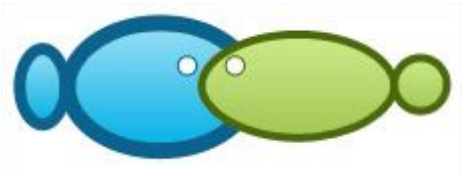

# Zeolite as a major control factor of water quality problems arising from stocking density of European sea bass (*Dicentrarchus labrax*) juveniles

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**Abstract**. Improperly managed ammonia levels alter growth, survival and creates unfavorable conditions on fish health in production systems. This experiment was designed to determine the impact of natural zeolite on removing ammonia from water, growth enhancement and survival rate of European seabass, *Dicentrarchus labrax* juvenile. *D. labrax* with initial body weight of 4.5±0.06 g fish$^{-1}$ were stocked at three different densities, namely 1kg m$^{-3}$ (low density), 2 kg m$^{-3}$ (medium density) and 3 kg m$^{-3}$ (high density) with three levels of zeolite (5, 7.5 and 10 ppt) and a control group (without zeolite) forming twelve treatments for 120 days in winter season. Thus, 24 hapas with an average area 0.5 m$^3$ were fixed in twelve concrete ponds (4x8x1.5 m) to form two replicates for each treatment. The results revealed that water quality parameters improved significantly ($p < 0.05$) with increasing zeolite level controlling the negative impact of densities on pH and dissolved oxygen which improved from 7.05 and 4.8 mg L$^{-1}$ to 8.0 and 6.35 mg L$^{-1}$ respectively. Ammonia removal efficiency significantly ($p < 0.05$) improved with increasing zeolite level with all density levels. The best ammonia removal rate (76.60%) was obtained at Z10 treatment (71.2±0.445) with low density. Fish survival rate was significantly ($p < 0.05$) high among treatments, the best survival rate obtained was at low density with high zeolite level (94%), while the lowest (76%) obtained was at high density with Z0 (control) treatment. Growth performance was significantly ($p < 0.05$) higher at Z10 for all densities when compared with Z0, Z5 and Z7.5 treatments. Despite of the negative effect of stocking density, feed conversion ratio (FCR), protein efficiency ratio (PER) and protein productive value (PPV) showed significant ($p < 0.05$) improvement with increasing zeolite level. However, significant ($p < 0.05$) increase of body protein was observed, while fat and ash content decreased with increasing zeolite that diminished the adverse effect of increased density. The present study highlights the important role of zeolite (Z10) level in improving water quality, ammonia removal rate, growth performance and survival rate in stocking juveniles of European seabass.
**Key Words**: clinoptilolite, growth rates, ammonia removal, intensification, European sea bass juveniles.

**Introduction**. New intensified production methods are required to meet the rising demand of population development. Offered space for aquaculture expansion, along with the freshwater shortage and the constricted wastewater regulations are the leading impairments for persistent traditional systems (Badiola et al 2012; Dalsgaard et al 2013). The European seabass, *Dicentrarchus labrax* as one of the most important Mediterranean cultured euryhaline fish species in Europe (Turkey, Greece, Spain, Italy and Croatia) as well as Egypt, are the largest producers in the Mediterranean region (FAO 2016). Fish density is a limiting major factor that could be greatly responsible for negative impacts on fish performance and welfare, thus the fish produced running costs (Ellis et al 2002). In farmed seabass, a wide range of densities are commonly used according to the type of rearing system and size (Ellis et al 2002). Generally, high stocking densities could amplify energy demand and alter digestive enzyme activities (Liu et al 2016), along with body composition (Toko et al 2007). An earlier study revealed the concurrent impact of high

density and impaired growth (Lupatsch et al 2010). Another studys on fish welfare distinguished between specific effects of biomass increase per se and those caused by the deprivation of water quality (Person-Le-Ruyet et al 2008). The study of Björnsson & Ólafsdóttir (2006) implied that impaired growth, mortality and nutritional condition could be negatively correlated with rearing system, and the ammonia is the limiting factor in recirculating system than the flow through system. Stocking densities along with dissolved oxygen, water temperature, pH, ammonium, nitrate and carbon dioxide contents are the most influencing factors in pond ecology resulting in fish body weight fluctuation during the production cycle (Fivelstad et al 1995, 1998). Fish stocking density impacts greatly the growth, survival, health, water quality and production (Costa et al 2013). In intensive systems fish performance is greatly affected by ammonia levels in water parameter after dissolved oxygen concentration (Francis-Floyd et al 2009). Levels of ammonia as low as 0.2 mg $L^{-1}$ (Randall & Tsui 2002) and average pH of (8.0), temperature (21.8°C), salinity (37.0 ‰) are not considered toxic unless long-term exposure (Lemarié et al 2004). Ammonia toxicity at relatively low levels could have detrimental impact on fish tissues such as gill impairment and physiological factors such as reduced growth, higher oxygen consumption and more susceptible to bacterial infections (Piper & Smith 1984; Francis-Floyd et al 2009) and can restrict yields in intensive fish culture (Colt & Armstrong 1981). However, ammonia removal from pond water with high fish density eliminates the drastic effect on fish health which in turns the fish welfare is enhanced. One of the most effectively used method for ammonia exclusion from polluted water is the ion exchange with natural zeolite (Tchobanoglous et al 2011). Zeolite has strong capabilities to eradicate ammonia and to adsorb and desorb molecules that permit rapid endorsement and loosing of charged particles (Rahmani & Mahvi 2006; Filipponi & Sutherland 2013). Besides removing ammonia, zeolite has high adsorbing capability to toxic gases, regulate pH level, provide micro nutrients, adsorb odors, bacteria, suspended solids, waste and organic matter in fish ponds (Aly et al 2016; Abdel Rahim 2017). Consequently, it is necessary to evaluate the impact of water quality on health status and production of European sea bass in flow through system. Therefore, the present study examined the effect of water quality on growth performance of *Dicentrarchus labrax* stocked in three different densities reared in concrete tanks treated with three levels of zeolite.

## Material and Method

***Experimental design***. Approximately 6,800 European seabass juveniles were obtained from the Marine Fish Rearing Unit, general authority for fish resources and development (Alexandria, Egypt) and kept as a fish stock in 3 large scaled open system (5 $m^3$ each) at the marine aquaculture facility (El-Max Research Station, National Institute of Oceanography and Fisheries (NIOF), Alexandria, Egypt). The experimental facility was composed of 12 rectangular (3 x 8 x 1 m) concrete tanks (24 $m^3$ each). The tanks used were part of open system of the laboratory of larval rearing unit. Twelve treatments were performed to evaluate the effectiveness of adding zeolite with different levels on growth performance of seabass juveniles stocked with different densities and reared in saline underground water with fluctuated content of ammonia. Juveniles were randomly distributed among 24 hapas with an area of 0.5 $m^3$ each (two hapas per tank), fixed in the concrete tanks at three stabilized rearing densities: 1 kg $m^3$ ($D_1$), 2 kg $m^{-3}$ ($D_2$) and 3 kg $m^{-3}$ ($D_3$). Each fish density was exposed to 3 levels of zeolite (5, 7.5 and 10 g $L^{-1}$) and expressed as follows: low zeolite (LZ), medium zeolite (MZ), and high zeolite (HZ). In addition, a control treatment with the same mentioned densities exposed to the saline underground water with no zeolite. All tanks were covered with galvanized metal sheets ceiling to protect the fish from external visual stress. A particle trap channel (50 L x 0.5 H x 0.5 W) was fitted outside all tanks and crossed by part of the outlet flow to remove solid waste (faeces and uneaten feed). Light intensity was according to natural day and night photoperiod at 12 h light – 12 h dark, including 30 min of dawn and dusk. For every tank in all treatments, at day 0, 24, 48, 72, and 96, the fish, fasted for 24 h, and were anaesthetized (using a solution containing 20 mg $L^{-1}$, clove oil, Eugenol). Fish density was reduced every 24 days and randomly removed in order to avoid oxygen

concentration depletion due to increased biomass, collected individuals were weighed to the nearest 0.01 gram to establish the initial weight of the stocking density.

***Experimental fish***. For the experimental trials 5,800 European seabass (1450 per treatment) with an individual initial body weight of approximately 4.5±0.06 g were randomly distributed among the 24 separate experimental hapas fixed in 12 tanks and adapted to the experimental conditions for 15 days. The average stocking density (three densities per treatment) was 1±0.08 (125 individual hapa$^{-1}$ x 2), 2±0.15 (250 individual hapa$^{-1}$ x 2), and 3±0.09 (350 individual hapa$^{-1}$ x 2) kg per m$^3$. During the adaptation phase the dead and morbid fish were replaced. The experiment duration was 120 days (from November 2016 to February 2017), during which, the dead fish were replaced by fish with an equal weight to keep the stocking density adjusted. The cumulative mortality rate (in % of the initial fish number) was calculated for each density group. The newly stocked fish were marked after sedation by clipping the operculum. All marked fish were excluded from final individual-based analysis (individual weight, individual average daily gain (ADG), and individual specific growth rate (SGR) and the determination of the survival rate in order to reassure that only fish exposed to the corresponding examined conditions over the whole experimental trial were considered for final analysis of the mentioned parameters. At the end of the experimental period, ten fish were taken randomly from each hapa, and were used for carcass proximate analyses. Fish of each hapa used for proximate carcass analyses were minced together and lyophilized. The percent dry weight was determined by oven-drying portions of the minced fish at 105°C for 24 h. Crude protein (Nx6.25) was determined by the Micro-Kjeldahl method, lipids by Soxhlet extraction and ash contents by burning in a muffle furnace at 550°C for 12 h. Food conversion ratios (FCR) were calculated as dry food (-12% moisture) per body weight increase.

***Growth parameters and feed utilization***. At the end of the experiment, final body weight (FW), weight gain (WG), average daily gain (ADG), specific growth rate (SGR), survival rate, feed conversion ratio (FCR), and daily feed intake (DFI), were documented and calculated according to the following equations:

Weight gain (WG, mg/fish): WG = Wt - W0. Where: Wt: final weight - W0: initial body weight;
Average daily gain (ADG, mg/fish/day): ADG = Wt - W0/n. Where: n duration period;
Specific growth rate (SGR, %/day): SGR = 100 × (ln Wt - ln W0)/ days. Where: ln: natural logarithm
Survival (S, %): S% = (no. of fish at the end/no. of fish at the start) × 100;
Feed intake (FI, g): This is the amount of feed given or supplied during the experimental period;
Feed conversion ratio (FCR, g): FCR = dry matter intake (g)/body weight gain (g);
Protein efficiency ratio (PER): PER = weight gain (g)/ protein intake (g);
Protein productive value (PPV, %): PPV % = 100 × (final body protein content – initial body protein content)/protein consumed (g).

***Experimental diets***. Fish were given a dry pelleted commercial feed prepared and formulated at El-Max Research Station feed mill of the following composition: moisture 12%, crude protein 42%, crude fat 21%, crude fiber 2%, ash 8% and NFE 16%. The daily amount of food was introduced by hand to *adlibitum* three times daily, six days per week.

***Installation of natural zeolite as ammonia removal product.*** The natural clinoptilolite (zeolite) was purchased and used as an adsorbent for ammonia. Table 1 shows the chemical composition of Clinoptilolite imported from Yemen (http:// alixzeolite.com/en/) with a unit price of 0.9 US $/kg. Natural zeolite were placed inside three separated concrete ponds in three concentration levels (250 g L$^{-1}$, 375 g L$^{-1}$ and 500 g L$^{-1}$) to maintain concentration of zeolite 5, 7.5 and 10 ‰ . The underground water supply three zeolite tanks with the former concentrations then the fish tanks takes its water from the zeolite tanks. Zeolite was removed and washed with fresh tap water every week.

Table 1

Chemical composition of Yemen natural zeolite

| *Element* | *%* | *Element* | *%* | *Element* | *%* |
|---|---|---|---|---|---|
| $SiO_2$ | 62.22 | $Fe_2O_3$ | 4.033 | BaO | 0.085 |
| $Al_2O_3$ | 11.096 | $K_2O$ | 3.266 | $P_2O_5$ | 0.033 |
| $Na_2O$ | 0.78 | $TiO_2$ | 0.339 | ZnO | 0.025 |
| MgO | 0.599 | ZrO | 2 0.112 | SrO | 0.047 |
| CaO | 3.583 | Cl | 0.025 | MnO | 0 |

***Water quality parameters***. . El- Max research station, where the experiment has taken place, with ground well as a source of saline water (about 100 m depth). Unfortunately, analysis of water samples demonstrated that the concentrations of ammonia and heavy metals were slightly higher than the recommended concentrations for marine fish hatcheries (Moretti et al 1999). The measured values of ammonia were (1.062 mg $L^{-1}$), $NO_3$ (0.036 mg $L^{-1}$), and $NO_2$ (0.0011 mg $L^{-1}$). Water temperature, dissolved oxygen (DO), total ammonia nitrogen (TAN), pH and salinity were monitored daily (Table 2). The temperature and pH were measured using portable pH Meter (pH-8424-HANNA Instrument). DO was measured by HI-9142 (HANNA Instrument). The concentration of total ammonia nitrogen (TAN) was analyzed using YSI 9300 photometer and YSI Professional Plus. The concentration of un-ionized ammonia was calculated from $NH_4$ using pH, temperature and salinity according to the USEPA (1999). The unionized ammonia ($NH_3$), and nitrite ($NO_2$) concentrations were measured every 15 days to ensure no limiting levels (Table 2) might affect the experimental density. Therefore, the percentage of ($NH_3$) to $NH_4$ was given by the following equation:

$$\%NH_3 = 100/[1+10(\log K^{-1} - pH)]$$

$$\text{with } \log K1 = -0.467 + 0.00113 \times S + 2.887.9 \times T^{-1}$$

where $K^{-1}$ is the dissociation constant, S (in g $L^{-1}$) the salinity and T the temperature (°K)

Also, salinity was measured using YSI EcoSense (EC300 Conductivity/Salinity Meter). Water temperature range (11-18°C), and salinity level was 28.3±0.96 ‰ in all experimental tanks. Additional oxygenation was provided by compressed air through air stones. Water exchange was performed by pumping or using gravity. The water exchange rate was fixed to 30% of the tank volume per day to ensure tank cleaning and waste removal of particles. Collected data were analyzed at the end of experiment.

***Statistical analysis***. The results obtained were analyzed by analysis of variance, using two-way ANOVA (Tuckey test at 0.05 probability level) according to Assaad et al (2015). ANOVA was used to evaluate the effect of ammonia removal products on growth parameters, feed utilization parameters, body carcasses composition, the water quality parameters, and survival rate (%) of European seabass juveniles. The final results in tables are expressed as means ± SEM.

## Results

***Water quality and ammonia removal efficiency***. Concentrations of water quality parameters were almost constant in all the experimental tanks and in the range of recommended levels for farmed sea bass. Final results of water quality were displayed in Table 2. Changes in temperature, DO, pH, nitrite, total ammonia and un-ionized ammonia presented in (Table 2; Figure 1). It was indicated that changes in DO, pH, $NO_2$ , $NH_4$ and un-ionized ammonia due to: (a) as stocking density increases, DO decreases and ammonia increases; (b) increasing zeolite concentration led to higher DO and pH and lower ammonia measurements; (c) there was a significant interaction between stocking density (SD) and zeolite concentration level for pH and ammonia measurements ($NH_4$ and $NH_3$). The results pointed out that there were no significant ($p > 0.05$) differences in temperature (11 to 15°C) and salinity (28±1.23) parameters throughout the experimental period. However, the results of pH and DO showed statistically significant deprivation differences among the tested groups. Data obtained showed that pH increase significantly ($p < 0.05$) from 7.35 with control treatment to 7.72 and 7.78 with increasing

zeolite level to 7.5 and 10 ‰, however it decreased significantly ($p < 0.05$) from 7.7 ‰ in D3 to 7.48 ‰ in $D_1$. Also, DO enhanced significantly ($p < 0.05$) with adding zeolite and decrease of density. Generally, the concentration of DO was within the safe limits (6.5 ppm to 7.4 ppm).

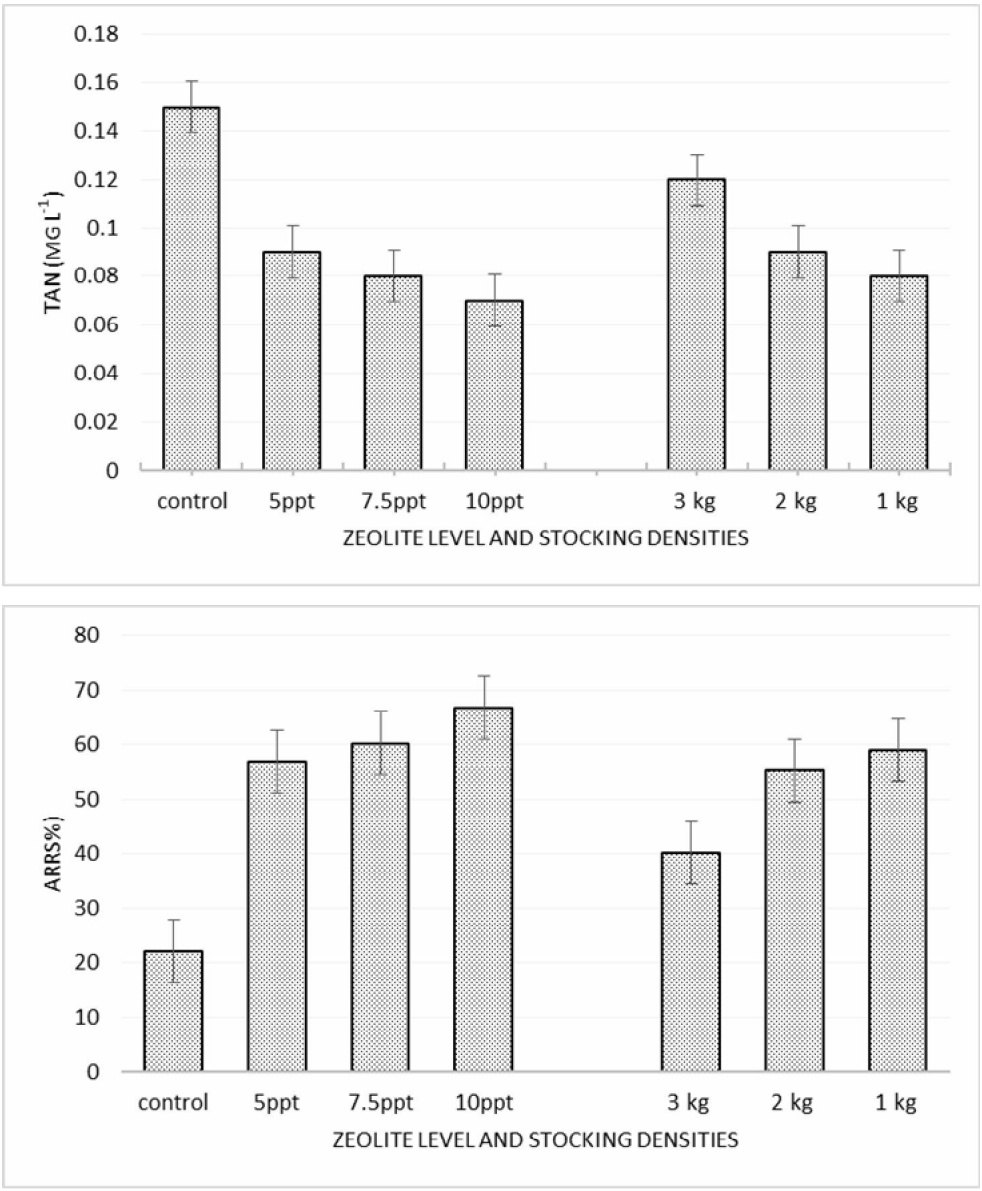


Figure 1. Total ammonia nitrogen in water (upper); Ammonia Removal Rate; as % of the Source (ARRS) = (TAN source – TAN treatment) * 100/TAN source; Ammonia reduction as a percentage from source (lower), with different stocking densties (kg $m^{-3}$) throughout the study period.

The overall results of the total ammonia nitrogen ($NH_4$-N), unionized ammonia $NH_3$ and nitrite ($NO_2$) throughout the study period (Table 2) were significantly high ($p < 0.05$) between the examined treatments and the control. An inverse relation was observed between all ammonia measurements, stocking densities and natural zeolite levels. However, highly significant ($p < 0.05$) differences in the average final content of TAN and $NH_3$ were observed between the tested treatments and the control. The results can be arranged from the best lowest to the worst highest as follows: Z10 with stocking density $D_3$, $D_2$, and $D_1$, then Z7.5 with stocking density $D_3$, $D_2$, and $D_1$, followed by Z5 with stocking density $D_3$, $D_2$, and $D_1$ and finally the control with stocking density $D_3$, $D_2$, and $D_1$. Values of ammonia removal rate as percentage of the source water revealed that the tested zeolite achieved better results than the control and the best result was achieved at Z10 with ammonia removal rate 71.2% with low stocking density. The bi-weekly results

of total ammonia nitrogen, (TAN) during the experimental period have been shown in Table 3. During the experimental weeks, there were highly significant ($p<0.05$) differences between the tested treatments and the control. The lowest TAN was observed for high zeolite with $D_3$, $D_2$ and $D_1$, followed by medium zeolite then low zeolite and the highest TAN was recorded for the control treatment, respectively. The value of ammonia removal rate as percentage of the source water increases with increase of zeolite level and stocking density.

***Growth performance***. Final body weight (FBW), weight gain (WG), average daily gain (ADG), and specific growth rate (SGR, %) were shown in Table 4 and Figure 2. Data of growth performance showed significant ($p < 0.05$) differences between treatments where the peak growth was achieved at high zeolite treatment with the FBW of 20.2, 20.4 and 20.8 g $fish^{-1}$ for the high ($D_1$), medium ($D_2$), and low ($D_3$) stocking densities, respectively. While the lowest growth rate was observed for the control treatment (11.6, 12.2, and 15 g $fish^{-1}$) for $D_1$, $D_2$, and $D_3$ respectively, with no significant differences between stocking densities and the other zeolite treatments (medium zeolite and low zeolite). As for WG, ADG and SGR% followed the same pattern as FBW. The data on the survival rate showed there was highly significant difference ($p < 0.05$) between treatments. Enhanced survival rates were significantly ($p < 0.05$) different with the increasing zeolite level and decreasing stocking density (Table 4; Figure 2). Also, the cumulative mortality rate was increase significantly with $D_1$ (1.17%) verses 1.08 and 1.06% for $D_2$ and $D_1$ respectively, however it decrease significantly from 1.16% with control treatment to 1.07% with highest level of zeolite. The feed conversion ratio (FCR), protein productive value (PPV %) and protein efficiency ratio (PER) revealed that FCR, PPV and PER were significantly ($p < 0.05$) affected by stocking density and zeolite levels and revealed the best results at the lowest stocking density and with high zeolite level (Table 4). The best FCR, PER and PPV was recorded with high zeolite treatment and $D_3$, and the worst values were found to be with the control treatment and $D_1$ stocking density. The results clearly showed that adding zeolite has a great influence on the growth performance and survival rate of seabass juveniles.

Table 2

Water quality parameters for European seabass rearing tanks tested with different densities and zeolite levels

| Variable | Control | | | Z 5‰ | | | Z 7.5‰ | | | Z 10‰ | | | P-value | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | HD | MD | LD | HD | MD | LD | HD | MD | LD | HD | MD | LD | Col1 | Groups | C×G[1] |
| TAN* (ppm) | 0.209± 0.001[a] | 0.133± 0.0006[b] | 0.122± 0.0004[c] | 0.0977± 0.006[d] | 0.0811± 0.0009[f] | 0.077± 0.0006[f] | 0.0894± 0.002[e] | 0.077± 0.0002[f] | 0.0691± 0.0003[g] | 0.0769± 0.0009[f] | 0.063 ± 0.002[g] | 0.057 ± 0.001[h] | <0.001 | <0.001 | <0.001 |
| $NH_3$ (mg $L^{-1}$) | 0.0051± 0.00[a] | 0.0032± 0.00[b] | 0.003± 0.00[c] | 0.0024± 0.0002[d] | 0.002± 0.00[f] | 0.0019 ±0.00[f] | 0.0022 ±0.00[e] | 0.0019 ±0.00[f] | 0.0017 ±0.00[g] | 0.0019 ±0.00[f] | 0.0016 ±0.00[g] | 0.0014 ±0.00[h] | <0.001 | <0.001 | <0.001 |
| ARRS** | -5.46± 0.473[h] | 33± 0.29[g] | 38.6± 0.189[f] | 50.6± 3.09[e] | 59± 0.442[c] | 61.1± 0.316[c] | 54.8± 0.786[d] | 61.1± 0.114[c] | 65.1± 0.167[b] | 61.1± 0.473[c] | 68± 1.18[b] | 71.2± 0.45[a] | <0.001 | <0.001 | <0.001 |
| $NO_2$ (mg $L^{-1}$) | 0.125± 0.005[a] | 0.115± 0.005[a] | 0.1±0[b] | 0.069± 0.002[c] | 0.064± 0.003[c] | 0.049± 0.003[d] | 0.042± 0.01[d] | 0.021±0[e] | 0.017± 0.0035[e] | 0.037± 0.005[d] | 0.01± 0[ef] | 0.001± 0[f] | <0.001 | <0.001 | 0.213 |
| pH | 7.05± 0.05[f] | 7.35± 0.05[e] | 7.65± 0.05[cd] | 7.55± 0.05[d] | 7.55± 0.05[d] | 7.65± 0.05[cd] | 7.65± 0.05[cd] | 7.7±0[cd] | 7.8±0[bc] | 7.65± 0.05[cd] | 7.9± 0.1[ab] | 8±0.1[a] | <0.001 | <0.001 | 0.017 |
| DO (mg $L^{-1}$) | 4.8± 0.1[f] | 5.2± 0.1[e] | 5.6± 0.1[cd] | 5.11± 0.09[e] | 5.55± 0.05[cd] | 5.75± 0.05[bc] | 5.35± 0.05[de] | 5.8± 0.1[bc] | 5.98± 0.06[b] | 5.75± 0.15[bc] | 6.25± 0.05[a] | 6.35± 0.05[a] | <0.001 | <0.001 | 0.725 |

Values are means±SEM, n = 2 per treatment group; [a-h] Means in a row without a common superscript letter differ ($p < 0.05$) as analyzed by two-way ANOVA and the DUNCAN test; [1]C × G = Col1 × groups interaction effect; * Total ammonia nitrogen (TAN) content of the source water was 0.2±0.01 ppm; **Ammonia Removal Rate; as % of the Source (ARRS) = (TAN source – TAN treatment) * 100/TAN source.

Table 3

The biweekly results of total ammonia nitrogen (TAN) in the experimental tanks of European seabass juvenile tested with different zeolite levels with different densities

| Variable | Control | | | Z 5‰ | | | Z 7.5‰ | | | Z 10‰ | | | P-value | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | D1 | D2 | D3 | D1 | D2 | D3 | D1 | D2 | D3 | D1 | D2 | D3 | Col1 | Groups | C×G1 |
| Sample 1 | 0.14± 0.02[a] | 0.09± 0.00[b] | 0.09± 0.0[b] | 0.11± 0.02[ab] | 0.09± 0.00[b] | 0.08± 0. 0[b] | 0.08± 0.00[b] | 0.08± 0.00[b] | 0.07± 0.00[b] | 0.08± 0.00[b] | 0.08± 0.00[b] | 0.07± 0.0[b] | <0.001 | 0.002 | 0.053 |
| Sample 2 | 0.19± 0.01[a] | 0.13± 0.01[b] | 0.13± 0.0[b] | 0.11± 0.02[bc] | 0.09± 0.00[bd] | 0.08± 0.00[bd] | 0.09± 0.00[bd] | 0.07± 0.00[cd] | 0.07± 0.00[cd] | 0.08± 0.00[bd] | 0.07± 0.00[cd] | 0.06± 0.0[d] | <0.001 | 0.001 | 0.153 |
| Sample 3 | 0.27± 0.01[a] | 0.15± 0.00[b] | 0.15± 0.1[b] | 0.10± 0.00[c] | 0.09± 0.00[cd] | 0.08± 0.00[de] | 0.09± 0.00[cd] | 0.08± 0.00[d] | 0.08± 0.00[d] | 0.08± 0.00[cd] | 0.07± 0.00[de] | 0.06± 0.00[e] | <0.001 | <0.001 | <0.001 |
| Sample 4 | 0.26± 0.01[a] | 0.17± 0.01[b] | 0.15± 0.0[b] | 0.09± 0.00[c] | 0.09± 0.00[cd] | 0.08± 0.00[cd] | 0.09± 0.00[c] | 0.08± 0.00[cd] | 0.07± 0.00[cd] | 0.08± 0.00[cd] | 0.07± 0.01[cd] | 0.05± 0.00[d] | <0.001 | <0.001 | <0.001 |
| Sample 5 | 0.21± 0.01[a] | 0.12± 0.02[bc] | 0.14± 0.0[b] | 0.11± 0.01[bc] | 0.08± 0.00[cd] | 0.08± 0.00[cd] | 0.09± 0.00[cd] | 0.08± 0.00[cd] | 0.06± 0.00[d] | 0.08± 0.00[cd] | 0.06± 0.00[d] | 0.06± 0.0[d] | <0.001 | <0.001 | 0.001 |
| Sample 6 | 0.19± 0.01[a] | 0.13± 0.01[b] | 0.13± 0.03[bc] | 0.09± 0.0[bd] | 0.08± 0.00[d] | 0.08± 0.00[d] | 0.09± 0.0[bd] | 0.08± 0.0[cd] | 0.07± 0.00[d] | 0.07± 0.00[d] | 0.07± 0.00[d] | 0.05± 0.0[d] | <0.001 | 0.001 | 0.056 |
| Sample 7 | 0.20± 0.01[a] | 0.13± 0.01[b] | 0.10± 0.0[c] | 0.09± 0.00[cd] | 0.08± 0.0[cde] | 0.08± 0.0[cde] | 0.09± 0.00[cd] | 0.07± 0.00[df] | 0.07± 0.00[df] | 0.08± 0.0[cde] | 0.06± 0.00[ef] | 0.05± 0.0[f] | <0.001 | <0.001 | <0.001 |
| Sample 8 | 0.21± 0.01[a] | 0.14± 0.011[b] | 0.10± 0.00[bd] | 0.09± 0.00[cd] | 0.08± 0.0[cde] | 0.08± 0.00[cde] | 0.10± 0.01[bc] | 0.07± 0.0[cde] | 0.06± 0.0[cde] | 0.07± 0.0[cde] | 0.03± 0.03[e] | 0.05± 0.0[de] | <0.001 | <0.001 | 0.002 |

Values are means±SEM, n = 2 per treatment group; [a-f] Means in a row without a common superscript letter differ ($p < 0.05$) as analyzed by two-way ANOVA and the TUKEY test; [1]C × G = Col1 × groups interaction effect.

Table 4

Growth performance and nutrient utilization of European sea bass at the end of the experiment

| Variable | Control | | | Low zeolite | | | Medium zeolite | | | high zeolite | | | P-value | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | D1 | D2 | D3 | D1 | D2 | D3 | D1 | D2 | D3 | D1 | D2 | D3 | Col1 | Groups | C×G[1] |
| IBW | 4.42± 0.02 | 4.48± 0.02 | 4.46± 0.06 | 4.43± 0.02 | 4.5± 0.02 | 4.5± 0.06 | 4.45± 0.02 | 4.47± 0.01 | 4.54± 0.02 | 4.47± 0.01 | 4.48± 0.04 | 4.49± 0.07 | 0.715 | 0.117 | 0.875 |
| FBW | 11.6± 0.93$^{e}$ | 12.2± 0.056$^{de}$ | 15± 0.6$^{bcd}$ | 13.7± 0.71$^{ce}$ | 12.7± 0.6$^{de}$ | 16.1± 0.34$^{bc}$ | 15.9± 0.5$^{bc}$ | 16.3± 0.2$^{bc}$ | 17.2± 0.8$^{b}$ | 20.2± 0.05$^{a}$ | 20.4± 0.43$^{a}$ | 20.8± 0.45$^{a}$ | <0.001 | <0.001 | 0.089 |
| S % | 76±1$^{f}$ | 90±1$^{d}$ | 93.5± 0.5$^{abc}$ | 82±1$^{e}$ | 93.5± 0.5$^{abc}$ | 93±0$^{ad}$ | 90.5± 0.5$^{cd}$ | 94.5± 0.5$^{ab}$ | 95±0$^{a}$ | 91.5± 0.5$^{bd}$ | 93±0$^{ad}$ | 94±0$^{ab}$ | <0.001 | <0.001 | <0.001 |
| Gain | 7.17± 0.95$^{e}$ | 7.7± 0.08$^{de}$ | 10.5± 0.6$^{bcd}$ | 9.3± 0.7$^{ce}$ | 8.24± 0.6$^{de}$ | 11.6± 0.4$^{bc}$ | 11.4± 0.5$^{bc}$ | 11.8± 0.2$^{bc}$ | 12.6± 0.8$^{b}$ | 15.7± 0.03$^{a}$ | 16± 0.4$^{a}$ | 16.3± 0.52$^{a}$ | <0.001 | 0.001 | 0.096 |
| ADG | 0.06± 0.008$^{e}$ | 0.064± 0.001$^{de}$ | 0.09± 0.005$^{bcd}$ | 0.08± 0.006$^{ce}$ | 0.07± 0.005$^{de}$ | 0.097± 0.003$^{bc}$ | 0.1± 0.004$^{bc}$ | 0.1± 0.002$^{bc}$ | 0.11± 0.007$^{b}$ | 0.13± 0.0003$^{a}$ | 0.13± 0.003$^{a}$ | 0.14± 0.004$^{a}$ | <0.001 | 0.001 | 0.096 |
| SGR | 0.8± 0.07$^{e}$ | 0.83± 0.01$^{de}$ | 1.01± 0.04$^{cd}$ | 0.94± 0.04$^{ce}$ | 0.9± 0.04$^{de}$ | 1.06± 0.03$^{c}$ | 1.06± 0.02$^{c}$ | 1.08± 0.01$^{bc}$ | 1.11± 0.041$^{ac}$ | 1.26± 0.0006$^{ab}$ | 1.26± 0.01$^{ab}$ | 1.28± 0.03$^{a}$ | <0.001 | 0.002 | 0.072 |
| FCR | 3.81± 0.4$^{a}$ | 3.12± 0.03$^{ab}$ | 1.71± 0.008$^{cd}$ | 2.54± 0.24$^{bc}$ | 2.57± 0.3$^{bc}$ | 1.56± 0.053$^{d}$ | 1.97± 0.13$^{cd}$ | 1.73± 0.02$^{cd}$ | 1.4± 0.05$^{d}$ | 1.5± 0.04$^{d}$ | 1.35± 0.001$^{d}$ | 1.1± 0.03$^{d}$ | <0.001 | <0.001 | 0.003 |
| PER | 0.66± 0.06$^{g}$ | 0.8± 0.008$^{g}$ | 1.46± 0.006$^{cde}$ | 0.1± 0.1$^{fg}$ | 0.1± 0.12$^{fg}$ | 1.61± 0.06$^{be}$ | 1.27± 0.08$^{ef}$ | 1.44± 0.01$^{de}$ | 1.8± 0.06$^{bc}$ | 1.67± 0.04$^{bd}$ | 1.85± 0.002$^{b}$ | 2.27± 0.05$^{a}$ | <0.001 | <0.001 | 0.148 |
| PPV | 6.04± 1.4$^{f}$ | 9.97± 0.762$^{f}$ | 19.7± 1.33$^{cd}$ | 10.6± 1.52$^{ef}$ | 11.9± 1.35$^{ef}$ | 23.8± 0.204$^{bc}$ | 16.7± 1.5$^{de}$ | 22.5± 1.47$^{cd}$ | 29.7± 1.68$^{b}$ | 25.1± 0.678$^{bc}$ | 30.3± 0.712$^{b}$ | 41.9± 0.174$^{a}$ | <0.001 | <0.001 | 0.292 |

Values are means±SEM, n = 2 per treatment group; [a-g] Means in a row without a common superscript letter differ ($p < 0.05$) as analyzed by two-way ANOVA and the TUKEY test; [1]C × G = Col1 × groups interaction effect; IBW - initial body weight, FBW - final body weight, WG - body weight gain, FCR - feed conversion ratio, SGR - specific growth rate, PER - protein efficiency ratio, PPV - protein productive value, ER - energy retention, K - condition factor, and S - survival.

Table 5

Whole-body proximate composition of European sea bass, *Dicentrarchus labrax* after 120 experimental days

| Variable | Control | | | Low zeolite | | | Medium zeolite | | | high zeolite | | | P-value | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | D1 | D2 | D3 | D1 | D2 | D3 | D1 | D2 | D3 | D1 | D2 | D3 | Col1 | Groups | C×G[1] |
| Moisture | 70.7± 1.11 | 69.9± 1.05 | 70.8± 1.07 | 70.5±1 | 70.4± 0.422 | 69.7± 0.603 | 70.7± 0.175 | 69.2± 1.1 | 70.6± 0.167 | 70.2± 0.0845 | 70.3± 0.344 | 69.8± 0.592 | 0.946 | 0.595 | 0.805 |
| Protein | 44.3± 0.065$^{f}$ | 49.3± 0.5$^{e}$ | 51.8± 0.11$^{ce}$ | 45.4± 0.88$^{f}$ | 49± 1.12$^{e}$ | 52.7± 0.345$^{cd}$ | 50.5± 0.33$^{de}$ | 53.7± 0.19$^{c}$ | 58.1± 0.595$^{b}$ | 53.5± 0.05$^{c}$ | 57± 0.16$^{b}$ | 61.6± 0.33$^{a}$ | <0.001 | <0.001 | 0.412 |
| Lipid | 27.7± 0.68$^{a}$ | 25.4± 0.275$^{ac}$ | 24.6± 0.26$^{c}$ | 27.4± 0.705$^{ab}$ | 25.2± 0.81$^{bc}$ | 23.3± 0.22$^{cd}$ | 23.1± 0.195$^{cd}$ | 21.9± 0.04$^{de}$ | 20.1± 0.22$^{ef}$ | 21.7± 0.355$^{de}$ | 21.3± 0.25$^{de}$ | 18.9± 0.135$^{f}$ | <0.001 | <0.001 | 0.239 |
| Ash | 17.9± 0.745$^{a}$ | 15.2± 0.225$^{ce}$ | 13.5± 0.15$^{ef}$ | 17.2± 0.175$^{ab}$ | 15.7± 0.31$^{bcd}$ | 13.9± 0.125$^{de}$ | 16.3± 0.135$^{ac}$ | 14.3± 0.15$^{de}$ | 11.7± 0.375$^{f}$ | 14.8± 0.305$^{ce}$ | 11.6± 0.41$^{f}$ | 9.36± 0.465$^{g}$ | <0.001 | <0.001 | 0.126 |

Values are means±SEM, n = 2 per treatment group; [a-g] Means in a row without a common superscript letter differ ($P < 0.05$) as analyzed by two-way ANOVA and the TUKEY test; [1]C × G = Col1 × groups interaction effect.

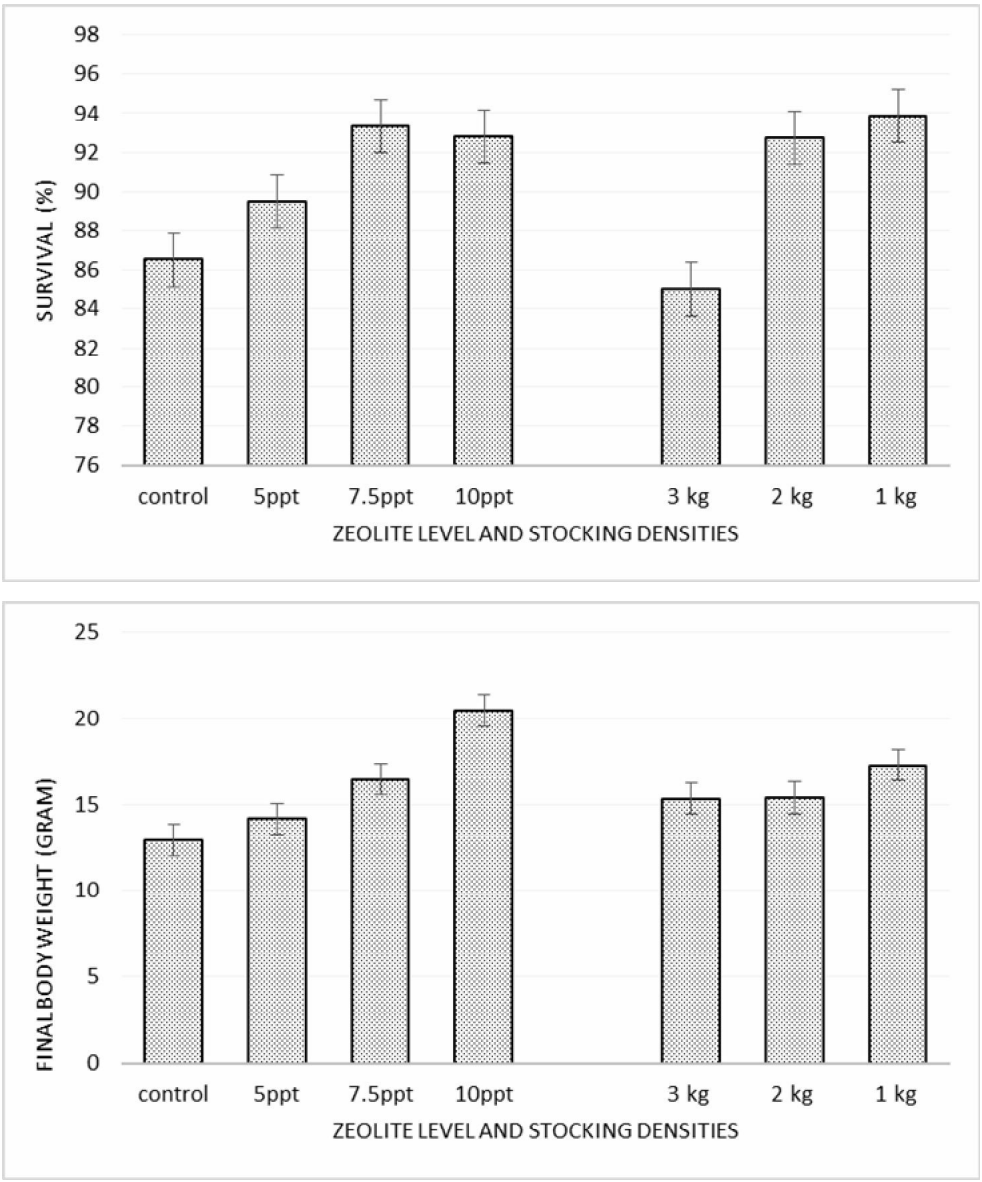


Figure 2. Survival rate for the European seabass juveniles (upper); final body weight of *D. labrax* (lower), throughout the study period.

***Carcass composition***. The proximate body analysis of European seabass juveniles (Table 5) demonstrated a significant decrease in body lipid and ash contents with the increasing content of body protein ($p < 0.05$), being significantly lower in fish of $D_3$ in high zeolite than those of $D_1$ in control treatment ($p < 0.05$). On the contrary, the increase in natural zeolite level led to a concomitant increase of whole body protein from 44.3% in fingerlings of $D_1$ in control treatment to 61.6% in $D_3$ of high zeolite treatment ($p < 0.05$). However, the whole body moisture levels did not noticeably differ between the studied treatments

**Discussion**. Stocking densities along with DO, water temperature, pH and salinity are the most influencing factors in pond ecology resulting in fish body weight fluctuation during the production cycle. Numerous factors are known to suppress growth in high stocking densities, for example reducing food consumption (Oppedal et al 2011), inhibiting social interactions (Sloman et al 2000), and worsening water quality (Fivelstad et al 1995, 1998). Increased stocked density induces food and habitat competition that usually impairs animal production (Huang & Chiu 1997). In other words, when growth is negatively affected with stocking density it is considered density dependent, such as the cases found for shrimp, *Litopenaeus vannamei* Boone (Velasco et al 1999; Gaber et al 2012).

In this experiment, population density clearly affected water quality and with increasing density, high TAN and low DO appeared. However, ammonia removal from pond water with high fish density eliminates the drastic effect on fish health which in turns the fish welfare is enhanced. There are many methods used to reduce ammonia from polluted water, but the ion exchange method with natural zeolite and biological nitrification/denitrification was by far the most effective (Tchobanoglous et al 2011). From the first week of the present experiment, the effectiveness of zeolite in ammonia removal was very obvious that there were significant ($p < 0.05$) differences between the control and zeolite treatments especially with high fish density groups. However, significantly ($p < 0.05$) lower TAN removal was seen at Z5 and Z7.5 than for Z10 treatment group. This could be ascribed to the high activity of nitrification or microbial in the experimental ponds exceeding the adsorption ability of low zeolite doses. And also it might be because of the daily water exchange rate and/or due the decrease of zeolite activity after prolonged water exposure comprising high levels of TAN. These findings are in consensus with the results reported by Kedziora et al (2014) who found that using levels of zeolite at 10 g $L^{-1}$ and higher is more effective in removing ammonia. Also, Aly et al (2016) revealed that Z10 reduce TAN and UNA more than Z5. Burgess et al (2004) marked a direct connection between the mass of zeolite and amount of total ammonia removed. However, Saeed et al (2015) showed that, application of zeolite significantly decreased all the inorganic dissolved nitrogen, namely total ammonia ($NH_3$, $NH_4$-N), nitrite ($NO_2$) and nitrate ($NO_3$) content in the water. This agrees with our results whereas, $NO_2$ decrease significantly with increasing zeolite level. This finding might have been principally due to the adsorption capacity of zeolite as it attracted nitrate ions and improved nutrient retention from water (Mumpton 1999). Polat et al (2004) mentioned that although the negative charges of zeolite retains valuable nitrogen, but also allows for the adsorption of some positively charged ions like $NH_4$. Also, earlier researches claimed that zeolite could be effectively applied to lower the ammonium concentrations in freshwater (Durhan et al 1993; Besser et al 1998). However, results herein showed that the density of fish had significant influence on water quality and a high density culture may result in high concentrations of ammonia nitrogen, nitrite nitrogen, and low concentrations of DO and pH in the tank water. Oppositely it was observed when zeolite was applied, significant increase ($p < 0.05$) in pH was linked with the increased zeolite levels. Leinonen & Lehto (2001) explained this observation by implying that the weakly acidic nature of zeolite is hydrogen selective causing pH to elevate when dilute electrolyte solvent is equilibrated with sodium-form exchangers. The pH range mostly desirable by many fish species for optimum is 7-9 (Boyd 1998).

However in the current study, the DO increased significantly ($p < 0.05$) with increased zeolite level treatment, while in control group acquired lower value. The virtuous water quality conditions caused by zeolite increased significantly ($p < 0.05$) the DO content as previously reported by Ferdous et al (2013) and Saeed et al (2015). However, Boyd (1998) confirmed that 5.0 mg $L^{-1}$ the lower recommended DO level fulfill the needs of fish welfare. Ammonia removal rate for the control treatment was -5.46, 33 and 38.6% for high, medium and low density treated with zeolite compared with the source water. The water movement up to the header tank containing zeolite and down to the inlet pipes and supplemented artificial aeration may assist in removing a percentage of ammonia in water. This explanation is compatible with Tchobanoglous et al (2011) whom stated that using air stripping as a common method of ammonia removal, but in high density crowded fish, feces and wasted feeds increase ammonia over source value.

Generally, the recorded values of pH, TAN, and un-ionized ammonia in this experiment were within the acceptable range of seabass hatcheries (Lemarié et al 2004; Munday et al 2009). Water quality significantly changed among different densities, and the differences in TAN and DO may have been responsible for the significant differences of fish mortality (Liu et al 2016). Also, nitrite values decreased significantly ($p < 0.05$) with increasing zeolite levels and with decreasing stocking density. In general, nitrate prejudices various number of physiological factors and impairs the survival of most fish species (Van Bussel et al 2012). European seabass showed density-dependent growth performance in the present study as the highest body weight was observed in the lowest

density and vice versa. Although, Liu et al (2016) noticed that growth rate of rainbow trout (*Oncorhynchus mykiss*) is greatly affected with high density, but also the energy demand and metabolism increased. Additionally, chronic stress produces a series of defense mechanisms that can divert energy from being used for growth to other stress-responsive and energy-consuming processes (Lupatsch et al 2010).

In the study herein, higher stocking densities resulted in both significantly lower body weight and standard length at the end of the experiment (3 kg $m^{-3}$), suggesting that 3 kg $m^{-3}$ is a point in which additional energy is used for body defense mechanisms, reducing the resources available for body growth. The majority of energy from food is converted into net energy for maintenance (including basic metabolism and adaptive processes for stressful situations) and net energy for production (including factors such as growth performance, body fat, and reproduction) in teleost fish (Fan et al 2008). Intensive social interactions caused by dense rearing condition might contribute to increased basic metabolism demands, arrested growth, and low food intake in fish (Larsen et al 2012; Liu et al 2014). Concurrently, in the present experiment the FCR of high density was significantly enhanced ($p < 0.05$) with 3 kg $m^{-3}$ density. This significant up-regulation of FCR indicates that density stress reduce the ratio of growth energy from food energy. Moreover, FCR also demonstrated significant increases within two higher density groups. Although, FCR improved significantly with increasing zeolite levels in all stocking density and it might be due to improved water quality with adding zeolite and reducing energy that demand to face stress. The proper conditions of water quality in zeolite treatments may be a reason for higher harvest weights than those reared in control ponds (Aly et al 2016). The data in the present study matches the conclusions of Xia et al (2009) and Saeed et al (2015) who reported that natural zeolite aids in the prevention of disease occurrence and enables growth promotion and survivability and thus higher profits.

Moreover, natural zeolite aids in ammonia reduction in pond water of European sea bass positively induced growth rate in the present experimental trial. Fayed et al (2019) reported that adding liquid *Yucca schidigera* extract significantly reduced the ammonia in pond water and noticeably increased the growth rate of *D. labrax* juveniles.

Previous studies have found that stocking densities can affect the body composition of African catfish, *Clarias gariepinus*, and vundu catfish, *Heterobranchus longifilis* (Toko et al 2007). In present experiment, high density clearly affected crude fat and crude protein when density increases from 1 to 3 kg $m^{-3}$. Rainbow trout effectively convert protein and fat, but not glucose, into energy for growth and adaptation to stressful situations (Fan et al 2008). In teleost fish, net energy for production consists of growth performance, body fat store and reproduction (Elliott 1976). Redundant energy within this category in a dense environment of poor growth performance facilitates its conversion into high body fat stores within the high density group. Moreover, significant up-regulations crude fat content within the high density group may also suggest that fat accumulated in viscero-somatic mass. However, crude protein increased significantly ($p < 0.05$) when zeolite levels increased from 7.5 ‰ , even though crude fat decreased. Saeed et al (2015) demonstrated that, application of zeolite had no significant effect on the proximate composition of Nile tilapia (*Oreochromis niloticus*), because fish in all ponds fed artificial feed. But earlier finding by Hanley (1991), inferred that both endogenous and exogenous factors affect the body composition of fish. But in the current study, differences in body composition may be results of increasing zeolite levels and its effect on water quality, then enhances body composition although raising stocking density. Results of the current study revealed that PPV%, and PER were greatly ($p < 0.05$) affected by stocking density and zeolite levels and exhibited preeminent results at low stocking density and high zeolite rate.

**Conclusions**. The present study conclusively highlights the effectiveness of natural zeolite application on the growth performance and survival of European sea bass juveniles. The enhancement of water quality by 10 ‰ zeolite triggers positively the growth and survival and decreases obviously the amount of ammonia in water. The natural zeolite could be a revitalizing method of water quality that is used safely to

remove total ammonia nitrogen (TAN) and may in turns improves the ability of *D. labrax* to use offered feed and maintain high growth rates along with elevated survival rate.

**Acknowledgements**. We are grateful for the close collaboration between National Institute of Oceanography and Fisheries and Faculty of Agriculture (Saba basha) Alexandria University in completing this study.

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Received: 24 March 2019. Accepted: 29 May 2019. Published online: 30 June 2019.
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How to cite this article:
Fayed W. M., Sallam G. R., Khalid A. E., Kashuit A. A., Aly H. A., Omar E. A., 2019 Zeolite as a major control factor of water quality problems arising from stocking density of European seabass (*Dicentrarchus labrax*) juveniles. AACL Bioflux 12(3):953-967.