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The inhibitory effects and positive contributions of live foods on protease activities of meagre, *Argyrosomus regius* (Asso 1801) larvae *in vitro* assay

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Abstract: The aim of this study was to gather preliminary data about the potential inhibitory effects and contributions of live foods used from 3 to 32 days after hatching (DAH) in routine feeding protocols on protease activities of meagre, *Argyrosomus regius* (Asso 1801) larvae, using in vitro techniques. Enriched rotifer, *Artemia* nauplii and *Artemia* metanauplii were tested in the present study. The highest values of protease activities of meagre larvae at 7 DAH in 2013 and 2014 years were established. The lowest values at 15 DAH in 2013 and at 20 DAH in 2014 were observed. Protease activities of enriched rotifer, *Artemia* nauplii, and *Artemia* metanauplii were 21.76±0.31, 36.00±1.48–29.33±0.93, and 416.44±19.7–403.53±11.85 U/mg protein, respectively ($p< 0.05$). The highest inhibitions of live foods were observed at 7 DAH. The positive contributions of live food *Artemia* metanauplii's on protease activities of meagre larvae were significant ($p< 0.05$). The inhibitory effects and positive contributions of live foods on survival and growth rates of meagre larvae, should be taken into account in meagre and other marine fish larvae's future studies. Cysteine protease activities of *Artemia* sp. should be investigated to provide the higher growth and survival ratio from the feeding protocols used in marine fish larvae.

Keywords: meagre; *Argyrosomus regius*; protease activity; inhibitions; live food; *in vitro*

1. Introduction

It is an important and controversial subject of larval nutrition to can provide sufficient amount of nutrients to the marine fish species whose initial feeds are 35–100 μ, that affects the development of rearing [1,2,3]. Although microdiets have been used with satisfactory results in the fish species such as salmonid fishes and African catfish, *Clarias gariepinus*, larvae, the growth rate and survival were lower than those obtained in larvae fed on *Artemia* nauplii [4,5]. However, the larval stages of marine fish larvae require live food organisms for optimal growth and survival. Cahu and Zambonino Infante, indicated that the survival and growth of marine fish larvae fed solely on microdiet are known to be very poor, but supplementation with live foods usually results in a marked improvement [6]. To explain the success of live food over microdiets, some authors showed that fish larvae had insufficient digestive enzyme capacity for the digestion of exogenous food [7,8,9]. Additional information in this regard needs to define the factors that influence the better utilization of live food by fish larvae when compared to microdiets. Therefore, recent studies have been focused on the contribution of digestive enzymes from *Artemia* and rotifers commonly used as the major food source in aquaculture [10,11,]. García–Ortega et al., showed that the contribution of digestive enzymes from *Artemia* to the total digestion of food by the catfish larvae was less than 1% of the total amount of the proteolytic activity measured in the larval gut [11]. Some studies demonstrated that proteases derived from live food had only a small contribution to the enzymatic activity measured in sea bass and sardine larvae [10,12]. Although the results of the above studies, there still exist some unresolved issues concerning the contribution of

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exogenous enzymes to digestion. For a sustainable aquaculture, studies on determining effects of live foods supported the protease activity of larvae in critical feeding periods are the key tools to understand and solve nutritional problems. In this point, inhibitory effects of the live foods used in the larval feeding must be investigated to solve the nutritional problem. Studies carried out until now focused on the changes in the biochemical compositions and enzymatic activities observed through the different developmental stages and the different periods such as the enrichment and the starvation of live foods [13,14].

Enzymatic differences in live foods and the fact that *Artemia* is a feed source due to environmental and seasonal effects and that the copepod culture has not been achieved at industrial level despite its superiority in nutrient and enzyme activities is in the problems of live food nutrition [15–23]. Live foods especially in *Artemia*, it has been reported that the external enzymes in *Artemia* help larval digestion or activate existing zymogens in the larval gut [24–26]. Recent studies have found that live food does not stimulate enzyme production or secretion in the intestinal lumen, there's lack of pancreatic and intestinal enzyme levels in larvae fed on live food or micronutrients, and that the contribution of the external enzyme is limited by the otolithic process of live food on the larval intestine [25,26]. It has also been understood that the contribution of external digestive enzymes to the total digestion capacity of larvae is negligible in many species at negligible levels [26]. *Artemia*, on the other hand, has been reported to stimulate endocrine response, affecting digestion, boosting the bombesin hormone and activating endocrine factors that increase utilization of microdiets [25].

There are studies dealing with the production of meagre, *Argyrosomus regius* an important species for the Mediterranean Basin [27–34]. It has been found that the larvae of meagre are showing rapid development in these studies and in the study carried out. This can be considered as an important advantage for larval feeding protocols. However, it is important to determine the changes in the ontogenetic and enzymatic activities of the larvae as well as larval development. In relation to this topic, there are also studies dealing with the enzymatic changes of the meagre [31,33–36]. On the other hand, the lack of studies and the inadequacy of practical applications to determine the potential of evaluation of live food sources due to changes in larval development and enzymatic activities have been determined. And currently, a study on the inhibitory effects of live foods on protease activities of meagre larvae is not available. The studies carried out on this subject will sort out on the scientific evaluation as well as on the quest for industrial development.

Therefore, the aims of this research were (i) to revealed data about the potential inhibitory effects of live foods commonly used in the routine feeding procedure of marine fish larvae such as enriched rotifer (*Brachionus plicatilis*), *Artemia* nauplii and *Artemia* metanauplii on protease activities of meagre larvae using *in vitro* techniques (ii) to investigate the contribution of digestive proteases from live foods to the total digestion of food during critical larval stages of meagre larvae.

2. Materials and Methods

2.1. Larval rearing and sampling

Larval rearing and sampling stage of the present study was carried out at the EGEMAR Aquaculture Food Industry and Commercial Incorporated Company. Larval rearing protocol and sampling are given at Table 1.

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2.2. Analytical methods

2.2.1. Extracts of larvae

The samples were rinsed in distilled water after thawing and then the extracts of whole larvae were homogenizationed and centrifuged (16,000 g, 30 minute 4ºC).

Table 1. Meagre larvae's rearing protocol

DAH	Practice
	Hormone injection (GnRH; 20 µg kg-1 ♀ and 10 µg kg-1 ♂) **Eggs incubition** (conical fiberglass tank; 23.6±0.5&22.0 ±0.2 ºC at 2013&2014 year, respectively)
0-15	**Larva tank** (7 m^3 ellipsoidal fiberglass tanks with black walls; 75-80 larvae/L)
15/16-32	**Weaning tank** (raceway 27&15 m^3 tank at 2013&2014 year, respectively and 10-12 larvae/L)
0-32	**Sea water** (sand, bag and UV filters; 20.8-24.1&20.8-22.2ºC temperature, 27.0-40.0 g/L salinity, 8.4-14.4&7.8-14.7 mg/L oxygen levels, 7.5-7.9&7.7-8.1 pH; 18L-light:6D-dark at 2013&2014 year, respectively)
	Green water
3-15	(Sanolife GWS; INVE Aquaculture, NV Hoogveld, 91 9200, Dendermonde, Belgium or ω3 Algae®; Bernaqua, NV Hagelberg, 3 B-2250, Olen, Belgium&*Nannochloropsis occulata* at 2013&2014 year, respectively)
16-26	Sanolife GWS&*Nannochloropsis occulata* at 2013&2014 year, respectively
	Live food
3-9&3-8	Rotifer, *Brachionus plicatilis* Culture 25 ºC temperature and 28 g/L salinity (Algamac Protein Plus; Aquafaune Bio-Marine Inc. Hawthorne USA and Sparkle; INVE Aquaculture) Enrich 26 ºC temperature and 28 g/L salinty (Spresso; INVE Aquaculture) 10-15 prey/mL enriched rotifer (**R**) at 2013&2014 year, respectively
6-11&7-11	*Artemia* nauplii 29 ºC temperature and 28 g/L salinty (AF 480; INVE Aquaculture&Artemia Cysts; Vinh Chau-Bac Lieu Artemia Co. at 2013&2014 year, respectively) 2-4&4-6 prey/mL *Artemia* nauplii (**A0**) at 2013&2014 year, respectively
10-32	*Artemia* metanaupli (*Artemia* EG; 250.000 npl/g Salt Lake Artemia Great Salt Lake Brine Shrimp Cooperative, Inc.&*Artemia* SepArt EG >250.000 npl/g INVE Aquaculture Salt Lake City Utah/USA at 2013&2014 year, respectively) Culture 29 ºC temperature and 28 g/L salinty Enrich 24 h, 26 ºC temperature and 28 g/L salinty (Algamac 3050; Aquafaune Bio-Marine Inc., Red Papper; Bernaqua, Spresso; INVE Aquaculture&Spresso; INVE Aquaculture at 2013&2014 year, respectively) 1.5-6&1.5-5 prey/mL enriched *Artemia* (**A1**) at 2013&2014 year, respectively
	Microdiets
16/17-32	(Orange Start-S,100-200 µ, Orange Start-L, 200-300 µ, Orange Nurse-XS, 300-500 µ, Orange Grow-S, 300-500 µ, Orange Grow-L, 500-800 µ; INVE Aquaculture&Gemma Micro 150, 100-200µ; Skretting AS Sjøhagen 15, 4016 Stavanger/P.b. 319 Sentrum, 4002 Stavanger, Norway, Caviar 200-300µ; BernAqua, Caviar 300-500µ; BernAqua, Perla Larva Proactive 4.0 300-500µ; Skretting AS at 2013&2014 year, respectively)
Sampling	
3-32	Before the morning feeding and stored in liquid nitrogen (-196 ºC)

2.2.2. Extracts of live foods

Five live foods enriched rotifer (*B. plicatilis*; **R**), *Artemia* nauplii (AF 480, INVE Aquaculture; **A0**–AF), *Artemia* nauplii (*Artemia* Cysts, Vinh Chau-Bac Lieu Artemia Co.Op; **A0**–AC), *Artemia* metanauplii (EG, Salt Lake *Artemia* Great Salt Lake Brine Shrimp Cooperative, Inc.; **A1**–SL) and *Artemia* metanauplii (EG, INVE Aquaculture Salt Lake City Utah/USA; **A1**–EG) were tested with *in vitro* techniques in the present study. Extracts of live foods prepared by homogenization (100 mg/mL in distilled water) followed by centrifugation

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(15,000 g, 10 minute) were used in protease inhibition analyses.

2.2.3. Determination of protease activities of larvae and live foods

Total protease activities of meagre larvae and live foods were measured as described by [37], using casein (10 mg/mL) in 50 mM Tris–HCl buffer at pH 8.5 as the substrate. The mixtures including extracts of larvae-substrate and live food-substrate were incubated and then the reaction was stopped by addition of 500 µL trichloroaceticacid (TCA) (120 g/L). One unit of enzyme activity was defined as 1 µg of tyrosine release per minute. The soluble protein concentrations of meagre larvae and live foods were determined according to Bradford [38].

2.2.4.Effects of live foods on protease activities of larvae

The inhibitory effects of live foods on protease activities of meagre larvae were determined by measuring the reduction in protease activity of extracts using a modification of the method described by García–Carreño [39]. The method is based on the measurement of residual protease activity remaining after preincubation with live foods such enriched rotifer, *Artemia* nauplii and *Artemia* metanauplii.

2.2.5.Statistical methods

Fish total lenght and wet weight were calculated and are given on as mean±standard error (SE). The experimental data (protease activities of meagre larvae and inhibitios and contributions of live foods on protease activities of meagre larvae's) were subjected to one–way ANOVA and mean±standard error (SE) differences were made by Duncan test at P=0.05 content level by using SPSS software statistical package [40]. All measurements were carried out in triplicates.

This research study was approved by Süleyman Demirel University Local Ethical Committee on Animal Experiments (Ref. Number: B.08.6.YÖK.2.SD.0.05.0.07.00-22).

3. Results

The present study results reveal the inhibition effects and the digestive protease contributions of live foods such as enriched rotifer, *Artemia* nauplii and *Artemia* metanauplii on protease activities of meagre larvae from 3 to 32 DAH. Also, the growth parameters (the total length and wet weight values) of meagre larvae from 3 to 32 DAH were determined in the study. Total length and wet weight of meagre larvae values from 3 DAH to 32 DAH were 3.19±0.02–21.61±0.22 mm and 0.53±0.02–118.00±1.09 mg at 2013 year and 3.22±0.02–20.95±0.30 mm and 0.54±0.02–89.21±0.91 mg at 2014 year, respectively (Figure 1).

The changes observed in protease activities, inhibitions and digestive protease contributions of live foods are given in Table 2. Figure 2 and Figure 3 show protease activities of meagre larvae, inhibition values and contributions of live foods on protease activities of meagre larvae at 2013 and 2014 years, respectively. The differences observed in protease activities of meagre larvae from 3 to 32 DAH were statistically significant ($p < 0.05$). The highest value of protease activities of meagre larvae at 7 DAH were 211.21±12.56 and 393.97±7.9 U/mg protein at 2013 and 2014 years, respectively. The lowest values of protease activities of meagre larvae at 2013 and 2014 years were 5.95±0.60 U/mg protein at 15 DAH and 9.64±1.25 U/mg protein at 20 DAH, respectively. Results revealed that the fluctuations in

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protease activities of the larvae feed of live food at the larva unit were sharper than those of the live food cofeed with the microdiet in the weaning unit.

Table 2 Protease activities of live foods and inhibitions and contributions of live foods on protease activities of meagre larvae

	Rotifer	*Artemia* nauplii		*Artemia* metanauplii	
	R	**A0**-AF	**A0**-AC	**A1**-SL	**A1**-EG
	Protease activity (U/mg protein means±SE)				
	21.76±0.31^{B}	36.00±1.48^{B}	29.33±0.93^{B}	416.44±19.70^{A}	403.53±11.85^{A}
	Inhibition and contributions degres (% means±SE)				
DAH	**Larvae unit**				
3	–44.87±0.23^{a}	–35.02±0.27^{ab}	–34.97±0.27^{a}	–23.98±0.32^{a}	–37.31±0.26^{a}
5	–28.76±0.18^{b}	–21.38±0.20^{cde}	–17.49±0.21^{bc}	18.21±0.30^{bc}	**2.91±0.26**^{bc}
7	–51.60±0.97^{a}	–38.46±1.23^{a}	–40.29±1.20^{a}	–32.46±1.35^{a}	–43.57±1.13^{a}
10	**23.06±6.77**^{f}	–14.41±4.71^{def}	**19.72±6.59**^{f}	**199.31±16.48**^{g}	**162.94±14.48**^{fg}
12	**14.91±4.01**^{f}	–12.18±3.07^{ef}	–6.70±0.65^{d}	**173.80±9.57**^{g}	**133.87±8.17**^{f}
15	–16.64±4.27^{cd}	–14.99±4.36^{def}	–17.02±4.25^{bc}	**83.19±9.39**^{ef}	**50.17±7.70**^{de}
	Weaning unit				
17	–28.10±1.66^{b}	–25.80±1.71^{bcd}	–17.49±1.90^{bc}	**1.46±2.34**^{ab}	–24.43±1.74^{ab}
20	**19.05±0,00**^{f}	–20.42±9.14^{cdef}	**18.30±0.00**^{f}	**247.55±39.92**^{h}	**174.94±31.58**^{g}
22	**3.50±2.46**^{e}	–23.37±3.50^{bcde}	–13.32±1.90^{cd}	**114.80±9.82**^{f}	**79.44±8.20**^{e}
25	**17.14±1.81**^{f}	–9.11±1.78^{f}	**24.52±2.44**^{f}	**89.64±3.71**^{ef}	**80.07±3.53**^{e}
27	–13.74±3.26^{cd}	–27.98±2.72^{abc}	–14.46±3.24^{cd}	**51.77±5.74**^{cde}	**531.53±23.88**^{h}
30	–22.10±3.11^{bc}	–30.05±2.79^{abc}	–25.51±2.97^{b}	**23.34±4.92**^{bcd}	**12.54±4.49**^{bcd}
32	–8.00±3.30^{d}	–8.28±3.29^{f}	**7.36±1.12**^{e}	**62.46±5.83**^{de}	**29.11±4.63**^{cd}

Differences between different uppercase and lowercase letters in the same column (mean±SD) are significant ($p< 0.05$). The dark colored columns show the values of the live foods in the period and the dark colored numbers show the contributions of live foods.

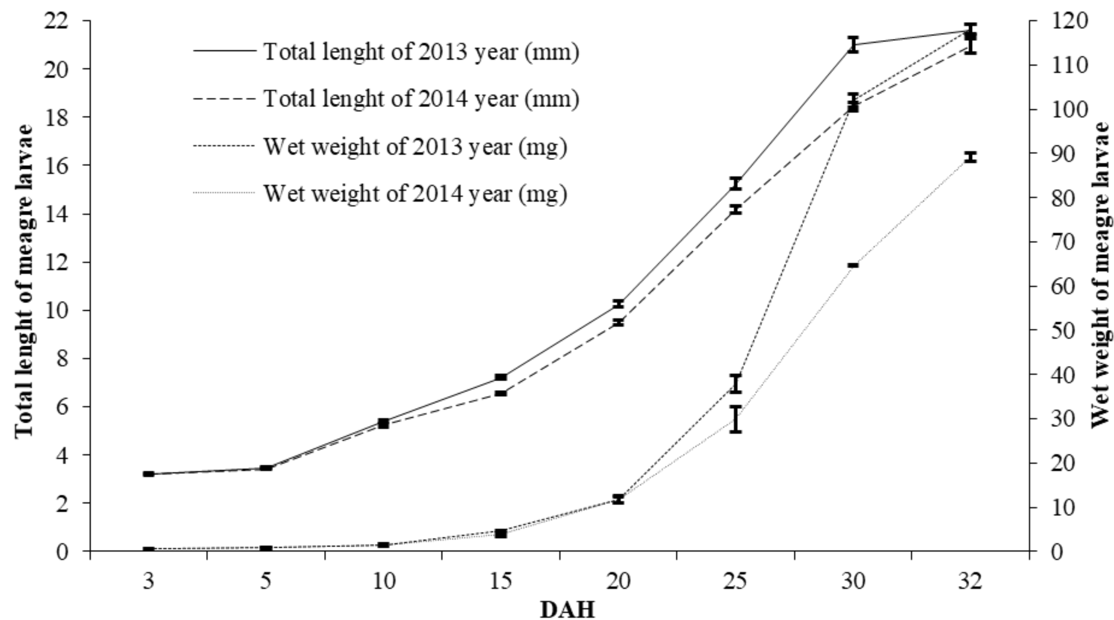

Figure 1 Total lenght and wet weight changes of meagre larvae (mm and mg mean±SE, n=30)

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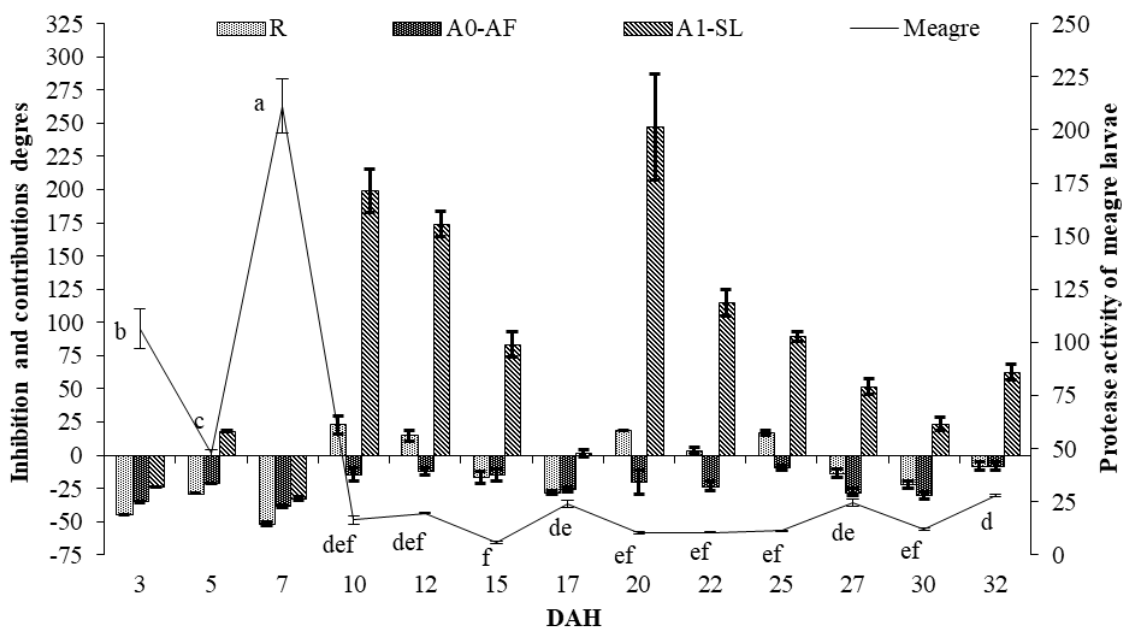

Figure 2 Protease activities of meagre larvae (U/mg protein mean±SE) ($p < 0.05$) and inhibitios and contributions of live foods on protease activities of meagre larvae's of 2013 year (% mean±SE). Line–larva protease and bar–live food inhibition and contributions

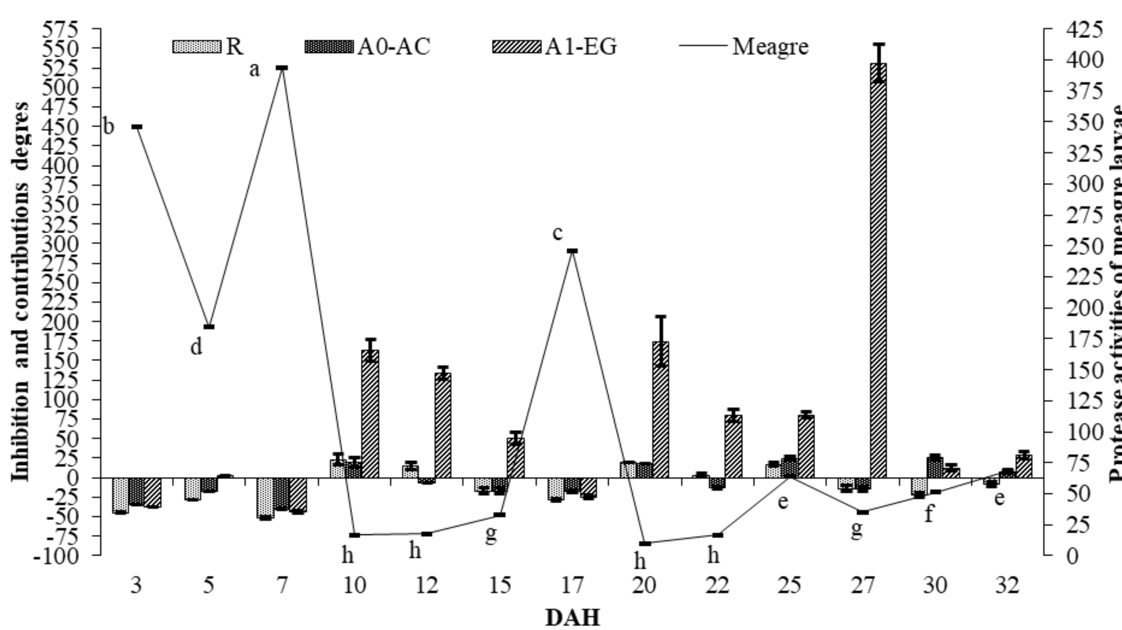

Figure 3 Protease activities of meagre larvae (U/mg protein mean±SE) ($p < 0.05$) and inhibitions and contributions of live foods on protease activities of meagre larvae's of 2014 year (% mean±SE). Line–larva protease and bar–live food inhibition and contributions

The differences observed in protease activities of live foods were statistically significant ($p < 0.05$) (Table 2). Protease activities of live foods tested in the current study such as enriched rotifer, *Artemia* nauplii and *Artemia* metanauplii were 21.76±0.31, 36.00±1.48 and

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29.33±0.93, and 416.44±19.70 and 403.53±11.85 U/mg protein, respectively. The differences observed between rotifer and *Artemia* nauplii were not statistically significant (p> 0.05). Also, the differences in *Artemia* metanauplii tested were not statistically significant (p> 0.05). The highest and lowest protease activities of live foods were found in *Artemia* metanauplii (A1–SL; 416.44±19.70 U/mg protein) and enriched rotifer (R; 21.76±0.31 U/mg protein), respectively.

According to the results of the inhibitory effects of live foods on protease activities of meagre larvae, the high inhibitions of protease activities were obtained when extracts were incubated in the presence of solutions prepared with live foods used in the present study. The highest inhibitions of live foods tested in the present study were observed in enriched rotifer (51.60±0.97%), *Artemia* nauplii (38.46±1.23% and 40.29±1.20%) and *Artemia* metanauplii (32.46±1.35% and 43.57±1.13%) at 7 DAH.

On the other hand, the positive contributions of live foods such as enriched rotifer, *Artemia* nauplii, and *Artemia* metanauplii on protease activities of meagre larvae were determined during the sampling period. The highest contributions of enriched rotifer, *Artemia* nauplii and *Artemia* metanauplii on protease activities of meagre larvae were 23.06±6.77% (10 DAH), 24.52±2.44% (25 DAH) and 247.55±39.92% (20 DAH)–531.53±23.88% (27 DAH), respectively. *Artemia* metanauplii from 3 to 32 DAH has positive contributions from 1.46±2.34% to 531.53±23.88% during the study except for the above mentioned inhibitions. Digestive protease contributions of rotifer and *Artemia* nauplii were only identified in 10 DAH included in the live food period while the digestive protease contributions of *Artemia* metanauplii were observed in all feeding periods.

In contrast, different live food contributions have been identified on different days of larval proteases, which are still low in 2014 (p< 0.05). In the first day (3 DAH) when the mouth opened, inhibitory effects were observed in live feed sources. In addition, attention should be paid to the effects of inhibition of live feed in the first ten days (3–10 DAH). Even in this period, *Artemia* metanauplli inhibition effects were identified.

4. Discussion

The aim of the present study was to determine the potential effects of live foods on protease activities of meagre larvae. Also, the growth parameters (the total length and wet weight values) of meagre larvae from 3 to 32 DAH were determined in the study. The fluctuations in the protease activities of meagre larvae through the current study were observed. Zambonino Infante and Cahu indicated that the decline observed in specific activities of enzymes is not due to a diminution in enzyme synthesis but is the result of an increase in tissue proteins [41].

Rotifer (*B. plicatilis*) protease activity level was lower than Naz and Yúfera [19], higher than Diken et al. [22], and similar to Haközü [20]. Also, Munilla–Moran et al. [7] showed that the rotifer had the lowest enzymatic activity compared to *Artemia* sp. and copepods. On the other hand, *Artemia* nauplii protease activity level was similar to Haközü [20] and Diken et al. [22]. *Artemia* metanauplii's protease activity level was lower than Diken et al. [22], higher than Haközü [20] and Diken et al. [23], and similar to Naz and Yúfera [19]. The statistical differences between the protease activity of enriched rotifera and *Artemia* metanauplii and also between *Artemia* nauplii and *Artemia* metanauplii [18] support the results of the study. Munilla–Moran et al. [7] indicated that the enzymatic activity of *Artemia* changed depending on the nutritional status and developmental stage. The results found on protease activities of live foods showed that protease activity of *Artemia* metanauplii (A1–SL) was higher than those of rotifer and *Artemia* nauplii tested in the study while A1–SL and A1–EG had similar protease activities. In addition, protease activity values of enriched rotifer and

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Artemia nauplii were similar to each other. These differences may be the result of the developmental status as the mentioned by Naz [14] and Munilla–Moran et al. [7]. As indicated by Naz [14], current study results revealed that the highest digestive protease contribution from live food to fish larvae used commonly in marine fish culture provide by *Artemia* metanauplii.

At the same time, Warner and Shridhar [42] determined that a large part of *Artemia* eggs was from the cystine protease group, which will also affect the contribution of the external enzyme which is effective on the breakdown of the proteins. According to the observations of Yufera et al. [43], sea bream larvae that during the first month post-hatching food is digested mainly by the action of alkaline proteases and the gastrointestinal pH is alkaline. Therefore, in fish without a functional stomach at the larval stage, the importance of the contribution of digestive enzymes from *Artemia* is decrease. Cysteine proteases are not active at alkaline pH [42]. This process may be neutralized when such proteases are in contact with the alkaline contents of larval gut. Thus, the supply of these proteases might be meaningless to the fish larvae if the pH in the larval intestine in around 8.

Our results revealed that live foods such as enriched rotifer and *Artemai* nauplii caused a significant inhibitions on protease activities of meagre larvae. However, *Artemia* metanauplii had the lower inhibitions on protease activities of larvae. Warner et al. [44] showed that encysted embryos and larvae of the brine shrimp *Artemia franciscana* contain a cysteine protease which represents over 90% of the protease activity in these organisms. It is known that cysteine proteases active in acid mediums but not in alkali mediums. Therefore, the inhibitions observed in live foods such as enriched rotifer and *Artemai* nauplii may be the result of cysteine protease which represents over 90% of the protease activity found in *Artemia* embryos and larvae. The high positive contributions determined in live foods such as *Artemia* metanauplii may be the result of the decrease in cysteine proteases followed the developmental status.

Cahu and Zambonino Infante [6] indicated that the survival and growth of marine fish larvae fed solely on microdiet are known to be very poor, but supplementation with live foods usually results in a marked improvement. Current study results together with Diken et al. [23], Naz and Yúfera [19] showed that the contribution of *Artemia* metanauplii was high while the contribution of rotifer (*B. plicatilis*) and *Artemia* nauplii were low. Yúfera et al. [43] reported that the contribution of rotifer proteases to larval intestines in feeding the gilthead seabream larvae was not significant, supporting the situation of rotifer feed contributions in protease activities of meagre larvae. Therefore, live food contributions and inhibitions on the digestive proteases of marine fish larvae should be taken into account during the optimization of feeding procedures.

According to current study 10–32 DAH *Artemia* metanauplii contributions to larvae of meagre was higher than 34–81 DAH weaning period's *Artemia* metanauplii's contributions to larvae of gilthead seabream [23]. *Artemia* metanauplii contributions in 34 DAH larvae of gilthead seabream at the beginning of the weaning [23] was higher in 30–32 DAH larvae of meagre at the end of the weaning according to current study. The *Artemia* metanauplii contributions in the larvae of 34 DAH gilthead seabream [23] were similar to the 15 and 25 DAH and higher than the 17 DAH of larvae of meagre in present study. *Artemia* metanauplii contributions in 35–67 DAH weaning period larvae of European seabass were higher than weaning period sea bream larvae [23]. Outside 67 DAH according to larvae of 35, 46, and 57 DAH European seabass [23], *Artemia* metanauplii contrubitions to the 10–32 DAH larvae of meagre on the 10, 12, and 20 DAH of the present study were determined to be high. According to the Haközü [20], the protease contribution of rotifer on the gilthead seabream at 25 DAH was higher than that of the protease contribution of rotifer on the meagre larvae at 10 DAH while the protease contribution of *Artemia* nauplii on the gilthead seabream larvae at 5

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DAH was lower than the protease contribution of the meagre larvae at 25 DAH. On the other hand, the protease contribution of *Artemia* metanauplii on the gilthead seabream larvae at 25 DAH was higher than the protease contribution of *Artemia* metanauplii on meagre larvae at 20 DAH and 27 DAH. Consequently, protease activities of larvae and live foods should be discussed together with protease activities and evaluated with live food contributions and inhibitions. Therefore, in fast growing and in especially feeding periods before weaning in species in which live food contributions and inhibitions are different due to the fluctations observed in the protease activities according to the developmental status. The results reveal the importance of species–specific studies.

Results of the study Kolkovski et al. [8], Cahu and Zambonino Infante [45] and Koven et al. [25] suggested that *Artemia* digestive enzyme contributions would be more effective in microdiet and enhance microdiet performance. According to Munilla–Moran et al. [7] determined that live food contributions, especially protease contributions, support the contribution of live foods to the results that positive effects of live foods are determined on days when protease activities of larvae of meagre are low. According to Kurokawa et al. [10], the addition of rotifer (*B. plicatilis*) proteases to sardine larvae, García–Ortega et al. [11] with *Artemia* feedstocks decapitated in the larvae of the African catfish, Zambonino Infante and Cahu [46] European sea bass, Cahu and Zambonino Infante [47] and Moyano et al. [48] *Artemia* contributions to the larvae of gilthead sea bream support the live food contributions of the study results.

5. Conclusion

The present study provides information about the potential effects of live foods used in the feeding of meagre larvae. When such data becomes available, they will serve the replacement of live foods with microdiets for sustainable aquaculture. The protease contributions of live foods *Artemia* metanauplii tested in the study were higher than those of rotifer (*B. plicatilis*) and *Artemia* nauplii. The inhibitory effects and positive contributions of live foods used in the routine feeding protocols on survival and growth rates of meagre larvae should be taken into account in future studies. Also, cysteine protease activities of *Artemia sp.* should be investigated to provide the higher growth and survival ratio from the feeding protocols used in marine fish larvae.

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