Riboflavin enrichment throughout the food chain from the marine microalga *Tetraselmis suecica* to the rotifer *Brachionus plicatilis*and to White Sea Bream (*Diplodus sargus*) and Gilthead Sea bream (*Sparus aurata*) larvae

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Abstract

The riboflavin enrichment of the marine microalga *Tetraselmis suecica* and the transfer of this vitamin to higher trophic levels of the aquatic food chain such as the rotifer *Brachionus plicatilis* and the larvae of two species of sparids: white sea bream and gilthead sea bream were studied. The preliminary experiment consisted of determining the concentration of riboflavin added to *T. suecica* cultures to achieve a maximum quantity of this vitamin in the microalgal cells. Seven concentrations were tested in triplicate: 0 (control), 2.5, 5, 10, 20, 40 and 80 ng ml^{-1}; the results showed that the 10 ng ml^{-1} was the optimum concentration that the microalgae accumulated 4.29 ± 0.19 pg cell^{-1}, 21.2 ± 0.35 ng ml^{-1} and 19.4 ± 0.56 μg g^{-1} (dry weight) of riboflavin. Control and enriched microalgal cultures were used for feeding the rotifer *B. plicatilis*. Control and enriched rotifers were used for feeding white sea bream and gilthead sea bream larvae. Rotifers fed on enriched microalgal cultures accumulated significantly ($P < 0.05$) more riboflavin than those fed the control culture after 24 h of enrichment (17.7 ± 1.3 and 13.7 ± 1.2 μ g g^{-1} (dw), respectively) and after 24 h of starvation (10.2 ± 44 1.1 and 5.6 ± 0.4 μg g^{-1} (dw), respectively). In both species of sparids, those larvae fed enriched rotifers contained significantly more riboflavin than those fed control rotifers, the vitamin B2 content in control and enriched white sea bream larvae was 21.7 ± 2.7 and 29.2 ± 1.3 μg g^{-1} (dw), respectively, and in control and enriched gilthead sea bream larvae it was 5.5 ± 1.0 and 7.3 ± 0.05 μg g^{-1} (dw), respectively. Significant differences in length and survival of white sea bream larvae were observed. In the present study, riboflavin enrichment of microalgal cultures resulted in higher levels of this vitamin in both rotifers and fish larvae.

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Keywords

Microalga; Rotifer; Fish larva; Riboflavin; Aquaculture

1. Introduction

Microalgae are photosynthetic unicellular microorganisms that constitute the basis of the aquatic food chain. They are consumed by filter molluscs, crustacean larvae as well as by zooplanktonic species such as the rotifer *Brachionus plicatilis* and the microcrustacean *Artemia* sp., both reared as food for different species of crustacean and fish larvae in aquaculture. Despite the efforts made to replace microalgae with inert diets, aquaculture still depends on the production and use as live food for commercially important aquatic animals. The marine micoalga *Tetraselmis suecica* (Kylin) Butch is one of the live foods most frequently employed in aquaculture systems.

Microalgae generally contain high-levels of water-soluble vitamins but there is high variation within and among algal species (Seguineau et al., 1993, Seguineau et al., 1996 and Brown et al., 1999). Their vitamin content depends on genetic traits, culture conditions and the vitamin concentration in the growth medium (Seguineau et al., 1996, Brown et al., 1999 and Carballo-Cárdenas et al., 2003).

Riboflavin is a water-soluble vitamin and its principal metabolic role is as a component of two flavoprotein co-enzymes, flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN). These co-enzymes act as prostetic groups of many oxidation–reduction enzymes and are needed to maintain cellular respiratory functions, especially in little irrigated tissues such as the eye cornea. Inadequate supply of riboflavin, especially in fish, results in several gross deficiency signs such as reduced growth, appetite and feed conversion efficiency, cornea and eye lens opacity, darkening of body pigmentation (Fernández and Borrás, 1993), photophobia, cataract formation, eye haemorrhage, anemia and poor coordination (Halver, 1989).

Dietary vitamin supplementation in aquafeeds is achieved through various methods, such as vitamin premixes, vitamin emulsions and microalgal diets. Dietary supplementation of vitamins and other compounds (hormones, fatty acids, amino acids, etc) has been accomplished by using microencapsulated diets (Walford et al., 1991, Alarcón et al., 1999, Langdon, 2003, Yúfera et al., 2003 and Yúfera et al., 2003), lipid spray beads (Önal and Langdon, 2004 and Nordgreen et al., 2007) and phospholipid vesicles (Lai et al., 2004). A large amount of vitamins are lost during feed and microcapsule production (Langdon et al., 2000,Langdon, 2003, Yúfera et al., 2003, Önal and Langdon, 2004 and Langdon et al., 2007) and due to the leeching of specific components of formulated diets in water, especially water-soluble vitamins (Fernández and Blasco, 1993 and Gadient and Schai, 1994).

The culture of marine fish larvae is largely dependent on the supply of live diets such as rotifers to ensure successful larval development. In the present work, riboflavin enrichment of the marine microalga *T. suecica*and its transfer from the microalgae to rotifers and fish larvae were

studied. Microalgal cells were enriched by adding the appropriate concentration of riboflavin to the culture medium; rotifers and fish larvae fed directly or indirectly on the enriched microalgae were enriched in this vitamin as well.

2. Materials and methods

2.1. Experiment 1: riboflavin enrichment of *T. suecica* cultures

2.1.1. Microalgal culture

The first experiment consisted of determining the concentration of riboflavin added to *T. suecica* cultures to achieve maximum concentration of this vitamin in the microalgal cells. Cultures of the marine microalga *T. suecica* (Kylin) Butch were grown in 0.5 l flasks, maintained at 18 ± 1 °C and illuminated on 12:12 h light:dark (L:D) cycle. Natural seawater was enriched with Algal-1 medium to obtain a final concentration of: 4 mM $NaNO_3$; 0.2 mM NaH_2PO_4; 2 μM $ZnCl_2$; 2 μM $MnCl_2$; 2 μM Na_2MoO_4; 0.2 μM $CoCl_3$; 0.2 μM $CuSO_4$; 40 μM ferric citrate; 70 μg l − 1 thiamin; 10 μg l− 1 biotin; 6 μg l− 1 vitamin B12; 52.8 μM EDTA (Fábregas et al., 1984). When cultures had reached late-logarithmic growth phase, semicontinuous cultures were established with a daily renewal rate of 30%. Riboflavin was added to the microalgal cultures daily for five days. Previous studies demonstrated that dissolved riboflavin in the culture medium without microalgae exposed to the same conditions cited above experimented a 50% decrease after 24 h due to vitamin photolability. Seven riboflavin concentrations were tested in triplicate: 0 (control), 2.5, 5, 10, 20, 40 and 80 ng ml^{-1}. At the end of the experiment, samples of 200 ml of each culture were harvested by centrifugation and freeze-dried for riboflavin analysis. Microalgal cell density was measured by counting culture aliquots in a Neubauer haemocytometer using an Eclipse E400 microscope (Nikon, Japan). The dry weight was determined according to Utting (1985).

2.2. Experiment 2: riboflavin transfer from microalgae to rotifers and fish larvae

2.2.1. Microalgal culture

In the second experiment, enriched and control microalgae were used for feeding the rotifer *B. plicatilis*. Cultures of *T. suecica* were grown in 2 l Erlenmeyer flasks, maintained at 20 ± 1 °C and illuminated on 14:10 h L:D. As with the first experiment, natural seawater was enriched with Algal-1 medium (Fábregas et al., 1984) and when cultures had reached late-logarithmic growth phase, semicontinuous cultures were established with a daily renewal rate of 30%. Triplicate cultures with the optimum riboflavin concentration in the culture medium (obtained in the experiment 1) and control cultures (without riboflavin) were grown for five days. At that time, microalgae were used as food for rotifers and samples of 200 ml of each microalgal culture were harvested by centrifugation and freeze-dried for subsequent analysis.

2.2.2. Rotifer culture

Prior to the start of the experiment ("rotifers 0 h"), rotifers were starved for 24 h. Subsequently, cultures of the rotifer *B. plicatilis* were carried out in 1 l Erlenmeyer flasks (500 ml of culture) in a constant temperature chamber at 26 ± 1 °C. Rotifers were cultured at a density of 1000 rotifers ml^{-1} and fed enriched and control microalgal cultures for 24 h. Treatments were carried out in triplicate.

Aliquots of rotifer culture needed to feed fish larvae were removed ("rotifers 24 h"). The remaining quantities of rotifers were starved for an additional 24 h ("rotifers 48 h"). Samples of " rotifers 0 h", as well as samples of rotifers used as food for fish larvae ("rotifers 24 h") and samples of rotifers with the 24 h additional starvation ("rotifers 48 h") were collected using a 63 μm nylon screen and freeze-dried in order to analyse their riboflavin content.

2.2.3. Fish larvae culture

Two species of sparids were used in this study: *Sparus aurata* L. (gilthead sea bream) and *Diplodus sargus* L (white sea bream). Eggs were obtained from broodstock reared in tanks in the Estaçao de Piscicultura (IPIMAR) in Olhão (Portugal), and incubated in 200 l tanks at 19 ± 1 °C.

The same procedure was followed with both fish species. When larvae had opened their mouths, they were moved to 20 l tanks. The tank water temperature was 18 ± 1 °C with 110 ml min^{-1} of renewal flow-rate and they were illuminated on 14:10 h L:D cycle. Water temperature and dissolved oxygen were measured daily. The fish larvae were stocked at a density of 50 larvae l^{-1}, with a total of 1000 larvae per tank. In three of these tanks, larvae were fed enriched rotifers and in three other tanks with control rotifers, at a density of 5 rotifers ml^{-1}, two or three times per day (according to dietary larval needs) for five days.

In order to study the effect of riboflavin enrichment in fish larvae, data of initial and final length (30 larvae per tank) and survival were reported daily. Survival rate was calculated by a daily counting of death larvae in each tank in relation to initial density of larvae (1000 larvae per tank). Samples were collected at the beginning (before being fed) and end of the experiment and then freeze-dried for vitamin analysis.

2.3. Riboflavin analysis

Freeze-dried samples were extracted by the method of Dawson et al. (1988) with modifications. Samples were hydrolysed with 0.5 N HCl (121 °C for 30 min), cooled to room temperature, adjusted to pH 4.0 to 4.5 with 2 M sodium acetate and incubated with a mixture of α-amylase and papain at 42 °C for 3 h. Samples were deproteinated with 50% trichloroacetic acid at 100 ° C for 10 min. Samples were filtered and analysed on a Hewlett Packard HPLC (High Performance Liquid Chromatography) using a reversed-phase 250 × 4.6 mm Eclipse XDB C_{18} (5 μm) column with a fluorescence detector (FLD) at an excitation maximum at 450 nm and an emission maximum of 530 nm.

2.4. Statistical analysis

Statistical tests were performed using SPSS/PC+ version 11.0 (SPSS Inc.) program. Data were analysed by an overall one-way analysis of variance (ANOVA) and, when differences observed were significant, means were compared by the Duncan's multiple range test.

3. Results

3.1. Riboflavin enrichment of *T. suecica* cultures

Riboflavin concentration in microalgal cultures did not affect culture growth. Media of cellular densities of each culture were compared and no significant differences were found (Fig. 1). The marine microalga *T. suecica* accumulated riboflavin as the vitamin concentration in the culture medium increased, until reaching a maximum at which point it began to decrease. This maximum was achieved when the vitamin concentration in the culture medium was 10 ng ml^{-1}. With higher concentrations of vitamin in the culture medium, the intracellular riboflavin content decreased (Fig. 2 and Fig. 3).

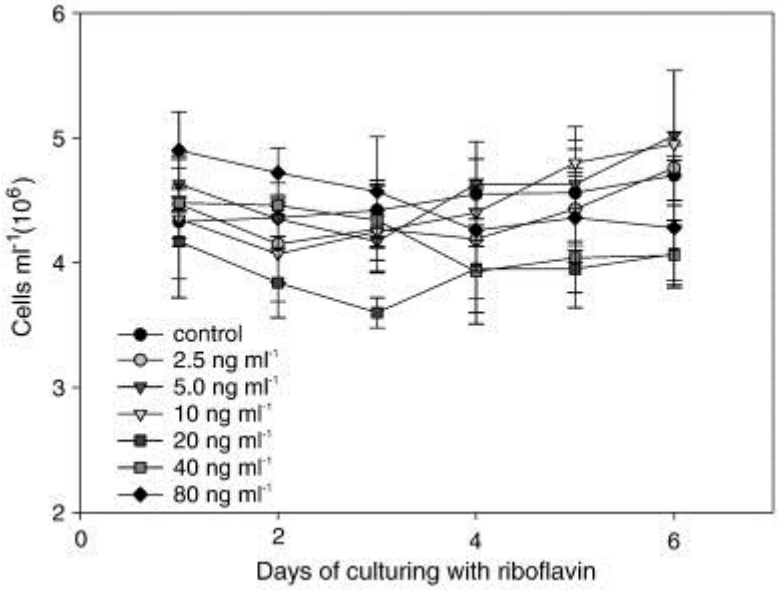

Fig. 1.

Cell density of *Tetraselmis suecica* cultures during the period of enrichment. Cultures were in semicontinuous after reached late logarithmic phase. Data are given as mean ± SD.

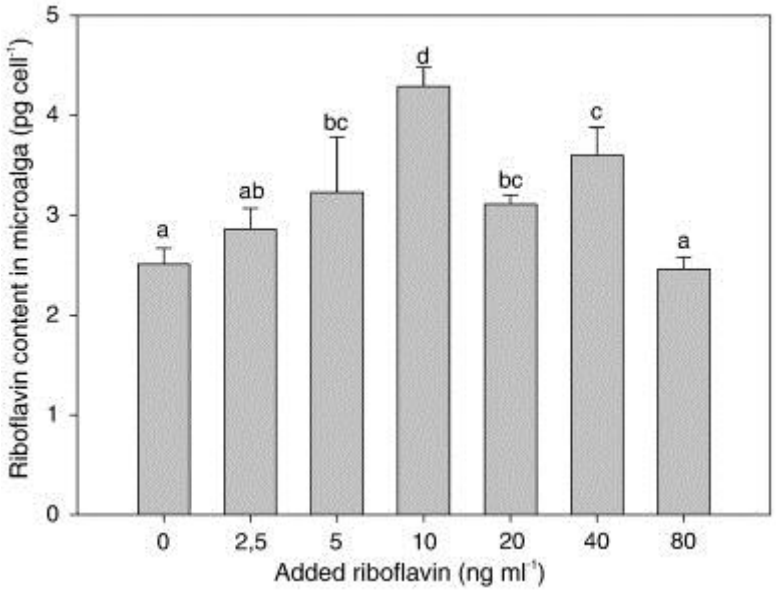

Fig. 2.

Riboflavin content in *Tetraselmis suecica* expressed as pg cell^{-1} at different concentrations of added vitamin. Data are given as mean ± SD. Microalgal cultures sharing the same letters are not significantly different from each other (ANOVA, Duncan's test, $P < 0.05$).

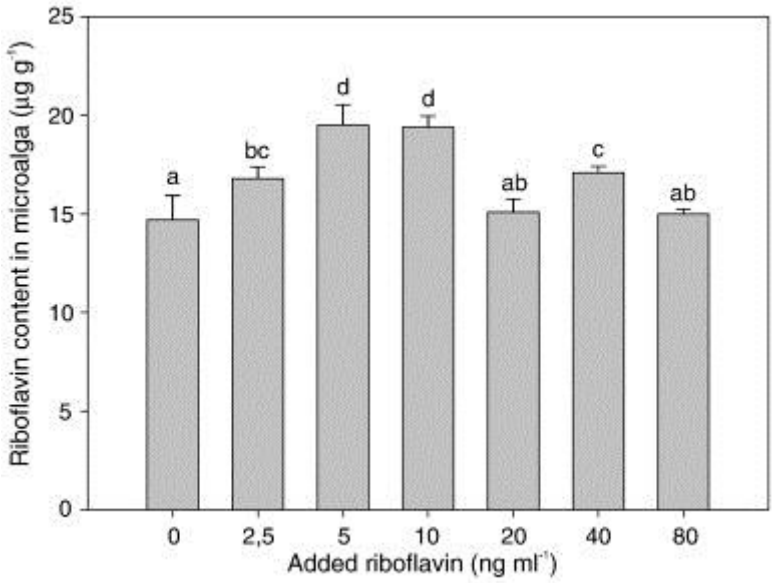

Fig. 3.

Riboflavin content in *Tetraselmis suecica* expressed as µg g^{-1} of dry weight at different concentrations of added vitamin. Data are given as mean ± SD. Microalgal cultures sharing the same letters are not significantly different from each other (ANOVA, Duncan's test, $P < 0.05$).

When the riboflavin content in *T. suecica* is expressed as pg cell^{-1} (Fig. 2), the maximum vitamin content was found in cultures with 10 ng ml^{-1} of added vitamin (4.29 ± 0.19 pg cell^{-1}). This value was significantly ($P < 0.05$) higher than the vitamin content of the remaining cultures. However, there were no difference between control cultures (2.51 ± 0.16 pg cell^{-1}) and cultures with 80 ng ml^{-1} of vitamin (2.46 ± 0.12 pg cell^{-1}).

Similar results were obtained when the riboflavin content in microalgae is expressed as ng ml^{-1}. Cultures with 10 ng ml^{-1} of added vitamin had higher ($P < 0.05$) vitamin concentration

(21.2 ± 0.35 ng ml^{-1}) than the remaining cultures. However, there were no differences between control cultures (11.8 ± 0.69 ng ml^{-1}) and cultures with 80 ng ml^{-1} of vitamin (10.8 ± 0.68 ng ml^{-1}).

When the riboflavin content in microalgal cells is expressed as μg g− 1 (dw) (Fig. 3), cultures with a vitamin concentration of 5 and 10 ng ml^{-1} had higher ($P < 0.05$) vitamin content than the remaining ones, reaching values of 19.5 ± 1.04 and 19.4 ± 0.56 μg g^{-1} (dw), respectively, but there were no difference between them. In control cultures, the riboflavin content in *T. suecica* cells was 14.7 ± 1.23 μg g^{-1} (dw). The concentration of 10 ng ml^{-1} of riboflavin in the culture medium was chosen as the optimum to achieve a maximum enrichment for marine microalga *T. suecica*.

3.2. Riboflavin transfer from microalgae to higher trophic levels

3.2.1. *T.suecica → B. plicatilis*

Microalgal cultures with a riboflavin concentration of 10 ng ml^{-1} and control cultures (without added vitamin) were used for feeding the rotifer *B. plicatilis* for 24 h. There were no significant differences between the riboflavin content of microalgae used to feed rotifers and that observed in microalgae of the preliminary experiment with 10 ng ml^{-1} of riboflavin added to the culture medium.

Rotifers fed enriched microalgal cultures accumulated significantly ($P < 0.05$) more riboflavin than those fed the control ones after 24 h (“rotifers 24 h”) of enrichment. The riboflavin enrichment in the rotifer after 24 h were 2.1 and 2.7 for the rotifer feed with control and enriched microalgae, respectively. Even after being starved an additional 24 h (“rotifers 48 h”), a higher ($P < 0.05$) riboflavin concentration was observed in enriched rotifers. After 24 h of starvation the rotifers lost 59% and 42% of their riboflavin content, rotifers feed with control and enriched microalgae, respectively. (Table 1).

Table 1.

Riboflavin content in *Brachionus plicatilis* fed with control and enriched microalgal cultures

Brachionus plicatilis	Control (μg g^{-1})	Enriched (μg g^{-1})
0 h	6.49 ± 0.7	6.49 ± 0.7
24 h	13.7 ± 1.2	17.7 ± 1.3
48 h	5.6 ± 0.4	10.2 ± 1.1

Samples of rotifers were collected to analyse their riboflavin content at 0 h (beginning of the experiment), 24 h (rotifers after 24 of feeding with control and enriched microalgae) and 48 h (rotifers fed with control and enriched microalgae for 24 h and starved for an additional 24 h). Data are given as means values ± standard errors of the means.

3.2.2. *B. plicatilis → D. sargus*

White sea bream larvae fed enriched rotifers showed significantly ($P < 0.05$) enriched levels of riboflavin in comparison with those fed the control ones, in which the riboflavin content at the end of the experiment was similar to the initial state (Table 2).

Table 2.

Riboflavin content in white sea bream and gilthead sea bream larvae fed with control and enriched rotifers

		Control (μg g^{-1})	*Enriched* (μg g^{-1})
White sea bream	Initial	23.3 ± 0.8	23.3 ± 0.8
	Final	21.7 ± 2.7	29.2 ± 1.3
Gilthead sea bream	Initial	2.5 ± 0.5	2.5 ± 0.5
	Final	5.5 ± 1.0	7.3 ± 0.05

Samples of fish larvae were collected to analyse their riboflavin content when larvae had opened the mouth (Initial) and after 5 days of feeding with control and enriched rotifers (Final). Data are given as means values ± standard errors of the means.

Significant differences ($P < 0.05$) in length and survival of fish larvae were observed (Table 3). The final length of larvae fed enriched rotifers was 3.94 ± 0.01 mm while larvae fed the control rotifers was 3.85 ± 0.02 mm, the initial length of larvae being 3.63 ± 0.01 mm. Larval survival of those fed riboflavin enriched rotifers was 74.6% in contrast to the 61.3% observed in control larvae (without riboflavin).

Table 3.

Length and survival of white sea bream and gilthead sea bream larvae fed with control and enriched rotifers

		Length (mm)		Survival (%)	
		Control	Enriched	Control	Enriched
White sea bream	Initial	3.63 ± 0.01	3.63 ± 0.01	61.3 ± 1.2	74.6 ± 1.4
	Final	3.85 ± 0.02	3.94 ± 0.01		
Gilthead sea bream	Initial	3.44 ± 0.1	3.44 ± 0.1	73.4 ± 0.8	68.4 ± 4.5
	Final	3.81 ± 0.1	3.84 ± 0.05		

Data are given as means values ± standard errors of the means.

3.2.3. *B. plicatilis → S. aurata*

Gilthead sea bream larvae fed enriched rotifers accumulated higher ($P < 0.05$) content of riboflavin than larvae fed control rotifers, reaching values of 7.3 ± 0.05 and 5.5 ± 1 μg g^{-1} (dw), respectively (Table 2). However, there were no difference between enriched and control larvae in either their length or survival (Table 3). The final length of control larvae was 3.81 ± 0.1 mm while in enriched larva was 3.84 ± 0.05 mm. Initial length of larvae was 3.44 ± 0.1 mm. In this case, no difference was observed in larval survival, which was 73.4% in the controls and 68.4% in enriched ones.

4. Discussion

In the present work, riboflavin enrichment in microalgal cultures was observed as the vitamin concentration in the culture medium increased. The maximum vitamin content in microalgae was achieved at a concentration of 10 ng ml^{-1} of added vitamin. Higher riboflavin concentrations in the culture medium resulted in a decrease in the vitamin content in *T. suecica* cultures. Although little information is available related to vitamin enrichment of microalgal cultures, this

decrease could be due to vitamin excretion by microalgal cells. This degree of vitamin excretion varies with the phase of the growth cycle and with growth conditions (Aaronson et al., 1971, Odintsova and Shlapkauskaite, 1975 and Nishijima et al., 1979). Mutants of *Chlamydomonas eugametos*, which excreted from six to eight times more nicotine acid than the wild type, were isolated by Nakamura and Gowans (1964). This high level of vitamin excretion was not caused by an increase of vitamin synthesis but by a high excretion rate. It has been reported that an excretion of vitamins or other growth-promoting substances by algae was one of the theories used to explain the improvement in the production of larvae by adding microalgae to larval tanks (Brown, 2002). Further research on vitamin excretion by microalgae may help to explain the effect of growth conditions in microalgae cultures in order to increase the production of commercially produced vitamins.

The concentration of riboflavin observed in the control cultures of the marine microalga *T. suecica* (14.7 ± 1.23 μg g^{-1} dry weight) is lower than those published by other authors. Values of riboflavin content in *T. suecica* of 19.1 μg g^{-1} (Fábregas and Herrero, 1990), 42 μg g^{-1} (De Roeck-Holtzhauer et al., 1991) and 26 μg g^{-1} (Brown et al., 1999) have been reported. The riboflavin content in microalgae increases as the stationary phase begins (Brown and Farmer, 1994), so that the difference could be due to those specified in the studies mentioned above. The values were obtained at the end of the logarithmic phase or in the stationary phase, whereas the samples used in the present study were collected from a steady semicontinuous culture in the middle of the logarithmic growth phase. Other factors that could contribute to differences among studies include protocols for harvesting (centrifugation or filtration), processing and storage (freeze-dried or frozen at − 20 °C or lower), extraction and analysis (Brown et al., 1999).

In the current study a riboflavin enrichment of microalgal cultures was achieved, and as well in higher trophic levels such as the rotifer *B. plicatilis* and the *D. sargus* and *S. aurata* larvae fed them. There are several studies about the enrichment of zooplanctonic species using microalgal cultures. Brown et al. (1998)successfully enriched rotifers with vitamin C by feeding them microalgal diets compared to those fed baker's yeast. The degree of enrichment was directly related to the concentration of the vitamin in the microalgae. These results were similar to those obtained in the present study where the riboflavin enrichment of rotifers after 24 h is multiplied by 2.1 and 2.7 related with the riboflavin concentration of microalgae: control and enriched respectively. After 24 h of starvation the rotifer lost 59% and 42% of their riboflavin content for the both conditions tested, this lost is higher than the results with ascorbic acid (10%) reportedby Brown et al. (1998). Olsen et al. (2000) observed a vitamin C enrichment in *Artemia franciscana* using the microalga *Isochrysis galbana* as food. However, a decrease in vitamin B6 but no change in thiamin content in brine shrimp was detected. Those studies were based on the natural content of vitamin in the microalgae; in the present study, microalgal cells were enriched in riboflavin and then used to feed rotifers. The introduction of more vitamins from the first step in the aquatic food chain will lead to an increase in vitamin content in animals reared in aquaculture systems.

Although little information is available related to vitamin enrichment of zooplanctonic species using microalgae as food, there are reports related to the enrichment of microalgal cultures in other substances such as PUFA (polyunsaturated fatty acids) (Fidalgo et al., 1998) and astaxantin. Astaxantin enriched microalga was used to feed the *Artemia* sp. which gained a reddish pigmentation. By means of this food chain, this carotenoid can be transferred to higher trophic levels, providing the characteristic colour in salmonids and crustaceans (Orosa et al., 2001). Similarly, Fábregas et al. (2001) changed the protein, lipid and carbohydrate composition of *Artemia* sp. depending on the renewal rate of semicontinuous cultures of *T. suecica*.
In the present study, riboflavin enrichment of white sea bream and gilthead sea bream larvae was achieved by using riboflavin enriched rotifers as food. Limited literature is available related to riboflavin enrichment of fish or mollusc larvae using live food.

Seguineau et al. (1993) reported that the vitamin content in microalgae used to feed *Pecten maximus* larvae would be sufficient compared to data for vitamin requirements of coldwater fish, except for riboflavin. Furthermore, the content of riboflavin decreased during the first week of rearing and then was stabilized by ingestion. They also described the variation in the thiamin and riboflavin content in tissue of the scallop *P. maximus* and the Pacific oyster *Crassostrea gigas* during their reproductive cycle and they suggest an intense utilisation of those vitamins when mitoses in the gonads take place (Seguineau et al., 2001a and Seguineau et al., 2001b). The riboflavin composition of live feed (copepods, rotifers and enriched *Artemia*) compared with larval fish requirement (5 μg g^{-1}) was established by Hamre (2006). Van der Meeren et al. (2008) studied the biochemical composition of three s pecies of copepods to assess their nutritional value as food for juvenile and larvae of marine fish. They reported that riboflavin levels in copepods exceeded the recommended minimum requirements for fish, including juveniles, but there is little information available on the riboflavin requirements of marine fish larvae. The riboflavin requirement in fish species is relatively low estimated to be between 3 and 11 mg kg^{-1} of the diet (NRC, 1993). Deng and Wilson (2003)have demonstrated that the dietary riboflavin requirement were 4.1 mg kg^{-1} dry diet based on maximum weight. On the other hand, Fernández-Díaz et al. (2006) compared live and microencapsulated diets when feeding *Solea senegalensis* larva. They observed that those larvae that fed *Artemia* nauplii diets showed higher growth and a faster metamorphosis than those fed inert diets.
In the current study, it was observed that white sea bream larvae fed riboflavin enriched rotifers attained larger size and had higher survival than those fed control rotifers. However, in gilthead sea bream larvae the vitamin enrichment did not affect either growth or survival, indicating that the riboflavin content in rotifer feed with non enriched microalgae may be sufficient to the requirement of these larvae. There are few studies that indicate that riboflavin deficiency produces a decrease in survival and growth of fish, mollusc or crustacean larvae. Brønstad et al. (2002) reported that there were no difference in growth of Atlantic salmon parr as a result of riboflavin supplementation and no mortality was detected. Nevertheless, it has been observed that those parameters are most often affected by the vitamin C content in diet due to its

important role as an antioxidant as well as in the immune response (Merchie et al., 1995, Kolkovski et al., 2000, Smith et al., 2004 and Ai et al., 2006) Similar results in fish larvae have been reported in relation to dietary vitamin A (Fernández-Díaz et al., 2006 and Fontagné et al., 2006).

There is little information related to vitamin enrichment of the aquatic food chain. In the present study, by means of a riboflavin enrichment of microalgal cultures, higher levels of this vitamin were found in the aquatic food chain (rotifers and fish larvae). Further research on vitamin enrichment is needed in order to improve the quality of diets for larval culture systems.

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