus*) by larvae on zooplankton in natural assemblages. Mar. Ecol. Prog. Ser. 9, 245–253.

Chesson, J., 1978. Measuring preference in selective predation. Ecology 211-215.

Cohen, D.M., Inada, T., Iwamoto, T., Scialabba, N., 1990. FAO Species Catalogue. In: Gadiform fish of the world (Order Gadiformes). An Annotated and Illustrated Catalogue of Cods, Hakes, Grenadiers and Other Gadiform Fish Known to Date. FAO Fisheries Synopsis 125 Vol. 10 FAO, Rome (442 pp.).

Costas, D., Nande, M., Pérez, M., Casal, A., Lago, M.J., Costoya, N., Iglesias, J., Rodríguez, R., Palmeiro, O., Padrós, F., Carrason, M., Gómez-Gesteira, M., Presa, P., 2014a. First Feeding of the European Hake *Merluccius merluccius*: Influence of Light and Tank Background. Aquaculture Europe, Donostia, Spain, pp. 291–292.

Costas, D., Nande, M., Pérez, M., Casal, A., Lago, M.J., Costoya, N., Iglesias, J., Rodríguez, R., Palmeiro, O., Padrós, F., Carrason, M., Gómez-Gesteira M., Presa, P., 2014b. First feeding of the European hake *Merluccius merluccius*: selective prey and prey density, Aquaculture Europe, Donostia, Spain pp. 289-290.

Croy, M.I., Hughes, R.N., 1991. The role of learning and memory in the feeding behaviour of the fifteen-spined stickleback, *Spinachia spinachia* L. Anim. Behav. 41 (1), 149–159.

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 J. Mar. Sci. 60 (6), 1318–1327.

Dickmann, M., Möllmann, C., Voss, R., 2007. Feeding ecology of Central Baltic sprat (*Sprattus sprattus* L.) larvae in relation to zooplankton dynamics-implications for survival. Mar. Ecol. Prog. Ser. 342, 277–289.

Drillet, G., Frouël, S., Sichlau, M.H., Jepsen, P.M., Højgaard, J.K., Joarder, A.K., Hansen, B.W., 2011. Status and recommendations on marine copepod cultivation for use as live feed. Aquaculture 315 (3), 155–166.

Evjemo, J.O., Danielsen, T.L., Olsen, Y., 2001. Losses of lipid, protein and n − 3 fatty acids in enriched *Artemia franciscana* starved at different temperatures. Aquaculture 193 (1), 65–80.

Fariña, C., Abaunza, P., 1991. Contribución al estudio de los juveniles de merluza entre Cabo Villano y Cabo Prior (NW Galicia) mediante prospecciones pesqueras. Bol. Inst. Esp. Oceanogr. 7, 155–163.

Fielder, D.S., Bardsley, W.J., Allan, G.L., Pankhurst, P.M., 2002. Effect of photoperiod on growth and survival of snapper *Pagrus auratus* larvae. Aquaculture 211 (1), 135–150.

Fox, C.J., Harrop, R., Wimpenny, A., 1999. Feeding ecology of herring (*Clupea harengus*) larvae in the turbid Blackwater Estuary. Mar. Biol. 134 (2), 353–365.

Frimpong, E.A., Lochmann, S.E., 2005. Mortality of fish larvae exposed to varying concentrations of cyclopoid copepods. N. Am. J. Aquacult. 67 (1), 66–71.

Gago, J., Martins, T., Luis, O.J., Pousao-Ferreira, P., 2010. Survival and growth of selected marine fish larvae first fed with eggs and endotrophic larvae of the sea urchin *Paracentrotus lividus*. Aquacult. Res. 41, 96–108.

Garrison, L.P., Link, J.S., 2000. Diets of five hake species in the northeast United States continental shelf ecosystem. Mar. Ecol. Prog. Ser. 204, 243–255.

Gauld, D.T., 1959. Swimming and feeding in crustacean larvae: the nauplius larva. In: Proceedings of the Zoological Society of London. 132(1). Blackwell Publishing Ltd., pp. 31–50.

Hansen, M.H., 2011. Effects of feeding with copepod nauplii (*Acartia tonsa*) compared to rotifers (*Brachionus ibericus*, Cayman) on Quality Parameters in Atlantic cod (*Gadus morhua*) Larvae. Msc Thesis at the Department of Biology (NTNU), Trondheim.

Hunter, J.R., Macewicz, B.J., Lo, N.H., Kimbrell, C.A., 1992. Fecundity, spawning, and maturity of female dover sole *Microstomus pacificus*, with an evaluation of assumptions and precision. Fish. Bull. 90 (1), 101–128.

ICES, 2014. Report of the Benchmark Workshop on Southern Megrim and Hake (WKSOUTH). 3–7 February 2014, Copenhagen, Denmark. ICES CM 2014/ACOM pp. 40.

ICES, 2016a. Hake (*Merluccius merluccius*) in subareas 4, 6, and 7 and divisions 3.a, 8.a–b, and 8.d, Northern stock (Greater North Sea, Celtic Seas, and the northern Bay of Biscay). ICES Advice Book 9.3.32. http://www.ices.dk/sites/pub/Publication%20Reports/Advice/2016/2016/hke-nrtn.pdf (accessed 17.01.17).

ICES, 2016b. Hake (*Merluccius merluccius*) in divisions 8.c and 9.a, Southern stock (Cantabrian Sea and Atlantic Iberian waters). ICES Advice Book 7.3.21. http://www.ices.dk/sites/pub/Publication%20Reports/Advice/2016/2016/hke-soth.pdf (accessed 17.01.17).

Iglesias, J., Lago, M.J., Sánchez, F.J., Cal, R., 2010. Capture, transport and acclimation to captivity of European hake, *Merluccius merluccius* L.: preliminary data on feeding and growth. Aquacult. Res. 41, 607–609.

Iglesias, J., Lago, M.J., Otero, J.J., Sánchez, F.J., Cal, R., 2011. Tamaño de presa optimo en la primera alimentación de las larvas de la merluza europea, *Merluccius merluccius*. Actas of XIII Congreso Nacional de Acuicultura, Castelldefels, Spain.

Iglesias, J., Lago, M.J., Otero, J.J., Gómez, C., Cal, R., Sánchez, F.J., 2014. Effect of the lipid droplet adherence on growth and survival of the European hake (*Merluccius merluccius*) larvae. Aquacult. Res. 45 (11), 1754–1758.

Inada, T., 1981. Studies on the Merlucciid fishes. Bull. Far Seas Fish. Res. Lab. 18, 1–172.

Jolivet, A., De Pontual, H., Garren, F., Bégout, M.L., 2009. Effects of T-bar and DST tagging on survival and growth of European hake. In: Tagging and Tracking of Marine Animals with Electronic Device. Springer Netherlands, pp. 181–193.

Jolivet, A., De Pontual, H., Hervy, M., Paulet, Y.M., Fablet, R., 2012. Preliminary observations of survival and growth of European hake in captivity. Aquacult. Res. 43 (6), 949–954.

Karlsen, Ø., van der Meeren, T., Rønnestad, I., Mangor-Jensen, A., Galloway, T.F., Kjørsvik, E., Hamre, K., 2015. Copepods enhance nutritional status, growth and development in Atlantic cod (*Gadus morhua* L.) larvae can we identify the underlying factors? PeerJ 3, e902.

Katan, T., Nash, G.W., Rise, M.L., Hall, J.R., Fernandes, J.M.O., Boyce, D., Johnsen, C.A., Gamperl, A.K., 2016. A little goes long way: improved growth in Atlantic cod (*Gadus morhua*) fed small amounts of wild zooplankton. Aquaculture 451, 271–282.

Lloris, D., Matallanas, J., Oliver, P., 2003. Merluzas del mundo (Familia Merlucciidae): Catálogo comentado e ilustrado de las merluzas conocidas (No. 2). Food & Agriculture Organization, Rome.

Lubzens, E., Tandler, A., Minkoff, G., 1989. Rotifers as food in aquaculture. Hydrobiologia 186 (1), 387–400.

Mas-Riera, J., 1991. Changes during growth in the retinal structure of three hake species, *Merluccius* spp. (Teleostei: Gadiformes), in relation to their depth distribution and feeding. J. Exp. Mar. Biol. Ecol. 152 (1), 91–104.

Mazzocchi, M.G., Paffenhöfer, G.A., 1999. Swimming and feeding behavior of the planktonic copepod *Clausocalanus furcatus*. J. Plankton Res. 21, 1501–1518.

McLaren, I.A., Avendaño, P., 1995. Prey field and diet of larval cod on Western Bank, Scotian Shelf. Can. J. Fish. Aquat. Sci. 52 (3), 448–463.

Mehault, S., Domínguez-Petit, R., Cerviño, S., Saborido-Rey, F., 2010. Variability in total egg production and implications for management of the southern stock of European hake. Fish. Res. 104, 111–122.

Morote, E., Olivar, M.P., Bozzano, A., Villate, F., Uriarte, I., 2011. Feeding selectivity in larvae of the European hake (*Merluccius merluccius*) in relation to ontogeny and visual capabilities. Mar. Biol. 158 (6), 1349–1361.

Murua, H., Michael, L., 2010. The biology and fisheries of European hake, *Merluccius merluccius*, in the north-east Atlantic. Adv. Mar. Biol. 58 (10), 97–154.

Nande, M., Costas, D., Palmeiro, O., Lago, M.J., Casal, A., Iglesias, J., Costoya, N., Pérez, M., Paredes, E., Otero, J.J., Padrós, F., Carrasón, M., Gómez-Gesteira, C., Presa, P., 2014. First Feeding of the European Hake *Merluccius merluccius*: Growth under Natural Diets and Larval Fatty Acid Profile. Aquaculture Europe. Donostia, Spain, pp. 875–876.

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

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

Papandroulakis, N., Mylonas, C.C., Maingot, E., Divanach, P., 2005. First results of greater amberjack (*Seriola dumerili*) larval rearing in mesocosm. Aquaculture 250 (1), 155–161.

Pearre, S., 1986. Ratio-based trophic niche breadths of fish, the Sheldon spectrum, and the size-efficiency hypothesis. Mar. Ecol. Prog. Ser. 27 (299–3), 14.

Pepin, P., Penney, R.W., 1997. Patterns of prey size and taxonomic composition in larval fish: are there general size-dependent models? J. Fish Biol. 51 (Suppl A), 84–100.

Pérez, N., Pereiro, F.J., 1985. Aspectos de la reproducción de la merluza *Merluccius merluccius* de la plataforma Gallega y Cantábrica. Bol. Ins. Esp. Ocean. 2 (3), 39–47.

Peterson, W.T., Ausubel, S.J., 1984. Diets and selective feeding by larvae of Atlantic mackerel *Scomber scombrus* on zooplankton. Mar. Ecol. Prog. Ser. Oldendorf 17 (1), 65–75.

Petrik, C.M., Kristiansen, T., Lough, R.G., Davis, C.S., 2009. Prey selection by larval haddock and cod on copepods with species-specific behavior: an individual-based

model analysis. Mar. Ecol. Prog. Ser. 396, 123–143.
Piñeiro, C., Rey, J., De Pontual, H., Goñi, R., 2007. Tag and recapture of European hake (*Merluccius merluccius* L.) off the Northwest Iberian Peninsula: first results support fast growth hypothesis. Fish. Res. 88 (1), 150–154.
Pita, A., Leal, A., Santafé-Muñoz, A., Piñeiro, C., Presa, P., 2016. Genetic inference of demographic connectivity in the Atlantic European hake metapopulation (*Merluccius merluccius*) over a spatio-temporal framework. Fish. Res. 179, 291–301.
Preciado, I., Punzón, A., Velasco, F., 2015. Spatio-temporal variability in the cannibalistic behavior of European hake *Merluccius merluccius*: the influence of recruit abundance and prey availability. J. Fish Biol. 86 (4), 1319–1334.
Puvanendran, V., Brown, J.A., 2002. Foraging, growth and survival of Atlantic cod larvae reared in different light intensities and photoperiods. Aquaculture 214 (1), 131–151.
Rajkumar, M., 2006. Suitability of the copepod, *Acartia clausii* as a live feed for seabass larvae (*Lates calcarifer* Bloch): compared to traditional live-food organisms with special emphasis on the nutritional value. Aquaculture 261 (2), 649–658.
Rasdi, N.W., Qin, J.G., 2016. Improvement of copepod nutritional quality as live food for aquaculture: a review. Aquacult. Res. 47 (1), 1–20.
Roberts, D., Murphy, H.M., Jenkins, G.P., Fortier, L., 2014. Poor taxonomical knowledge of larval fish prey preference is impeding our ability to assess the existence of a "critical period" driving year-class strength. ICES J. Mar. Sci. 71 (8), 2042–2052.
Russo, T., Boglione, C., De Marzi, P., Cataudella, S., 2009. Feeding preferences of the dusky grouper (*Epinephelus marginatus*, Lowe 1834) larvae reared in semi-intensive conditions: a contribution addressing the domestication of this species. Aquaculture 289 (3), 289–296.
Sánchez, F., Gil, J., 2000. Hydrographic mesoscale structures and Poleward Current as a determinant of hake (*Merluccius merluccius*) recruitment in southern Bay of Biscay. ICES J. Mar. Sci. 57 (1), 152–170.
Sánchez, F.J., Otero, J., Cal, J.R., Lago, M.J., Gómez, C., Iglesias, J., 2012. The first spontaneous spawning of European hake *Merluccius merluccius* L.: characteristics of eggs and early larval stages. Aquacult. Res. 43 (11), 1729–1733.
Schmitt, P.D., 1986. Prey size selectivity and feeding rate of larvae of the northern anchovy, *Engraulis mordax* (Girard, 1854). 27. CalCOFI Rep.pp. 153–161.
Segers, F.H.I.D., Dickey-Collas, M., Rijnsdorp, A.D., 2007. Prey selection by North Sea herring (*Clupea harengus*), with special reference to fish eggs. ICES J. Mar. Sci. 64 (1), 60–68.
Shields, R.J., Bell, J.G., Luizi, F.S., Gara, B., Bromage, N.R., Sargent, J.R., 1999. Natural copepods are superior to enriched Artemia nauplii as feed for halibut larvae (*Hippoglossus hippoglossus*) in terms of survival, pigmentation and retinal morphology: relation to dietary essential fatty acids. J. Nutr. 129 (6), 1186–1194.
Shirota, A., 1970. Studies on the mouth size of fish larvae. B. Jpn. Soc. Sci. Fish 36 (4), 353–367.
Sumida, B.Y., Moser, H.G., 1980. Food and feeding of Pacific hake larvae, *Merluccius productus*, off southern California and northern Baja California. CalCOFI Rep., Work, 161.
Tandler, A., Mason, C., 1984. The use of 14C labelled rotifers (*Brachionus plicatilis*) in the larvae of gilthead seabream (*Sparus aurata*): measurements of the effect of rotifer concentration, the lighting regime and seabream larval age on their rate of rotifer ingestion. Eur. Maricult. Soc. 8, 241–259.
Tičina, V., Vidjak, O., Kačič, I., 2000. Feeding of adult sprat, *Sprattus sprattus*, during spawning season in the Adriatic Sea. Ital. J. Zool. 67 (3), 307–311.
Treasurer, J., Atack, T., 2013.Acquisition of a hake broodstock. Report. Scottish Aquaculture Research Forum (SARF), pp. 29. http://www.sarf.org.uk/ms-assets/documents/124752-670325.sarf063-02.pdf (accessed 01.09. 14).
Tucker Jr., J.W., 2012. Marine Fish Culture. (Springer Science and Business Media).
Vallés, R., Estévez, A., 2013. Light conditions for larval rearing of meagre (*Argyrosomus regius*). Aquaculture 376, 15–19.
van der Meeren, T., Naas, K.E., 1997. Development of rearing techniques using large enclosed ecosystems in the mass production of marine fish fry. Rev. Fish. Sci. 5 (4), 367–390.
Velasco, F., Olaso, I., 1998. European hake *Merluccius merluccius* (L., 1758) feeding in the Cantabrian Sea: seasonal, bathymetric and length variations. Fish. Res. 38 (1), 33–44.
Viitasalo, M., Flinkman, J., Viherluoto, M., 2001. Zooplanktivory in the Baltic Sea: a comparison of prey selectivity by *Clupea harengus* and *Mysis mixta*, with reference to prey escape reactions. Mar. Ecol. Prog. Ser. 216, 191–200.
Yúfera, M., Ortiz-Delgado, J.B., Hoffman, T., Siguero, I., Urup, B., Sarasquete, C., 2014. Organogenesis of digestive system, visual system and other structures in Atlantic bluefin tuna (*Thunnus thynnus*) larvae reared with copepods in mesocosm system. Aquaculture 426, 126–137.
Zar, J.H., 1999. Biostatistical Analysis, fourth ed. Prentice-Hall, New Jersey.