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Role of dietary inclusion of *Gracillaria arcuata* extract on growth performance and biochemical responses in grey mullet, *Mugil cephalus* (Linnaeus, 1758)

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Abstract

The present paper aims to examine the effects of dietary incorporation of *Gracillaria arcuata* extract (GE) on growth conduct and some blood biochemical parameters (Cholesterol (CHO), triglyceride (TG), total protein (TP) and glucose (GLU)) of the grey mullet, *Mugil cephalus*. Four experimental regimens were considered by including GE at the concentration of 0, 5, 10 and 15 mg kg^{-1}diet (GE0, GE5, GE10 and GE15 respectively). Each regimen was allocated randomly to three identical groups of fish (n=10 in each tank) with an introductory average weight of roughly 14.95g. Upon a 60 day feeding trial, fish fed with GE10 and GE15 diet had a considerable (p<0.05) final weight increase (FW), food intake (VFI), weight gain (WG), protein efficiency ratio (PER), lipid efficiency ratio (LER) and specific growth rate (SGR) compared with control group. While, there were no significant difference in FW, WG and LER among fish fed with GE5 diet and fish fed GE0 diet (p>0.05). No considerable difference, in terms of survival rate, was not shown between all of experimental treatments (p>0.05). The highest level of serum total protein was seen in fish on the GE15 diet (p<0.05). Fish fed GE10 and GE15 diets had lower Glucose, triglyceride and cholesterol concentrations than did fish fed GE0 and GE5 diet (p<0.05). The outcomes implied that dietary inclusion of *G. arcuata* extract caused positive influences on growth performance, feed utilization and biochemical responses in *M. cephalus*.

Keywords: *Gracillaria arcuata*, Grey mullet, Biochemical responses, Growth performance

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Introduction

Being a commercially influential species, grey mullet, *Mugil cephalus* has been bred for centuries in comprehensive and semi- intensive ponds in many Mediterranean countries. *M. cephalus* has an auspicious market in Europe, East and South Asia. Also, mullets are a vital aquaculture species in Iran. Consumer demand has expedited the aquaculture progression of these species in Asian countries (Yelghi *et al*. 2012; Akbary, 2019). Even though natural food may contribute required micronutrients to the bred mullet, the utilization of additional supplements caused higher yields and resulted in rich quality fish product (Wassef *et al*., 2001).

Seaweeds as a rich source of essential nutrient are considered for human and animal nutrition (Xuan *et al*., 2013). Even though nutritional aspects of macroalgae are not as prevalent compared to those of land plant raw sources, they contain low content in lipids in their chemical compostion (0.3–7.2 g100 g dry weight^{-1}). They contain moderate protein (10-30 g 100 g dry weight^{-1}), but have plenty of non-starch polysaccharides (consisting mostly of pectins, alginic acid, agar and carrageenan), minerals and vitamins (Wassef *et al*., 2001).. Contradictory growth results have been observed using of dietary seaweed supplementation in aqua feeds for fish. Mustafa *et al*. (1995) observed enhanced performance in red seabream, *Pagrus major* fed diets up to 5% level of *Ulva* sp, *Ascophyllum* sp and *Porphyra* sp supplementation. Soler-Vila *et al*. (2009) showed increased growth performance in rainbow trout, *Oncorhynchus mykiss* fed diets up to 10% level of *Porphyra* sp supplementation. In contrast, Valente *et al*. (2006) demonstrated that the use of the macro-algae *G. bursa-pastoris* at different inclusion levels (5-10 %) as an ingredient for European sea bass, *Dicentrarchus labrax* diets does not affect growth levels and feed utilization effectiveness. Also, Al-Asgah *et al*. (2016) reported that the utilization of the macroalgae *G. aucuata* at high incorporation levels (20-30%) for African catfish (*Clarias gariepinus*) regimens negatively affects growth performance and feed utilization efficiency. Xuan *et al*. (2013) demonstrated that the final body weights of black sea bream, *Acanthopagrus schlegelii* fed on red algae- *G.lemuneiformis* 5 and 10% diets indicated non-significant fluctuations while 20% determined a considerable depression in final weight from the control. Choi *et al*. (2014, 2015) showed that red algae extract of *Hizika fusiformis* and *Pyropia yezoensis* could have been positively influenced on growth and immunity in juvenile olive flounder. Preceding researches indicated that macroalgae may be utilized as a supplement in striped mullet, *M. cephalus* diets (Wassef *et al.*, 2001). Moreover, these studies only focused on the examination of growth performance, feed utilization or fish body composition, and did not pay attention to fluctuations in blood biochemistry incited by the utilization of macroalgae Until now, even with the aquaculture influence of the grey mullet, *Mugil cephalus*, no reports are

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available on the effects of macroalgae on the species' physiology.

Thus, the purpose of the current research was to investigate the effects of dietary incorporation of *Gracillaria arcuata* extract (GE) on growth performance and some blood biochemical parameters (Cholesterol (CHO), triglyceride (TG), total protein (TP) and glucose (GLU)) of the grey mullet, *Mugil cephalus*.

Material and methods

Fish

A total of 120 healthy fish, *M. cephalus* (mean weight 14.94 2.01 g mean length 21.10±1.06 cm) were naturally compiled from the Chabahar Coast in the southeast of Iran. The fish were then transferred to Offshore Fisheries Research Center in south of Iran and kept in a general tank prefilled with filtered seawater for 10 days. Fish were randomly divided (with equal densities) into twelve 60 L fiberglass tanks prefilled with 400 L of filtered well-aerated seawater (150 L h^{-1} of flow rate) with 10 fish per tank. The water was kept at 28-30 °C, dissolved oxygen 7.5±0.7 mg L^{-1}, pH 7.8±0.1, and salinity 33.3±0.30 g L^{-1}. Fish were administrated diets supplemented with 0, 5, 10 and 15 mg kg^{-1} GE as experimental groups in triplicates. All of them were hand-fed (3-5% of body weight) four times daily to apparent satiation for 60 days.

Experimental diet and herbal extract

The *G. arcuata* algae were gathered around the from Chabahar coast, Iran. Two kg of *G. arcuata* were dried at 60℃ in oven, powdered by mortar, pestle and strained. The powder (50 g) were added to 10 L (10%w/v) of methanol 99% in room temperature (24±1.2 ℃) within 48 h. The derived extract was concentrated to 300 ml via a rotary evaporator (IKA, Germany). The GE, which was diluted in 300 ml of distilled water, was then added to the staple diet to achieve the suitable concentrations of analysis and control at incorporation level of 0, 5, 10 and 15 mg kg^{-1} diet. Evaluated proximate compositions are shown in Table 1. The wet dough was pelletized at 1mm particle size via a chopper machine (National, Japan). The empirical regimens were dried, and strained into the required particle size (1.2 mm) and then kept at 4-8 °C until use (Choi *et al*., 2015).

Table 1: Ingredients (g kg^{-1}) and chemical composition (%) of the experimental diets.

	Diets			
Ingredients (g kg^{-1})	**GE0**	**GE5**	**GE10**	**GE15**
Gracillaria arcuata (g kg^{-1})	0	5	10	15
Fish meal	427	427	427	427
Soybean meal	192.5	192.5	192.5	192.5
Wheat flour	93	93	93	93
Dried yeast	37.5	37.5	37.5	37.5
Fish oil	55	55	55	55
Soy oil	27.5	27.5	27.5	27.5
Choline chloride	2	2	2	2
Bi calcium phosphate	3.7	3.7	3.7	3.7
Lecithin	28.15	28.15	28.15	28.15

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Table 1 continued:

Ingredients (g kg^{-1})	Diets			
	GE0	**GE5**	**GE10**	**GE15**
Premix[a]	9.4	9.4	9.4	9.4
Proximate composition(%)				
Crude protein	51.6	51.3	50.2	51.1
Crude lipid	11.9	11.2	11.8	11.4
Crude ash	12.1	12.2	12	12.3
Dry matter	92.2	92.3	91.8	92

[a]Premix (mg kg^{-1}) KI, 250; $MnSO_4 \cdot H_2O$, 2800; $ZnSO_4 \cdot H_2O$, 2350; vitamin K, 225; biotin 3500, (2%), niacin, 4850; calcium pantothenate, 11,000; folic acid, 2000; vitamin B1, 1500; vitamin B_2, 2000; vitamin B_6, 2000; and vitamin C, 50,000.

Growth performance

10 fish in each group and duplications) and the data were utilized to determine growth conduct according to Harikrishnan *et al*. (2012) and Akbary *et al.* (2011) as follows:

WG (%) = Wf−Wi/Wi ×100

:Where, WG was weight gain and mean final body weight (Wf) of every treatment was determined by dividing total fish weight in each tank by number of fish.

$$\text{VFI (g)} = \frac{100 \times \text{crude feed intake}/\ (\text{Wf} + \text{Wi}/2)}{t}$$

Where, VFI was voluntary feed intake

$$\text{DGR (g)} = \frac{[(\text{WG} \times 100)/(\text{Wi} + \text{Wf})/2)]}{t}$$

Where, DGR, Wi and Wf were daily growth rate, initial weight and final weight, respectively.

$$\text{SGR (specific growth rate)}(\%) = \left[\frac{\text{Ln(Wi)} - \text{Ln(Wf)}}{\Delta t}\right] \times 100$$

$$\text{FCR (feed conversion ratio)} = \frac{\text{Feed given (g.dry weight)}}{\text{body weight gain (g)}}$$

$$\text{PER (protein efficiency ratio)} = \frac{\text{WG (g.body weight gain)}}{\text{protein fed (g)}}$$

$$\text{LER (protein efficiency ratio)} = \frac{\text{WG (g.body weight gain)}}{\text{lipid fed (g)}}$$

$$\text{Surviva (\%)} = \frac{\text{final number of fish}}{\text{initial number of fish}} \times 100$$

Biochemical analysis

At the end of experiment, nine fish from each treatment were anesthetized (with clove oil at 5 mg L^{-1}) and blood samples were extracted after excising caudal peduncle and were poured in to un-heparinized sterile tubes 1–1.5 mL for the serum biochemical experimental objectives (Shaluei *et al.*, 2012; Akbary *et al*., 2016).

Serum glucose concentration was determined based on the approach provided By Trinder (1969). Serum total protein was measured using the approach explained by Wootton (1964). Triglyceride and cholesterol level measurements were taken place based on the approach explained by Sankar (2011). Biochemical estimation of blood glucose, protein, cholesterol and triglyceride were determined by means of standard analyses kits (Pars Azmon, Iran) using automatic analyzer (Furuno, CA-270, Japan).

Statistical analysis

All experiments were repeated twice. Data were assessed via one- way analysis of variance (ANOVA). Each group was assumed to be considerably different if $p<0.05$. When a considerable F value resulted from ANOVA the fluctuations among all groups were tested via the Duncan

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multiple comparisons test. SPSS was utilized for statistical purposes for windows versions 16. Data are determined as means±standard Error.

Results

Growth performance

The achieved results from varying mean growth and nutritional criterion have has been presented in Table 2. The addition of 10 and 15 mg of GE to the regimen resulted in a significant enhancement in final weight (FW), food intake (VFI), specific growth rate (SGR), protein efficiency ratio (PER) and lipid efficiency ratio (LER) in comparison with the control group (p<0.05). Peak levels of criterion mentioned previously were seen in fish fed on the GE15 regimen, while the FW, weight gain (WG) and LER in fish fed with GE5 regimen did not show considerable variances rather than those in control (p>0.05). No considerable difference was observed when it came to the survival rate of grey mullet on the experimental diets (p>0.05).

Table 2: Growth performance of grey mullet fed the experimental diets for 60 days.

Growth parameters	Diet (mg kg^{-1})			
	GE0	**GE5**	**GE10**	**GE15**
Initial weight (g fish^{-1})	14.98±1.12^{ns}	14.81±2.08^{ns}	14.85±2.10^{ns}	14.96±1.09^{ns}
Final weight (g fish^{-1})	37.86±2.90^{b}	41.87±1.08^{b}	47.55±1.89^{a}	48.68±1.03^{a}
Voluntary feed intake	1.64±0.13^{a}	1.27±0.04^{b}	1.18±0.06^{b}	1.26±0.06^{b}
Weight gain (%)	152.39±18.97^{b}	182.79±7.70^{b}	215.46±6.18^{a}	225.40±6.70^{a}
Daily growth rate (%)	6.26±0.82^{ns}	6.22±0.35^{ns}	6.84±0.42^{ns}	7.47±0.42^{ns}
Feed conversion rate	1.04±0^{ns}	1.03±0^{ns}	1.03±0^{ns}	1.04±0^{ns}
Specific growth rate (%)	1.46±0.13^{c}	1.72±0.04^{b}	1.91±0.03^{ab}	1.96±0.03^{a}
Protein efficiency ratio	12.08±1.59^{c}	16.61±1.07^{b}	19.01±1.52^{ab}	20.12±1.59^{a}
Lipid efficiency ratio	3.07±0.38^{b}	3.68±0.15^{b}	4.37±0.12^{a}	4.54±0.13^{a}
Survival (%)	100±0^{ns}	100±0^{ns}	99.79±0.03^{ns}	99.85±0.06^{ns}

Values (mean±SE of three replications), n=10. In each row not sharing a common superscript are significantly different (p<0.05).^{ns} =Not significant.

Biochemical responses

Blood criterion of fish on the experimental regimens are presented in Table 2. Data analysis in Table 2 implied that the dietary chlorella mostly influenced the criterion relevant to protein/ lipid metabolism of grey mullet. The serum total protein content was considerably (p<0.05) increased for fish fed GE10 and GE15 diets over the control group. The maximum total protein content was recorded in the fish on the GE15 regimen. There was considerable (p<0.05) decline in cholesterol, glucose and triglycerides levels in those fish on the GE diet over the control (Table 3). The lowest cholesterol and triglycerides levels of serum were observed in fish fed GE15. The lowest glucose level was observed among fish fed GE10 and GE15 diets.

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Table 3: Serum biochemical parameters of *Mugil cephalus* fed GE diet at different levels for 60 days.

	GE diet (mg kg $^{-1}$ feed)			
Parameter	**0**	**5**	**10**	**15**
Total protein (g dl^{-1})	4.42±0.43^{c}	4.80± 0.17^{bc}	4.96±0.12b	5.06±0.12^{a}
Glucose (mg dl^{-1})	55±1.57^{a}	24.86±1.40^{b}	17.16±1.01^{c}	15.13±1.24^{c}
Triglycerides (mg dl^{-1})	227.33±11.76^{a}	194.67±3.88^{b}	184±2.30^{c}	140.33±2.60^{d}
Cholesterol (mg dl^{-1})	105.20±2.37^{a}	99.33±1.88^{a}	82.66±3.71^{b}	59.66±2.60^{c}

GE diet, *G. aucuata* diet . Values (mean±SE of three replication). In each row not sharing a common superscript are significantly different (p<0.05)

Discussion

The possible utilization of macroalgae in fish feeds is dependent on relevant costs in their production, harvesting and processing before their incorporation in fish regimens. Valente *et al*. (2006) showed that the utilization of the macroalgae *G.bursa-pastoris* at different incorporation levels (5-10 %) as an ingredient for European sea bass, *Dicentrarchus labrax* diets does not affect growth conduct and feed utilization efficiency. Also, Al-Asgah *et al*. (2016) recorded that the utilization of the macroalgae *G. aucuata* at high incorporation levels (20-30%) for African catfish (*Clarias gariepinus*) diets suppresses growth performance and feed utilization efficiency. These complied with data achieved by Xuan *et al*. (2013) who showed that the final body weights of black sea bream, *Acanthopagrus schlegelii* fed on red algae- *G. lemuneiformis* 5 and 10% regimens showed non-significant discrepancies while 20% reported a considerable decrease in final weigh from the control. In contrast, in the our study, the use of the macroalgae *G. aucuata* extract (GE) at high inclusion levels (10-15 mg kg^{-1}) significantly promoted the growth and feed utilization in grey mullet, indicating that the omnivorous grey mullet well utilization plant protein sources. The effective composition of microalgae resulting in growth enhancement has not obviously defined, but the advantage has been connected to their content of vitamins and minerals, lipid mobilization and enhanced absorption and consumption efficiency ratios (Xuan *et al*., 2013). Increased VFI and PER were observed in grey mullet fed increasing GE diets, which may explain the reason for the increased growth in the present study. LER of GE10 and GE15 diet was considerably higher in comparison to GE5 and GE0. This may be due to *G. arcuata* extract supplements which influentially stimulated the lipid metabolism (Choi *et al*., 2015). Similarly Choi *et al*. (2015) discovered identical growth performances and protein usage efficiencies in olive flounder, *Paralichthys olivaceus* when on regimens containing 15 g of red algae, *P. yezoensis* extract kg^{-1}diet. These results revealed that the most suitable algae incorporation levels in fish diets may be dependent on the feeding manners of the fish and algae species.

5-15 mg GE in diets could significantly improve the blood parameters of grey mullet. In this research, considerably lower serum total cholesterol levels were noticed at all GE levels compared to the ones on the control diet. In congruence with our

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results, Casas- Valdez *et al*. (2006) recorded the significant minimization of serum cholesterol level in brown shrimp, *Farfantepenaeus californiensis* fed diets containing 4 % *Sargassum*. Also Akbary and Shahraki *et al*. (2017) observed the considerable reduction of serum cholesterol level in grey mullet fed regimens with 15g *Padina astralis extrac*t supplementation. kg^{-1} diet. Identical inclination of reducing blood cholesterol level also been obtained in olive flounder fed diets containing 15 g *P.yezoensis* extract kg^{-1} (Choi *et al*., 2015), suggesting that algae supplementation could stimulate hormonal control of lipid metabolism (Choi *et al*.,2015). In this study, significantly higher serum total protein concentrations was noticed at all GE levels compared to the ones on the control diet. Similar tendency of increasing the serum total protein also been achieved in olive flounder fed diets containing 5-20 g *P.yezoensis* extract kg^{-1} (Choi *et al*., 2015) suggesting that lipids are conversely utilized as an energy sources prior to protein. In the present study, significantly lower triglyceride and glucose levels were noticed at all GE levels compared to the ones on the control regimen. Similarly, Choi *et al*. (2015) indicated a decreeing tendency as dietary *P.yezoensis* extract level escalates. The same case also found by Akbary and Shahraki *et al*. (2017), They reported glucose and triglyceride levels in grey mullet fed wit 15g *P. astralis* extract kg^{-1} diet were significantly lower than the control. An inverse relationship of glucose concentration was detected when macroalgae level is increased. This fact might be an indirect consequence of the reduction in digestible carbohydrates as the incorporation level of algae was increased.

The outcomes of this research showed that marine macroalgae *G. arcuata* 10-15mg kg^{-1} incorporation levels have huge potential as possible ingredients in regimens for grey mullet with enhanced effects on growth conduct, feed utilization efficiency and blood biochemical criterion. Hence, this research implies that optimum dietary supplementation level of *G. arcuata* extract could be roughly 15 mg kg^{-1} of regimen for the positive influences on growth conduct and feed utilization efficiency with no blood biochemical criterion in grey mullet.

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References

Akbary, P., 2019. Growth yield, carcass traits, biochemical and non-specific immune parameters in grey mullet, *Mugil cephalus* Linnaeus, 1758 under cyclic starvation and L-carnitine supplementation. *Journal of Fisheries Sciences*, 18(**1**), 15-29.

Akbary, P., Hosseini, S.A. and Imanpoor, M.R., 2011. Enrichment of *Artemia nauplii* with essential fatty acids and vitamin C: effect on rainbow trout (*Oncorhynchus mykiss*) larvae performance. *Iranian*

[DOR: 20.1001.1.15622916.2020.19.1.30.2]

Journal of Fisheries Sciences, 10(**4**)**,** 557-569.

Akbary, P., Pirbeigi, A. and Jahanbakhshi, A., 2016. Analysis of primary and secondary stress responses in bighead carp (*Hypophthalmichthys nobilis*) by anesthetization with 2-phenoxyethanol. *International Journal of Environmental Science and Technology*, 13(**4**), 1009-1016.

Akbary, P. and Shahraki, N., 2017. Effect of dietary supplementation of *Padina astraulis* (Hauck) extract on biochemical response and digestive enzyme activities of grey mullet, *Mugil cephalus* (Linnaeus). *Iranian Journal of Fisheries Science*, (Under published).

Al-Asgah, N.A., Younis, E.S.M., Abdel-Warith, A.W.A. and Shamlol, F.S., 2016. Evaluation of red seaweed *Gracilaria arcuata* as dietary ingredient in African catfish, *Clarias gariepinus*. *Saudi Journal of Biological Sciences*, 23, 205-210.

Casas Valdez, M., Hernández Contreras, A., Marin, Álvarez, H., Aguila Ramírez, R.N., Hernández Guerrero, C.J., Sánchez Rodríguez, I. and Carrillo Domínguez, S., 2006. El alga marina *Sargassum* (Sargassaceae): una alternativa tropical en la alimentación de ganado caprino. *Revista de Biología Tropical*, 54(**1**), 83-92.

Choi, Y.H., Kim, K.W., Han, H.S., Nam, T.J. and Lee, B.J., 2014. Dietary *Hizikia fusiformis* glycoprotein-induced IGF-I and IGFBP-3 associated to somatic growth, polyunsaturated fatty acid metabolism, and immunity in juvenile olive flounder *Paralichthys olivaceus*. *Comparative Biochemistry and Physiology, A*, 167, 1–6.

Choi, Y.H., Lee, B.J. and Nam, T.J., 2015. Effect of dietary inclusion of *Pyropia yezoensis* extract on biochemical and immune responses of olive flounder *Paralichthys olivaceus*. *Aquaculture*, 435, 347–353.

Harikrishnan, R., Balasundaram, C. and Heo, M.S., 2012. Effect of *Inonotus obliquus* enriched diet on hematology, immune response, and disease protection in kelp grouper, *Epinephelus bruneus* against Vibrio harveyi, *Aquaculture*, 344, 48-53.

Mustafa, G., Wakamatsu, S., Takeda T.A., Umino, T. and Nakagawa, H., 1995. Effects of algae meal as feed additive on growth, feed efficiency and body composition in Red Sea Bream. *Fisheries Science*, 61(**1**), 25–28.

Sankar, G., Elavarasi, A., Sakkaravarthi, K. and Ramamoorthy, K., 2011. Biochemical changes and growth performance of black tigher shrimp larvae after using Ricinus communis extract as feed additive. *International Journal of PharmTech Research*, 3, 201-208.

Shaluei, F., Hedayati, A., Jahanbakhshi, A. and Baghfalaki, M., 2012. Physiological responses of great sturgeon (*Huso huso*) to different concentrations of 2-phenoxyethanol as an anesthetic.

[DOR: 20.1001.1.15622916.2020.19.1.30.2]

Fish Physiology and Biochemistry, 38, 1627-1634.

Soler-Vila, A., Coughlan, S., Guiry, M. and Kraan, S., 2009. The red alga *Porphyra dioica* as a fish-feed ingredient for rainbow trout (*Oncorhynchus* mykiss): effects on growth, feed efficiency, and carcass composition. *Journal of Applied Phycology*, 21(**5**), 617–624.

Trinder, P., 1969. Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor *Annals of Clinical Biochemistry*, 6, 24-27.

Valente, L., Gouveia, A., Rema, P., Matos, J., Gomes, E. and Pinto, I., 2006. Evaluation of three seaweeds *Gracilaria bursa-pastoris*, Ulva rigida and *Gracilaria cornea* as dietary ingredients in European sea bass *(Dicentrarchus labrax*) juveniles. *Aquaculture*, 252(**1**)**,** 85–91.

Wassef, E.A., ElMasry, M.H., Eissa, M.A. and Mikhail, F.R,. 2001. Evaluation of ®vesupplementaryfeeds for grey mullet *Mugil cephalus* L. fry. *Egyptian Journal of Nutrition and Feeds*, 4, 731-741.

Wootton, L.I., 1964. Micro-analysis in medical biochemistry in micrometer, 4th.ed. J &A Churchill, London. 264 P.

Xuan, X., Wen, X., Li, S., Zhu, D. and Li, Y., 2013. Potential use of macro-algae *Gracilaria lemaneiformis* in diets for the black sea bream, *Acanthopagrus schlegelii*, juvenile. *Aquaculture*, 412–413, 167–172.

Yelghi, S., Shirangi, S.A., Ghorbani, R. and Khoshbavar Rostami, H.A., 2012. Annual cycle of ovarian development and sex hormones of grey mullet (*Mugil cephalus*) in captivity. *Iranian Journal of Fisheries Sciences*, 11(**3**), 693-703.