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GENERAL INFORMATION

Abbassa International Journal for Aquaculture is Egyptian specific publication in aquaculture of the Egyptian society for water, aquaculture and environment. The journal is published in four volumes per year to include results of research in different aspects of aquaculture sciences. The journal publishes also special issues of advanced topics that reflect applied experiences of importance in aquaculture sector.

SEASONAL CHANGES IN WATER QUALITY AND THEIR EFFECTS ON CORTISOL AND LIPID CONTENTS IN SERUM AND TISSUE OF GILTHEAD SEA BREAM (SPARUS AURATA) CULTURED IN EARTHEN PONDS

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Abstract

The study was undertaken on sea bream fish to show the different water parameters (water temperature °C, dissolved oxygen mg/l, salinity ppt, pH, total ammonia, toxic ammonia. ortho phosphate, total alkalinity mg/l, and chlorophyll a. Phytoplankton org. /l and zooplankton org. /l) on some physiological measurements (glucose mg/dl, cortisol ng/ml, total protein g/dl, lipid composition in tissue and blood) and growth performance during the different seasons of culture. Water source was supplied from Manzala Lake which has a variable salinity. The main important factors in water quality were, salinity ranged from 25.5 to 36.5 ppt; temperature ranged from 16.1 to 31.1 °C, dissolved oxygen ranged from 4.1 to 7.6 mg/l and ortho phosphate from 0.11 to 0.36 mg/l from season to another. All water quality parameters in the suitable limits for growth expect water nutrients. The main important physiological factor was glucose (63.5:99.6 mg/dl) and cortisol (85.3:122.3 ng/ml). Serum cholesterol and triglycerides were recorded in different seasons and ranged from 141.2±4.6 to 120.5±3.2 mg/dl and 78.8±2.7 to 61.2±2.8 mg/dl respectively. Serum some electrolytes (sodium and potassium mmol/l) were measured. Also, hematological parameters; hemoglobin, erythrocyte count and hematocrite were recorded. The interaction between salinity and temperature affect many metabolic parameters in the tissues assessed, especially in liver and muscle, suggesting that such interaction is inducing an important metabolic cost for the animal. It was reflected on growth and some physiological parameters of Gilthead Sea

bream. The fish performance is affect by poor water quality. Sea bream had low daily growth compared as fresh water fish. The growth performance of Sea bream increased during summer and autumn. So the water quality and physiological states have direct effect on Sea bream growth performance.

Keywords: water quality; Sea bream; serum; cortisol ; glucose; cholesterol; triglycerides, Hb, RBCs; PCV, growth performance, final weight and weight gain.

INTRODUCTION

According to the FAO (2003a) aquaculture is growing more than 10% per year and this growth is expected to continue. A production of 47 million tons of aquaculture products, mainly fish, is estimated for the year 2010 (Dar, 1999). This increase is necessary to supply fish for a growing human population. Fish consumption is considered to be healthier than meat, and so its use is being promoted. Data from the FAO shows that wild capture fisheries seem to have reached its maximum yield (FAO, 2003b) making some farms of aquaculture necessary. Aquaculture is still the fastest growing food producing sector, compared to other food commodities with an annual increase approximately 12% (FAO 2004 and FAO 2009). Aquaculture practices are increasing all over the world due to progressive impoverishment of natural fish stock populations and worldwide increasing demand for fish-associated proteins (FAO, 2000). Paradoxically, farming activities have a strong negative feedback on natural fish populations as a consequence of two main reasons: (a) the enormous need of fish (from natural stocks) to be converted in farmed fish feed (Naylor et al., 2000) and (b) the deterioration of coastal areas (water, surface sediment and plant communities) due to high loads of organic matter and nutrients introduced by fish farms (Christensen et al., 2000). Wilcox, et al., (2006) found that the production of marine fish species increased with increasing natural feeds such as rotifers (Brachionus sp.) or brine shrimp (Artemia sp.). Also, they reported that a feed density of \geq 4000 rotifers/I gives better survival and growth than

lower densities. Euryhaline and eurytherm, teleost species present, the ability of living under different environmental conditions of salinity and temperature. Both variables are key factors in fish energy metabolism, inducing a metabolic reorganization to compensate salinity and temperature changes (Somero, 2004; Soengas et al., 2007). Salinity acclimation induces a metabolic reorganization in order to meet the energetic demands of osmoregulatory organs (gills, intestine and kidney) of teleosts (Soengas et al., 2007). Temperature also modulates osmoregulatory ability in different teleost species, inducing a clear disturbance when it is out of its optimal value range (Staurnes et al., 2001). In the earthen ponds, water salinity did not present important differences among seasons (ranging from 36 ppt in winter to 38 ppt in summer); however, water temperature showed big differences (24 °C in summer and 12 °C in winter (Vargas-Chacoff et al., 2009). The gilthead sea bream (S. auratus) belongs to the family Sparidae, order Perciformes. This order originated in the marine environment, but in general species are very euryhaline with some estuarine and freshwater families providing a good model for the assessment of physiological plasticity related to changes in environmental salinity. Polakof et al., (2006) they found that several aspects of acclimation of S. aurata to different osmotic conditions, demonstrating the existence of osmoregulatory, endocrine, and metabolic changes. The gilthead sea bream, Sparus aurata, is an euryhaline and eurytherm species important in Mediterranean aquaculture (FAO, 2000). Gilthead Sea bream, the most important finfish species in Mediterranean aquaculture, some studies have shown the effect of high rearing density on growth, survival and immune resistance (Montero et al., 1999), whereas others have focused on their essential fatty acid requirements (Ibeas et. al., 1994). Gilthead seabream (Sparus aurata) is a hermaphroditic species which undergoes sex reversal, has non-synchronous ovarian development and daily spawns large masses of eggs over a long period of time

(Zohar et al., 1995). As in other teleosts, the maturation cycle in gilthead seabream brood stocks requires large quantities of acronutrients such as lipids and proteins to be made available for transfer to the developing oocytes. Gilthead sea bream females feed throughout gonadal maturation and spawning and therefore, the nutrients and energy necessary for ovarian growth and other reproductive functions must be drawn from both dietary input and body stores (Almansa et al., 2001). Body composition of fish is well known to change in response to seasonal reproductive and environmental conditions (Dygert, 1990). Seasonal fluctuations in the levels of some metabolic enzymes have also been shown along the annual reproductive cycle of fish (Tripathi and Verma, 2004). Cholesterol is an essential structural component of cell membranes; it is the outer layer of plasma lipo-proteins and the precursor of all steroid hormones. The primary function of triglycerides is to store and provide cellular energy (Yang and Chen, 2003). Corticosteroid hormones are essential for the regulation of a wide variety of physiological processes. In teleost fish cortisol is the most important corticosteroid, playing a dual role as a glucocorticoid and mineralocorticoid hormone. Plasma cortisol has been used as the principal indicator of stress (Barton, 2002; Rotllant et al., 2003) as stress activates the hypothalamic pituitary internal axis (HPI) and the release of cortisol from the inter-renal cells located in the head kidney is the final hormone in this cascade (Rotllant et al., 2000). The effects of corticosteroid hormones in vertebrates are mediated through two intracellular receptors that act as ligand-dependent transcription factors.

The aim of the present research, completes the information provided by these authors, by studying the effect of changes in water quality levels in different seasons on gilthead Seabream plasma (total lipid in tissue and blood, T. cholesterol, triglycerides, total protein, glucose and cortisol) and growth performance (initial and final weight,

SGR, and weight gain) and their reflection on hemoglobin, erythrocytes count and hematocrit percentage.

MATERIAL AND METHODS

Animals and experimental conditions:

The experiment was designed in three earthen ponds (one hectare in area and one meter in water depth) cultured for two years with fingerlings of gilthead sea bream with average weight $(20.8\pm1.2 \text{ g})$. The water samples were taken monthly for 24 months then tabulated seasonally as means. The fish samples were taken in the 1st and second year for fish blood, tissue analysis and growth performance measurements. The initial weight of gilthead sea bream fish in the first year was 1.5±0.2 g. Investigations were performed on cultured gilthead sea bream (Sparus aurata), from three earthen fishponds have the same conditions of density and feeding, special fish farm in Damietta province, Egypt. The source of water intake was supplied from Manzala Lake. The experiment was performed to study the effects of seasonal changes in water quality on lipid content in tissue and blood, growth performance, and hematology of the gilthead sea bream (Sparus aurata). Fish were fed by commercial pellets, to apparent satiation, twice a day at a feeding rate 3% of fish body weight per day, 6 days a week. Growth performance, tissue and blood samples were taken from fish at the end of each season. While water samples were taken monthly and tabulated seasonally. Fish were fasted 24 hr before sampling.

Water quality:

Water samples for physico-chemical and biological analysis were monitored monthly during the study period; five samples were taken from fishpond then mixed with each other and take two litres from a mixture for water analysis and phytoplankton. Zooplankton samples were collected by filtering 100 liters of the fishpond water at each station through a small standard plankton net (mesh size 45µm) using a plastic container of ten litres capacity. The collected samples were preserved directly with 4% formalin solution. Phytoplankton was estimated according to methods reported in APHA (2000). Also, zooplankton species were identified according to Foissner and Perjer (1996). Dissolved oxygen, temperature, salinity and pH were measured in field. Dissolved oxygen and temperature were measured on field with an YSI model 58 oxygen meter (Yellow Spring Instrument Co., Yellow Springs, Ohio, USA). Water salinity was measured with a Conductivity Meter (YSI model 33, Yellow Spring Instrument Co., Yellow Springs, OH, USA). pH were measured using pH meter (Fisher Scientific, USA). Total alkalinity and total hardness were measured by titration methods as described by APHA (2000).

Blood sample collection:

At the end of each season, fish were individually captured from fishpond (20 fish were captured per pond). Fish were anaesthetized with diluted MS222 and blood was collected via the caudal vein into heparinized with 2 ml plastic syringe. Blood samples were immediately transferred to an Eppendorf tube coated with lithium heparin as anticoagulant. The blood sample were divided into two parts, first part as it is for Hb, RBCs and hematocrit measuring immediately, while the second part for the plasma was obtained by centrifugation at 3000 rpm for 20 min and stored at -20 °C prior to T. cholesterol (mg/dl), triglycerides (mg/dl), total protein (g/dl), sodium (mmol/l), potassium (mmol/l), cortisol (ng/ml) and glucose (mg/dl) determination.

Blood measurements:

Total protein concentration was determined by the Lowry technique (Lowry *et al.*, 1951), using BSA as the standard. Total lipid content was determined after extraction with chloroform/methanol (2:1 v/v) as described by Folch *et al.*, (1957). Cholesterol level was

determined by the method of Henry (1974). Triglycerides were analyzed by the method of Schettler and Nussel (1975). Cortisol levels in plasma were measured by immunological method (Sibar, Perugia, Italy) (Arakawa *et al.*, 1979). Glucose in plasma was carried out according to the method of Trinder (1969). Plasma potassium and sodium were analyzed by atomic absorption spectrophotometer (Thermo.400 with graffiti furnace (England) Thero. Comp. model 2005. Hematocrit was measured as packed cells volume by using a Haemofuge microcentrifuge (Heraeus-Christ, Osterode, Germany). Erythrocyte cells were counted by direct observation in a Neubauer chamber, after diluting a sample of fresh blood (1:20) with saline solution (Schreck and Moyle 1990).

Growth performance:

Growth performance was determined and feed utilization was calculated as follows:

Weight gain = W2 - W1;

Where W1 and W2 are the initial and final fish weight, respectively;

Daily weight gain = weight gain / T; where T is the number of days in the feeding period.

Statistical Analysis:

The obtained data was subjected to a two-way analysis of variance (ANOVA) to test the effect of seasonal changes on the some physiological parameters. Duncan's multiple range tests was used as a post-hoc test to compare between means at $P \le 0.05$. The software SPSS, version 10 (SPSS, Richmond, VA, USA) was used as described by Dytham (1999).

RESULTS

In Table (1) showed that ranges and means of water quality parameters during the experimental period (24 months) were not within suitable normal limits for fish growth performances for some parameters specially water temperature in winter season. Generally which means the level of measured water parameters were decreased in winter and increased in summer. Water temperature ranged 16.1 to 31.1°C, the water temperature were significantly differences among seasons during two years, but did not significantly differ in the same seasons during two years. The highest values of water temperature recorded in summer and lowest in winter. Dissolved oxygen was fluctuated from season to season depending on temperature, day light, nutrient and phytoplankton. The highest values of dissolved oxygen recorded in winter during two seasons and ranged from 7.2 -7.6mg/l. the lowest values recorded in summer and ranged from 4.1- 4.6mg/l. Salinity fluctuated from 25.5 to 36.5 g/l during four seasons in two years. The highest values recorded in summer during two years. Total alkalinity was ranged from 275 to 350 mg/l, in winter and summer season respectively. The average values of NH3 ranged from 0.03 to 0.09 mg/l in all seasons during two years. The highest vales recorded in summer and lowest values recorded in winter. The average concentration of ortho phosphate in experimental earthen ponds ranged from 0.11 to 0.36mg/l. also, similar trend was observed by ortho phosphate. The average values of chlorophyll a ranged from 36.52 to 134.12µg/l. generally, the water quality parameters were significantly increased in summer than others seasons and there is no significant difference between years in the same season.

Item season		Temp. °C	D.O mg/l	рН	Salinity g/l	Total alkal. mg/l	Ortho phosphate mg/l	NH ₃ mg/l	NO ₂ mg/l	NO ₃ mg/l	Chlorophyll a µg/l
	First	24.5±	$6.8\pm$	$8.6\pm$	$29.5\pm$	310±	$0.24\pm$	$0.05\pm$	$0.015\pm$	0.19±	$56.55 \pm$
Spring	year	0.4 ^b	0.2 ^b	0.3 ^b	0.6 ^b	12 ^b	0.01	0.003 ^b	0.003 ^b	0.03 ^b	5.5 °
	Second	25.2±	$6.4\pm$	$8.6\pm$	$30.5\pm$	332±	0.27±	$0.05\pm$	$0.017\pm$	$0.17\pm$	59.99±
	year	0.3 ^b	0.3 ^b	0.4^{b}	0.5 ^b	13 ^b	0.01	0.002 ^c	0.002 ^c	0.02 ^c	4.6 °
	First	30.2±	4.3±	9.2±	36.5±	275±	0.36±	$0.08\pm$	$0.036\pm$	0.31±	116.46±
Summer	year	0.5 ^a	0.2 ^c	0.4 ^a	0.5^{a}	11 ^c	0.01	0.002^{a}	0.002^{a}	0.03 ^a	10.22 ^a
	Second	31.1±	4.1±	9.2±	35.5±	286±	0.35±	0.09±	$0.039\pm$	0.36±	134.12±
	year	0.7 ^a	0.3 ^d	0.5 ^a	0.6 ^a	11 ^c	0.011	0.003 ^a	0.003 ^a	0.04 ^a	10.2 ^a
	First	22.6±	4.6±	8.8	29.5±	300±	0.0.3±	$0.065\pm$	$0.023\pm$	$0.22\pm$	86.74±
Autumn	year	0.2 ^c	0.2 ^c	0.3 ^b	0.4 ^b	13 ^b	0.01	0.003 ^b	0.003 ^b	0.02 ^b	7.02 ^a
	Second	23.2±	$4.8\pm$	$8.8\pm$	29.5±	290±	0.3±	0.065±	$0.024 \pm$	0.25±	79.36±
	year	0.3°	0.3 ^c	0.4^{b}	0.5 ^b	10 ^c	0.01	0.004 ^b	0.004 ^b	0.03 ^b	5.04 ^b
	First	16.5±	7.6±	8.3±	25.5±	350±	0.14±	0.033±	0.01±	$0.07\pm$	38.18±
Winter	year	0.3 ^d	0.3 ^a	0.3 °	0.3 ^c	15 ^a	0.01	0.002^{b}	0.002^{b}	0.04 ^b	1.8 ^d
	Second	16.1±	7.2±	8.2±	25.5±	370±	0.11±	0.03±	0.01±	0.09±	36.52±
	year	0.4 ^d	0.5ª	0.5 °	0.5°	12 ^a	0.01	0.003 ^c	0.003 ^c	0.02 ^c	1.94 ^d

Table (1): Total annual mean of some physico-chemical characteristics ofwater samples on experimental ponds during two years.

The means have the same superscript liters for the same column are not significant difference (P<0.05).

From the presented data in Table (2), show the fluctuated numbers of zooplankton and phytoplankton during four seasons in two years. The classifications of zooplankton and phytoplankton during study were to three groups for zooplankton and fours groups in phytoplankton. The zooplankton groups were Rotifera, copepoda and cladocera. The average number of rotifra were 88; 165; 162 and 128 organism/l in spring, summer, autumn and winter respectively in first year. Copepoda were 296; 435; 321 and 184org/l during same seasons and year respectively. Cladocera were 119; 296; 202 and 68org/l for the same respectively. The main group in zooplankton is copepoda. The average numbers of zooplankton groups did not significantly differed between two years, but were significantly differed among seasons. The phytoplankton groups were diatoms, blue green, green and bassilarophyce. The main group is diatom. The average numbers of diatom ranged from 5055 to 13615org/l

during four seasons in two years. Green algae ranged from 2155 to 5742org/l during the same period. Blue green algae ranged from 3490 to 7978org/l during the same period. Bassilarophyce ranged from 1088 to 3612org/l during the same period. Also, there were significant differed among seasons but did not significantly differed between years. The highest abundance of phytoplankton was diatoms followed by blue green and green . also, the highest number recorded in summer.

Items		z	ooplankton o	rg/ l	Phytoplankton org/ l				
Seasons	Year	Rotifera	Copepoda	Cladocera	Diatoms	Green algae	Blue green algae	Bacillarophyta	
a .	1rt	$\frac{88\pm}{5^{\rm b}}$	296± 3°	119± 2b	8722± 135b	3574± 135 ^b	$5584 \pm 224^{\rm b}$	2122± 2.3 ^b	
Spring	2nd	97± 9 ^b	327± 3°	125± 3	8950± 144 ^b	3665± 135 ^b	5632± 229 ^b	2355± 2.6°	
a	1rt	165± 6 ^a	435± 5a	296± 3 ^a	13378± 170 ^a	5742± 145 ^a	7818± 38 ^a	3465± 5.7 ^a	
Summer	2nd	182± 9 ^a	449± 7 ^a	282± 2	13615± 190 ^a	6116± 170 ^a	7978± 331 ^a	3612± 7.2 ^a	
	1rt	162 ± 6^a	321± 5 ^b	202 ± 2^a	10250± 133 ^a	4475± 169 ^a	6455 ± 40^{a}	2588 ± 6.3^{a}	
Autumn	2nd	178± 9 ^a	336± 6 ^a	194± 3	10545 ± 154^{a}	$\begin{array}{c} 4585 \pm \\ 182^{a} \end{array}$	4702± 42 ^a	2644± 5.2 ^b	
	1rt	128± 7 ^c	184 ± 4^{d}	68± 3 ^b	5465± 86°	2264± 150 ^c	3490± 24 ^c	1088± 1.1 ^b	
Winter	2nd	141± 6 ^c	196± 5b	53± 3	5055± 74 ^c	2155± 160 ^c	3666± 44°	1175± 1.3 ^c	

Table (2): Average of counting and identification of phytoplankton andzooplankton in the experimental ponds during two years.

The means have the same superscript liters for the same column are not significant difference (P<0.05).

In Table (3) showed that liver weight and hepatosomatic index percentage (HSI %) were related directly with the body weight of fish, so with increasing body weight increase the liver weight so, there is significant difference in HSI ratio in different seasons, HIS were recorded in high percentage at the end of second year (2.52 %) and the lowest ratio at the beginning of the first year (1.49 %).

Table	(3):	Liver	weight	(g),	hepatosomatic	index	(%)	and	lipid	
		composition of fish liver and muscle at different seasons of								
		Gilthea	d fish w	as rec	corded.					

season Item	Liver weight g	HIS	Muscle lipid %	Liver lipid %	Liver cholestero %	Liver triglyceri des %	S. Cholesterol	S. Triglycerides
Unit	g	%	dry weight	dry weight	dry weight	dry weight	mg/dl	mg/dl
Spring a	0.95 ± 0.03^{d}	1.49 ± 0.31 ^b	10.75± 0.53 ^b	30.83± 1.23°	3.26± 0.15 ^c	60.25 ± 1.92^{b}	138.6± 6.8a	74.6± 2.6a
Spring b	5.19± 0.41 ^c	2.32 ± 0.41^{d}	13.25± 0.21 ^b	34.56 ± 1.42^{b}	3.89± 0.21 ^{bc}	68.56± 3.33 ^{bc}	157.4± 7.8a	87.5± 3.3a
Summer a	2.12± 0.12c	1.85 ± 0.32 ^a	12.66± 0.32 ^a	36.72± 1.32ª	4.15± 0.21 ^a	70.25± 2.79 ^a	141.2± 4.6a	78.8± 2.7a
Summer b	7.35± 0.25 ^c	2.46 ± 0.31 ^a	15.34± 0.22ª	38.62± 1.31ª	4.79± 0.24 ^a	$\begin{array}{c} 78.95 \pm \\ 2.76^{a} \end{array}$	166.2± 6.6a	84.5± 3.2a
Autumn a	2.73± 0.21 ^b	1.88 ± 0.24^{a}	10.32± 0.15 ^b	33.65± 1.11 ^b	3.72± 0.18 ^b	68.92± 3.21ª	120.5± 8.2b	66.3± 2.3b
Autumn b	8.76± 0.31 ^b	2.51 ± 0.33 ^a	11.82± 0.11 ^c	35.21± 1.41 ^b	4.12± 0.31 ^b	72.44± 2.35 ^b	142.4+ 5.6b	71.4± 2.9b
Winter a	3.66± 0.24 ^a	2.11 ± 0.22 ^a	10.82± 0.45 ^b	31.92± 1.18 ^{bc}	3.32± 0.11 ^c	62.35 ± 1.25^{b}	122.6± 6.1b	61.2± 2.8b
Winter b	9.43± 0.35 ^a	2.52 ± 0.41 ^a	11.62± 0.21°	32.33± 1.31 ^{bc}	$\begin{array}{c} 3.52 \pm \\ 0.25^{c} \end{array}$	65.43± 2.46 ^c	139.5± 7.5b	65.7± 2.2c

The means have the same superscript liters (a-d) for the same year in the same column are not significant difference (P<0.05).

Spring, summer, autumn and winter a= first year.

Spring, summer, autumn and winter b= second year.

Muscle lipid was significant increase in summer seasons of two years. Muscle lipids were recorded in lowest value in $(10.32 \ \%)$ in autumn 1st year and the highest ratio in $(15.34 \ \%)$ summer of 2nd year. Whereas, liver lipid percentage has been significant increase in summer

seasons a two years, liver lipids was recorded in lowest value in (30.83 %) in spring 1st year and the highest ratio in (38.62 %) summer of 2nd year. The liver cholesterol percentage has been significant decrease in winter seasons a two years; liver cholesterol was recorded in lowest value in (3.26 %) in spring 1st year and the highest ratio in (4.79 %) summer of 2nd year. Liver triglyceride was significantly increased in summer (70.25%); autumn (68.92%) respectively whereas it was significantly decreased in spring (60.25%) and winter (62.35%). Whereas serum cholesterol and triglycerides were increased significant in spring (138.6 and 74.6 mg/dl) and summer (141.2 and 78.8 mg/dl) respectively. But they were recorded the lowest values in autumn (120.5 and 66.3 mg/dl) and winter (122.6 and 61.2 mg/dl) respectively.

In Table (4) showed that the hematological parameters (Hb, RBCs and PCV) were increased with increasing the body weight of fish and fish feeding, and some biochemical parameters in serum (total protein, cortisol, glucose, electrolytes sodium and potassium in serum) were be measured. So, hematological parameters (Hb, RBCs and PCV) were significantly increased, the highest values of Hb were recorded in (10.2 g/dl) winter of 2^{nd} year and the lowest values were recorded in (6.8 g/dl) spring of 1^{st} year. The highest numbers of RBCs were recorded in (3.162X10⁶/cmm) winter of 2^{nd} year and the lowest values were recorded in (2.322X10⁶/cmm) spring of 1^{st} year. The highest percentages of hematocrite were recorded in (29.72 %) winter of 2^{nd} year and the lowest percentages were recorded in (19.92 %) spring of 1^{st} year.

On the other hand in Table (4) also, some biochemical parameters in serum protein, cortisol and glucose levels were recorded. The highest value of serum protein was recorded in (6.8 g/dl) winter of 2^{nd} year and the lowest value was recorded in (5.2 g/dl) autumn of 1^{st} year; the highest value of serum cortisol was recorded in (122.3 ng/dl) winter of 2^{nd} year and the lowest value was recorded in (76.4 ng/dl) summer of 1^{st} year,

also, the highest value of serum glucose was recorded in (99.6 mg/dl) winter of 2^{nd} year and the lowest value was recorded in (56.2 mg/dl) summer of 1^{st} year. Electrolytes in serum sodium and potassium were measured seasonally and tabulated as showed in table (4). They were significant increased in autumn (168.3 and 4.35 mmol/l) and winter (175.6 and 4.53 mmol/l) respectively, but the lowest values of electrolytes were recorded in spring (144.2 and 3.87 mmol/l) and summer (149.3 and 4.01 mmol/l) respectively.

Item season	HB	RBCs	PCV	Total protein	plasma Cortisol	Plasma glucose	S. Na ⁺	S. K ⁺
Unit	g/dl	10 ⁶ /cmm	%	g/dl	ng/ml	mg/dl	mmol/l	mmol/l
Spring a	6.8 ± 0.2^{c}	2.322 ± 0.005^{d}	19.92± 1.21 ^b	5.8 ± 0.2^{a}	92.3± 2.6 ^b	65.2± 1.5°	145.6± 3.2°	4.21 ± 0.12^{a}
Spring b	9.6± 0.3 ^b	2.976 ± 0.008^{b}	26.92± 1.21 ^b	$6.5\pm$ 0.2^{a}	106.5± 2.1 ^b	76.5± 1.6 [°]	144.2± 2.5 ^b	3.87± 0.15 ^b
Summer a	7.4± 0.3 ^b	$2.462 \pm 0.004^{\circ}$	20.42± 1.31 ^b	$5.4\pm$ $0.3^{ m b}$	$76.4 \pm \\ 1.5^{\rm d}$	$56.2 \pm \\ 1.4^{d}$	144.2± 1.8°	$\begin{array}{c} 3.88 \pm \\ 0.19^{b} \end{array}$
Summer b	8.9± 0.2 ^c	$2.754 \pm 0.006^{\circ}$	23.81± 1.44 ^c	$6.1\pm0.1^{ m b}$	$\begin{array}{c} 85.3 \pm \\ 2.4^{d} \end{array}$	63.5 ± 1.5^{d}	149.3± 3.1 ^b	$\begin{array}{c} 4.01 \pm \\ 0.14^{b} \end{array}$
Autumn a	7.8± 0.3 ^b	2.505 ± 0.005^{b}	21.62± 1.35 ^a	5.2 ± 0.2^{b}	83.2± 2.3°	71.6± 1.2 ^b	155.9± 3.9 ^b	4.11± 0.18 ^a
Autumn b	8.4± 0.3 ^c	$\begin{array}{c} 2.604 \pm \\ 0.008^d \end{array}$	22.22± 1.21 ^c	$5.8 \\ \pm 0.2^{\rm b}$	96.3± 3.2 [°]	83.1± 1.7 ^b	168.3± 3.7 ^a	4.35 ± 0.11^{a}
Winter a	$\begin{array}{c} 8.4 \pm \\ 0.3^{a} \end{array}$	2.755 ± 0.006^{a}	23.62± 1.41 ^a	6.1± 0.3 ^a	109.2± 2.8 ^a	$\begin{array}{c} 83.2\pm\\ 1.8^{a}\end{array}$	168.2± 4.1 ^a	4.22± 0.21 ^a
Winter b	10.2± 0.2 ^a	3.162 ± 0.012^{a}	29.72± 1.31 ^a	$6.8\pm$ 0.2^{a}	122.3± 3.2 ^a	99.6± 2.3 ^a	175.6± 4.8 ^a	4.53± 0.13 ^a

Table (4): Plasma cortisol, glucose; serum sodium and potassium at different seasons of Gilthead fish.

The means have the same superscript liters (a-d) for the same year in the same column are not significant difference (P<0.05).

Spring, summer, autumn and winter a= first year

Spring, summer, autumn and winter b= second year

Fish production and growth performance are illustrated in Table (5) show that the seasonality changes affect on final weight, net gain and daily gain. The final weight of individual sea bream were 37.9, 88.1, 120.6 and 143.8g for four seasons (spring, summer, autumn and winter) in

first year respectively. In second year were 171.4; 208.9; 233.6 and 254.4g for four seasons (spring, summer, autumn and winter) respectively. The net gain was 36.4; 27.6; 50.2 and 37.5g for four seasons (spring, summer, autumn and winter) in first year respectively. In second year were 32.5; 24.7; 23.2 and 20.8g for four seasons (spring, summer, autumn and winter) respectively. The daily gain were 0.4; 0.31; 0.56 and 0.42g for four seasons (spring, summer, autumn and winter) in first year respectively. In second year were 0.36; 0.27; 0.26 and 0.23g for four seasons (spring, summer, autumn and winter) respectively.

 Table (5): Some growth performance parameters studied at different seasons on Sea bream.

Years items		Yea	ar 1		Year 2			
	spring	summ er	autum n	winter	spring	summ er	autum n	winter
Initial weight G	$1.5\pm 0.2^{\rm f}$	37.9± 1.5 ^e	88.1± 5.5 ^d	120.6 ± 5.1 ^c	143.8 ± 8.4 ^b	171.4 ± 8.6 ^b	$208.9 \\ \pm 9.9^{a}$	233.6 ± 12.7 ^a
Final weight g	37.9± 1.5 ^f	88.1± 5.5 ^e	120.6 $\pm 5.1^{d}$	143.8 ± 8.4 ^c	171.4 \pm 8.6^{c}	208.9 ± 9.9 ^b	233.6 ± 12.7 ^a	254.4 \pm 12.5^{a}
Weight gain G	36.4± 1.7 ^b	27.6± 1.2 ^c	$50.2\pm\\2.8^{\rm a}$	37.5± 2.3 ^b	32.5± 1.7 ^b	24.7± 1.6°	23.2± 1.6 ^c	$\begin{array}{c} 20.8 \pm \\ 1.1^{d} \end{array}$
Daily gain g	0.4 ± 0.02^{b}	0.31 ± 0.01^{b}	0.56± 0.01 ^a	0.42 ± 0.01^{b}	0.36 ± 0.01^{b}	$0.27 \pm 0.02^{\circ}$	$0.26 \pm 0.01^{\circ}$	$0.23\pm 0.01^{\circ}$

The means have the same superscript liters for the same raw are not significant difference (P<0.05).

DISCUSSION

The measurements of physico-chemical parameters in water under different experimental seasons and years are shown in Tables (1 and 2). The variation of pH could be explained by the photosynthetic uptake of CO_2 and bicarbonate that substituted hydroxyl ions. The pH values in two years were not significantly different. These results indicate that the increase of water temperature was significantly increased in pH. This

result is in agreement with Shaker et al. (2002) and Hamed and Hassan, (2011). Dissolved oxygen (DO mg/l) concentration in ponds did not vary significantly during two years, while the difference was significant among seasons. Several studies have shown the influence of different temperature regimes on fish growth and diet utilization as well as modifications in some enzyme activities related to energy metabolism in this species (Couto et al., 2008). The effects of cold temperature, related with the so-called "winter syndrome" that critically affect oxygen consumption, immune competence, growth and metabolism, have been also determined in Sparus aurata (Ibarz et al., 2007). The pH temperature and dissolved oxygen were the most influencing parameters in fish ponds, where their values in all ponds although fluctuated from time to time they stayed within the acceptable and favorable levels required for growth, survival and well being of the tested fish species.

The concentrations of the un-ionized ammonia (toxic form) NH₃-N in the present study was lower than those recorded in fertilized fish ponds (Hamed and Hassan, (2011). The increase of NH₃-N in the summer seasons compared to other could be explained by fish more activity, more eat and west and the decomposition of organic matter and via the direct excretion of ammonia by the large biomass of fish. The NO_2 and NO_3 concentrations in water followed the same trend of ammonia-nitrogen. The concentrations of NO_2 and NO_3 were also higher in summer seasons. These results may be due to the consumption of nitrate (which is an essential nutrient) by phytoplankton communities. Also, the increase of nitrate in summer seasons may be related to the increase of phytoplankton standing corps. It is of particular interest to notice a positive correlation between nitrate content and total phytoplankton which may be attributed to high consumption rate of NO₃-N by fish and more waste. These results are in harmony with those obtained by Shaker et al. (2009) and (Hamed and Hassan, (2011). The average concentrations of total alkalinity (T. alk.) were suitable for fish growth survival and well being. These results

are in agreement with those obtained by (Hamed and Hassan, (2011). The average concentrations of total phosphorus (T.P) and orthophosphate (O.P) were lesser in all ponds during all seasons in two years. These results may be due to the water exchange in ponds and added from Mazala lake that led to the decrease of organic matter in these ponds. The average concentration of chlorophyll "a" increased with increasing phytoplankton. Chlorophyll "a" in water ponds indicated the abundance of plankton in these ponds as a direct effect of pond management like fertilization of feeding. These results are in agreement with those obtained by Shaker *et al.* (2009).

The average numbers of phytoplankton and zooplankton decreased with the decreasing water temperature. These results clearly demonstrate that the total amount of phytoplankton and zooplankton decreased in winter seasons compared to summer seasons. Plankton population, chlorophyll a, net primary productivity (NPP) and gross primary productivity (GPP) remained low in the ponds indicating the incorporation of planktonic flora and fauna in plankton growth.

In the present studies both phytoplankton and zooplankton were high in ponds provided during summer seasons. Chlorophyll a concentration also coincided with the phytoplankton population. The same results were reported by Shaker *et al.*, 2009 and (Hamed and Hassan, (2011). There were significant difference in abundance in group of phytoplankton and zooplankton among the seasons. Abundance increased significantly over the experimental period. The highest recorded of phytoplankton and zooplankton was in summer seasons. These results may be due to the increase of water temperature and nutrients and perhaps also to the increase in productivity plankton. These results are in agreement with those obtained by (Hamed and Hassan, (2011).

The lower TAN concentrations during summer seasons others indicated higher ammonia uptake by phytoplankton as a nutrient (nitrogen

source). Thompson *et al.* (2002) reported that the attached diatoms and filamentous Cyanobacteria (periphyton) were responsible for the largest uptake of ammonium from the water in intensive shrimp culture ponds. Generally, from the water quality data it is clear that the water quality in all pond is poor during all seasons.

In fishponds, the pond bottom is the only substrate on which some larger benthic algae can grow. However, benthic algal mats seldom develop in highly eutrophic ponds due to shading by plankton blooms. In aquaculture systems, soil attached algae along with zooplankton and invertebrates can easily colonize the substrate in the water column. There were differences in abundance of plankton among seasons, suggesting that soil had a negative impact on phytoplankton production. But soil was added water by nutrient during all period. Zooplankton is important food sources for the larvae and some adult fish of many fish communities (Mavuti and Litterick, 1981). The highest feeding activity occurred at summer and spring. In spite of this predation on zooplankton, the production of zooplankter is still high; they are the natural trophic link between alga and zooplanktivorous predators such as larval fish (Nogrady *et al.*, 1993).

Physiological results showed that, when cortisol and glucose increased, this indicator for a certain stress, so, when cortisol and glucose were increased, this indicates that growth rate reduction or may be death rate was increased. But when total lipids in tissue or blood were depressed which means, the fish will be fasted or have certain stress to prevent it from eating, subsequently raise of cortisol, glucose and depression of lipids (total cholesterol, triglycerides in tissues and blood) must be due to stress. Increasing cortisol and glucose enhancement the lipase enzymes to increase and subsequently lipid analysis occur to use as source of energy for fish. Our suggested agreement with Pickering (1993) who found that reduction of growth is often considered to be a good indicator of chronic stress Rearing density has been reported to reduce growth in different cultured species including rainbow trout (Holm et al., 1990), This effect would be attributed to associated water quality deterioration (Kebus et al., 1992) or changes in thyroid hormones produced by a lower food intake (Vijayan and Leatherland 1988). Cholesterol is a steroid lipid found in the cell membranes of all body tissues and transported in the blood plasma. So, the cholesterol content in blood was significantly decreased after fasting in winter. The significant decrease in blood plasma cholesterol level of Gilthead Sea bream (Sparus aurata), after water temperature decrease or decrease of fish feeding, was similar those noticed in Heteropneustes fossilis after exposure to Oreochromis mossambicus after exposure aldrin and to urea (Balasubramanian et al., 1999). Also, the activity of triglycerides, after beginning of feeding in spring and summer seasons was significantly increased than those of autumn and winter seasons. A significant decrease in triglycerides content in the blood plasma of Cyprinus carpio by the action of gallium has been shown as an indication of its adverse effects on liver (Yang and Chen 2003), also they added triglycerides are used to evaluate nutritional status, lipid metabolism, and their high concentrations may occur with nephritic syndrome or glycogen storage disease. A similar effect of sub-lethal concentration of malathion has also been reported in Clarias batrachus (Lal and Singh, 1987).

The recorded data noticed that the increase of electrolytes sodium and potassium with water temperature decrease, these results maybe in agreement with the following studies, Waring *et al.*, (1996) there has been reported that plasma sodium and chloride levels display either a tendency to raise or a significant increase in seawater teleosts subjected to environmental stress due to water temperature descent. In low temperature-exposed gilthead sea bream, an increase or maintenance in plasma sodium and chloride levels have also been observed (Rotllant *et al.*, 2000). Instead, a decrease in sodium plasma concentration

occurred, a result that is consistent with some authors, such as Maetz and Evans (1972). These authors reported a reduction of plasma sodium levels in seawater fish as a result of temperature descent. The water temperature has strong relationship with plasma protein and glucose. These results are in agreement with the following studies; in farm animals the link between stress and susceptibility to diseases has long been acknowledged (Ladewing 1998). In this sense, exposure to winter temperatures may be stressful to gilthead sea bream, thus contributing to their susceptibility to developing the winter syndrome. Changes in the concentration of serum protein, albumin and globulin (Adham et al., 1997), as well as in the plasma glycaemia (Palti et al., 1999), have been used as indicators of stress response in fish.

The gain weight was noticed lowered in seasons of low water temperature; may be due to accessibility of fish for feeding. Due mainly to its commercial value, gilthead sea bream (Sparus aurata) farming has become a common practice along the Mediterranean coastline in the last 10 to 15 years. These data are in agreement with the following studies, Sarusic (1999) he found that during their first winter in the fattening farm, cultured gilthead sea bream maybe affected by a pathology termed "winter syndrome". This disease provokes chronic mortality during the coldest months and acute mortality episodes when the temperature rises at the beginning of the spring. Also, Padrós et al., (1999) they suggesting that gilthead sea bream refuse to feed when water temperature falls below 13 °C, they added 12 °C is a critical temperature with regard to their nourishment. The overall growth indices (specific growth rate ranged from 0.53 to 0.56%/day) were higher than those found by De Francesco et al. (2007) in seabream growing from 100 to 430 g.

These results indicated that the final weight and daily gain had positive relationship with water temperature, productivity, phytoplankton abundant and zooplankton abundant. These results are in harmony agreement with the presented data in Tables (1&2). These results are agreement with those obtained by Shaker *et al.*, (2009) and Hamed and Hassan, (2011).

Generally, the final weight, net gain, daily gain increased significantly with the increasing water temperature, phytoplankton, zooplankton, macronutrient (nitrogen and phosphors) and quality of feed. These results are in agreement with these obtained by Shaker *et al.*, (2009) and Hamed and Hassan, (2011) who reported that the growth performance of fish decreased with increasing water productivity.

These result indicated that the highest daily gain recoded first year. The daily gains during all seasons in two years were lowered than fresh water fish. These results may be due to sea bream species need big area for swimming and live. These results are agreement with those obtained by Shaker *et al.*, (2009).

CONCLUSION

Our data suggest that gilthead sea bream (*Sparus aurata*) respond differentially to changes in environmental salinity and temperature. As expected, the most affected tissues by salinity were those related with the osmotic acclimation, like muscle, and liver, as supplier of energetic substrates. The interaction between salinity and temperature affect many metabolic parameters in the tissues assessed, especially in liver and muscle, suggesting that such interaction is inducing an important metabolic cost for the animal. In general, the acclimation to extreme temperatures (especially low) alters the metabolic responses to different salinities thus suggesting that the energy demand of increased osmoregulatory work is not so important under extreme temperature conditions. The implications of these results are important for the culture of this species in earthen ponds in Egypt, where specimens can develop the called "winter syndrome" when environmental conditions of temperature are adverse.

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"قسم التفريخ والفسيولوجي. "فسم الليمنولوجي

المعمل المركزي لبحوث الثروة السمكية – مركز البحوث الزراعية – وزارة الزراعة ، مصر .

200 Seasonal Changes In Water Quality And Their Effects On Cortisol And Lipid Contents In Serum And Tissue Of ---

أجربت الدراسة على أسماك الدنيس لإظهار تأثير التغيرات الموسمية في خواص المياه المختلفة (درجة حرارة المياه ، الأوكسجين المذاب ، والملوحة جزء في الالف، ودرجة الحموضة، العسر الكلى والقلوية الكلية المغذيات النتيتوجينية والفوسفور والكلوروفيل ، العوالق النباتية والعوالق الحيوانية (على بعض القياسات الفسيولوجية) الجلوكوز ، الكورتيزول ، واجمالي تكوين البروتين و الدهن في الأنسجة والدم (، ومعدلات النمو خلال مواسم مختلفة من التربية. على مدار عامين كاملين. تم توفير مصدر للمياه من بحيرة المنزلة التي لديها نسبة الملوحة متغير .وكانت العوامل الرئيسية الهامة في نوعية المياه، والملوحة والتي لعبت التربة دورا هاما في الحفاظ على مستوياتها وعدم احداث تغيرات فجائية، تراوحت درجات الحرارة من.١٦.١حتى ٢١.١٦درجة مئوية، الأوكسجين المذاب تراوحت بين ٤.١ الى ٧.٦ ملجم/لتر ودرجة الحموضة من ٨.٢ الى ٩.٢ والملوحة من ٢٥.٥ الى ٣٦.٥ جم/لتر وشهدت درجة الملوحة اعلى ارتفاع اثناء موسم الصيف نتيجة لعمليات البخر وكذلك الاكسجين انخفاض اثناء الصيف لانخفاض الذوبانية بارتفاع الحرارة. اما بالنسبة للمغذيات سواء من النتروجين او الفوسفور او الكلوروفيل فقد دلت على فقر شديد في المياة وانها تحتاج الى تحسين كبير. كان من أهم العوامل الفسيولوجية الجلوكوز (63.5:99.6 ملغ / دل) و الكورتيزول (85.3:122.3) نانوجرام / مل . (ونسبة الكوليسترول في الدم والدهون الثلاثية في مواسم مختلفة، وتراوحت بين 3.2 ± 4،6-120،5 ± 2.2 ملغ / دل و 2،7 ± 8،8 حتى ٦١،٢ ± 2.8 ملغ / دل على التوالي .وتم قياس مصل الصوديوم والبوتاسيوم ملى مول / لتر .(أيضًا، هيموجلوبين الدم، وعدد كرات الدم الحمراء ونسبة الهيماتوكريت . وخاصبة في الكبد والعضلات، مما يشير إلى أن مثل

هذا التداخل مهم لعملية التمثيل الغذائي للحيوان .وقد انعكس ذلك على النمو وبعض المعا مالات الفسيولوجية للدنيس وتمتاز اسماك الدنيس بإنخفاض معدلات النمو اليومية مقارنة بأسماك المياة العذبة فقد اوضحت الدراسة انخفاض معدل النمو اليومى خلال جميع المواسم بإستثناء الصيف والخريف كان فيها النمو افضل الى حد ما .وبالتالي فإن نوعية المياه وحالات فسيولوجية يكون لها تأثير مباشر على أداء الدنيس. وعلية تنصح الدراسة فى متل هذة المزارع من ضرورة تحسين خواص التربة قبل موسم الزراعة وتحسين ادارة الاحواض والعمل على زيادة معدل تغيير المياة من البحر.