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Growth and gonadal maturation of Keeled mullet, *Liza carinata*, (Valenciennes, 1836) cultured at different salinities

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ABSTRACT

The growth experiment was conducted in fish culture outdoor cement ponds (5X4X1.2) under controlled conditions and filled with water (20 m^3). The experiment was carried out through 210 days. Every day, about 15-20% of water content was replaced to maintain water quality. Fish in all ponds were fed about 5% of total fish weight on artificial diet contains 25% fish meal. Experimental salinities (5, 15, 25, and 40 ‰) in four ponds (triplicate design) were obtained by diluting sea water with dechlorinated tap water (by passing through active charcoal). Fish at salinities 5 ‰ and 40 ‰ exhibited greater growth in length (cm) 10.77±0.097, 11.39±0.088 and weight (gm) 12.3±0.319, 15.02±0.44, respectively ($P<0.05$). Fishes at salinities of 15 ‰ and 25 ‰ represented lower growth in length 9.15±0.23, 9.1±0.10 and weight 7.41±0.53, 7.83±0.29, respectively ($P>0.05$). It was found that salinity does not affect significantly the condition factor of the individuals. Condition factor was ranging from 0.93 to 1.03 and there were no significant differences ($P<0.05$) between fish that had been subjected to different salinity treatments. Gonad somatic index (GSI) was significantly influenced by salinity ($P>0.05$). The GSI for both males and females have nearly the same fluctuation trends which reached to the maximum level at salinity of 40 ‰ for both sexes; 8.9 for female and 2.1 for male. The highest coefficient "b" of length-weight relationship were 2.839, 2.847 for fishes at salinities 40 ‰ and 5 ‰, respectively they closed to 3 it is indicated that isometric growth. The gonad maturation of salinity 5‰ reached to ripe stage for male and yolk vesicle stage for female but it reached to ripe stage at salinity 40‰ for both sex. These results suggest that *Liza carinata* is a suitable candidate for low salinity brackish water aquaculture. This could lead to significant cost savings when rearing fish inland.

Keywords: *Liza carinata,* salinity, growth, gonadal maturation

INTRODUCTION

Family Mugilidae is widely spread and representing an important species for cultivation in fish farms. *Liza carinata* commonly inhabit tropical and warm-temperate estuaries (Eisawy *et al*., 1974; McDowall, 1988; Blaber, 1997; Pombo *et al.*, 2005). Laffaille *et al.*, 1998, 2002; Torras *et al.*, 2000; Cardona, 2001; Almeida, 2003 indicated that mugilidae play a crucial ecological role where this fish community appears to be particulate organic matter transporter and could play a significant role in the global energy budgets of environment. Although this species always spawn at sea, they are highly euryhaline and thrive in a wide range of salinities (McDowall, 1988). Due to their euryhalinity, they are often stocked in brackish coastal lagoons to improve fisheries yields (Ravagnan, 1992) and are introduced into fresh water lakes and reservoirs to create new fisheries (Ben Tuvia *et al*., 1992). Growth of euryhaline species is often negatively affected by salinity because the energy used for osmoregulation is not available for growth (Brett, 1979; Wootton, 1990). Consequently, this species has an optimum salinity level at which the growth rate is highest and the cost of osmoregulation lowest, which may affect fish distribution in the wild (Blaber, 1997).

To determine the aquaculture potential of a species, a series of growth trials should be undertaken to help define the culture conditions for best growth (Deacon, 1997). Several interacting variables, such as temperature, light intensity, feeding intensity and feeding frequency can moderate fish growth (Brett, 1979). Salinity has been chosen for this study as it influences development and growth in marine fish (Iglesias *et al.*, 1987; Boeuf and Payan 2001 & Resley *et al*., 2006) through its effect on metabolic rate, food intake and food conversion ratio (FCR). For example, in a study testing a range of salinities on juvenile Atlantic croaker, *Micropogonias undulatus*, growth was improved at salinities below 35‰, with fastest growth at 5‰ (Peterson *et al*. 1999). Low salinity may reduce metabolic cost for homeostasis and ionic osmoregulation (Morgan and Iwama, 1996 & Woo and Kelly, 1999), for example in sea bream, *Sparus sarba*, and Atlantic cod, *Gadus morhua* (Woo and Kelly, 1999).

Several studies investigated the accumulatory response of marine fish species to salinity regimes lower than full-strength seawater. Those authors such as Lambert *et al*., (1994), Gaumet *et al*., (1995), Woo and Chung (1995) and Imsland *et al*., (2001& 2003) indicated to the growth rate of marine fish species was better at lower salinities than in full-strength seawater. The present study was conducted to compare growth and gonad maturation of *Liza carinata* at four different salinities throughout farming period.

MATERIAL AND METHODS

Liza carinata fry were collected from Suez Bay. They were transferred to the National Institute of Ocenography and Fisheries, Suez branch. The growth experiment was conducted in fish culture outdoor cement ponds (4X5X1.2) under controlled conditions and filled with water (20 m^3). Every day, about 15-20% of water content was replaced to maintain water quality. Fish in all ponds were fed about 5% of total fish weight on artificial diet contains 25% fish meal. Experimental salinities (5, 15, 25, and 40 ‰) in four ponds (triplicate design) were obtained by diluting sea water with dechlorinated tap water (by passing through active charcoal). Temperature, pH, salinity and dissolved oxygen (DO) concentrations were measured three times a week in all ponds. The dissolved oxygen was measured according to (Winkler, 1976). Salinity was adjusted using a Metter Toledo conductivity meter and the pH was measured using a Jenway 3505 pH meter. Samples for dissolved inorganic nutrient (Ammonia, Phosphate, Nitrate and Nitrite) analyses were collected weekly and analyzed immediately according to Parsons *et al*. (1984) using a Beckman DU-6 UV VIS spectrophotometer with blanks and standards.

Liza carinata fingerlings (25 fish/m^3) was used in this experiment with mean initial total length 4.3±0.7 cm; mean initial weight 0.6 ± 0.15g. At the end of the study, all fish were sacrificed to determine sex and maturity stage. Gonads were fixed in Bouin's solution for 24 hours and transferred to alcohol (70%). After conventional histological processing, sections of 5 μm thick were stained with haematoxylin and eosin and observed under a light microscope (Leica DM500). Assessment of gonadal development followed the description of Elhalfawy *et al*. (2007).

The condition factor (CF) was calculated using the formula: $CF=W/L^3 \times 100$
Where w = Total weight in g and L = total length in cm.

Length/weight relationship was calculated as W = a Lb, where a is the constant and b is the exponent of growth.

Gonadosomatic index (GSI) was calculated as GSI = G.wt / T.wt X 100

Where G. wt = gonad weight and T.wt. = Total weight

Where appropriate, one-way analysis of variance followed by Tukey's test was used, with significance level accepted at *P* = 5%.

RESULTS AND DISCUSSION

Chemical analysis

The physico-chemical variations of water in different salinities of fish ponds are shown in Table 1. The source of the dominant nutrient in the ponds was the fish food and possibly fish excretion. Ammonia-N was generally below 40 µg-at N/l and ranged between 14.06 and 35.85 µg-at N/l throughout the sampling period. Maximum allowable concentration of ammonia is 70 mg-at N/l for aquaculture water (Macdonald, 1994). Nitrate-N and nitrite –N concentrations in ponds did not exceed 5 and 2 µg-at N/l, respectively at any time during the study period. Phosphate-P concentrations were below 4 µg-at P/l and did not have so much fluctuation between ponds. The flow in the ponds was adjusted to control the concentrations of nutrients and minimize the eutrophication process (Krom and Van Rijn, 1989). This process which results from re-mineralization of detritus accumulated at the bottom of the ponds, it is often followed by unstable water quality that may retard fish growth and occasionally cause mass mortalities (Rimon and Shilo, 1982). After mentioned before, it can conclude that the water quality during the rearing period was suitable and had no harmful effect for all ponds.

Table 1: physico-chemical variations of water in different salinities of fish ponds.

Pond Parameter	Pond I 40 ‰	Pond II 25 ‰	Pond III 15 ‰	Pond IV 5 ‰
Temperature °C	23-31	23-31	22-32	23-31
pH	7.82-8.30	8.08-8.54	8.05-8.52	7.89-8.48
Dissolved Oxygen mg/l	6.55-7.65	6.00-6.68	7.77-8.55	7.53-7.83.
Ammonia µg-at/l	14.06-35.85	16.36-23.99	14.58-35.82	15.07-18.18
Phosphate µg-at/l	2.02-2.16	2.90-3.14	2.80-3.04	2.82-3.04
Nitrite µg-at/l	1.00-1.11	1.00-1.04	0.95-1.11	1.03-1.06
Nitrate µg-at/l	3.86-4.31	4.77-4.81	4.31-4.66	3.91-4.28

Growth

Average total weight and total length of the fish at the start of the study were 0.61 ± 0.19 g and 4.3 ± 0.54 cm, respectively. Salinity significantly affected growth of *Liza carinata* (Fig. 1). Fish reared at salinities 5 ‰ and 40 ‰ exhibited greater growth in length (cm) 10.77±0.097, 11.39±0.088 and weight (gm) 12.3±0.319, 15.02±0.44, respectively ($P<0.05$). While, fishes at salinities of 15 ‰ and 25 ‰ represented lower growth in length 9.15±0.23, 9.1±0.10 and weight 7.41±0.53, 7.83±0.29, respectively ($P>0.05$).

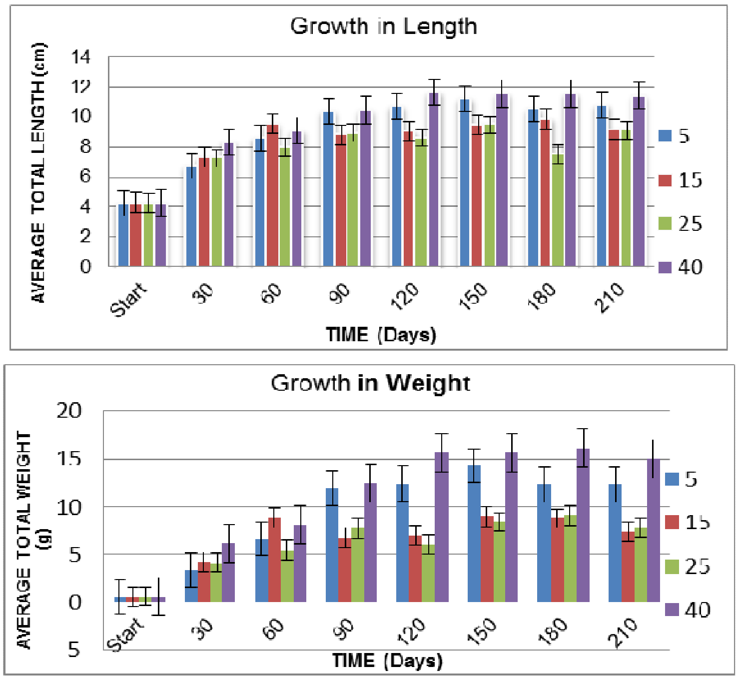

Fig. 1: Growth in total length (cm) and total weight (gm) of *Liza carinata* reared at four salinities degrees (5, 15, 25 and 40‰) for 210 days. The vertical bars represented 95% confidence intervals of average value per pond.

Our results demonstrated that the suitable salinities for growth in length and weight for *Liza carinata* was 40‰ and 5‰. Salinity of 40‰ represented the natural habitat of fish. While the low salinity (5‰) performed a considerable growth because of energy saving in osmoregulation process. This explanation was supported by Ferreira *et al*., 2008. This refers to the reduction of energy as the response of marine fish towards changes in salinity has not been consistent amongst species (Morgan and Iwama, 1996), the importance of salinity should be determined for each aquaculture species. Woo and Kelly (1999) and Boeuf and Payan (2001) suggested that at an iso-osmotic salinity of 15‰, growth is enhanced in stenohaline fish species, a group of marine species including silver kob. However, these effects are species-specific and need to be evaluated for each species or for fish from different origins. Euryhaline species can be reared at salinities below 35‰ due to their ability to move across salinity gradients (Deacon, 1997). Reduced salinity can decrease the energy demand for osmoregulation in some species and it may play a directing role for the growth of fish by improving their ability to digest and utilise food more efficiently (Resley *et al*., 2006). Turbot, *Scophthalmus maximus*, grew faster and their food conversion ratio improved when they were reared at salinities of 12–19‰ as compared to the 35‰ salinity of seawater (Lambert *et al*., 1994, Boeuf and Payan, 2001 & Imsland *et al*., 2001). Similarly, red drum, *Sciaenops ocellatus*, commercially produced under iso-osmotic conditions, grew better at a salinity of 11‰ than in seawater (Craig *et al*., 1995). In the stenohaline Atlantic cod, *Gadus morhua*, growth at 14‰ salinity was better than at 28‰ salinity (Lambert *et al*., 1994) and wild juvenile whitemouth croaker, *Micropogonias furnieri* (Sciaenidae), required relatively more metabolic energy at salinities above 20‰ when moving through a wide range of salinities (Aristizabal-Abud, 1992). Brown spotted grouper, *Epinephelus tauvina*, (Akatsu *et al*., 1983) and European seabass *Dicentrarchus labrax*, grew best at a salinity of

25‰ (Dendrinos and Thorpe, 1985). Ferreira *et al*., (2008) suggested that silver kob can be reared at this salinity (of 25‰) without negative effects on growth. Also, Saillant *et al*., (2003) who reported that sea bass growth is high at low salinities.

Condition Factor

At the end of the rearing period (210 day) the average condition factor did not differ among treatments over all ponds (Table 2). Condition factor was ranging from 0.93 to 1.03. Condition factor of fish was determined as this variable may reflect recent feeding conditions (Da Costa and Araujo, 2003). This has been suggested as a way to assess the health status of a fish species (Bolger and Connolly, 1989). Since there were no significant differences ($P<0.05$) in condition factor of fish that had been subjected to different salinity treatments, it is hypothesised that fish from all treatments consumed and utilized the artificial food equally well at all salinities.

Table 2: Condition factors (K) of *Liza carinata*, reared at different salinities for 210 days. Data are means±SE.

Pond salinity (‰)	Number of fish	K (g/cm^3)
5	44	0.98±0.016
15	25	0.93±0.012
25	36	1.03±0.011
40	25	1.01±0.011

Gonadosomatic Index (GSI)

It is obvious that GSI differs among treatments for male and female *Liza carinata*. Gonadosomatic index was significantly influenced by salinity ($P<0.05$). The GSI for both males and females have nearly the same fluctuation trends which reached to the maximum level at salinity of 40 ‰ for both sexes 8.9 for female and 2.1 for male. The variation of GSI is generally used to indicate the maturity stages and spawning of the fish (Fig. 2). When GSI reaches its maximum value, this gives a perfect indication about the time of spawning. Variations in GSI values were previously detected by Salem and Mohamed (1982) in lake Timsah, El-Boray (1993) in Suez bay, Mahmoud (1997) and El-Halfawy (2004) in different fish farms.

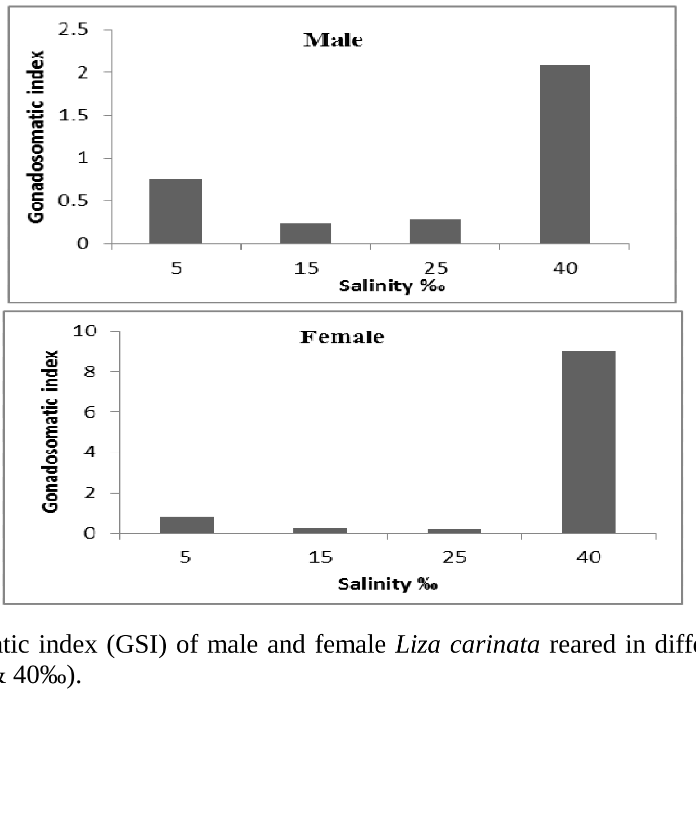

Fig. 2: Gonad somatic index (GSI) of male and female *Liza carinata* reared in different saline waters (5,15, 25 & 40‰).

Length-weight relationship

Length–weight relationships of fishes are crucial in the fisheries biology and assessment .The sample size, minimum and maximum length and weight, parameters of length-weight relationship (a&b) and coefficient of determination R^2, $\pm$ 95%CI of "b", values are shown in Table (3) and Figure (3). From this table it is evident that highest coefficient "b" were 2.839, 2.847 for fishes at salinities 40 ‰ and 5 ‰, respectively they closed to 3 it is indicated that isometric growth, while "b" less than 3 at salinities 25 ‰ and 15 ‰, values of "b" were 1.512 and 2.323, respectively that is allometric growth. It is well known that the function of "b" value represents the body form and it is directly related to the weight affected by ecological factors ,such as temperature, food supply, spawning conditions and other factors, such as sex, age, fishing time and area and fishing vessel (Ricker,1973). In this experiment we established all factors only salinity was change, so may be change in "b" value due to change in salinity and in range of total length and weight between ponds. This result coincides with Salem and Mohamed (1982) and Mahmoud (1997) as they found variations for b values of length weight relationship with different source of water salinity. Also, Sampaio *et al*., 2001, found that the values of b for the length weight relationship of Brazilian flounder were always near 3 which reared at different salinities.

Table 3: Length weight relationship of Liza carinata, at different salinities during 210 day.

Pond parameter	Log T.L Min - Max	Log T.wt Min - Max	Regression Ln a	b	S.E of" b"	95%of CI	R^2
Pond I 40‰	7.3 - 12.8cm	3.85-21.71gm	-1.8207	2.839	0.062	2.714-2.964	0.958
Pond II 25‰	4.7-11.3cm	7.9-12.33gm	-0.577	1.512	0.181	1.152-1.872	0.410
Pond III 15‰	4.5-11.5cm	0.91-14.11gm	-1.369	2.323	0.119	2.086-2.560	0.800
Pond IV 5‰	5.9-12.4cm	2.3-18.14 gm	-1.844	2.847	0.042	2.764-2.930	0.975

n= number of sample, Min=minimum, Max= maximum, a= intercept of regression line, b= slope of regression line, S.E = Standard Error, Cl= confidence level, R = regression coefficient.

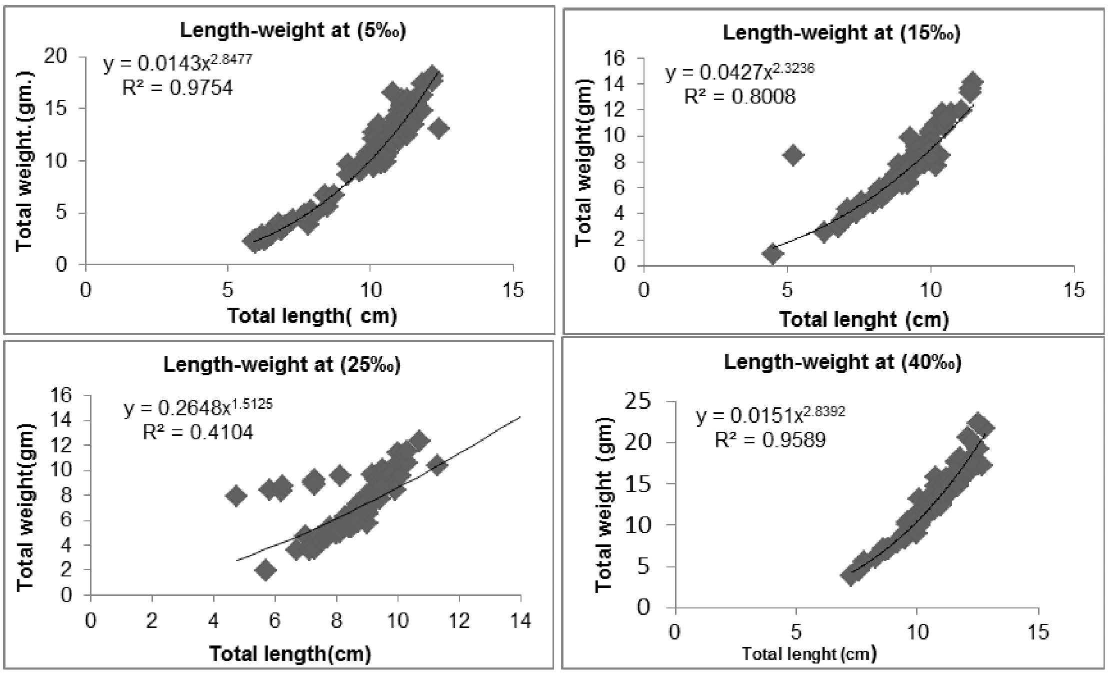

Fig. 3: Length-weight relationship of *Liza carinata* reared in different salinities.

Histological features of gonad maturation

The female:

1- Perinucleolar stage (Pond III, 15 ‰):

At the beginning of this stage, the oocytes are small and spherical or oval in shape. The cytoplasm increase and becomes greater in volume than the nucleus. Oocytes by the end of this stage vary between tetragonal and polygonal in shape. The nucleoli move to the peripheral position adjacent to the nuclear membrane (Table 4 & Fig. 4 A).

2- Yolk vesicle stage (Pond IV, 5‰ & Pond II, 25‰):

The oocytes were characterized by the appearance of yolk vesicles. As the oocytes develop, the yolk vesicles increase in number and size, gradually filling the cytoplasm from the periphery of the oocyte toward the center and arranged in raws (Table 4 & Fig. 4 B).

3- Ripe stage (Pond I, 40 ‰):

The yolk globules first appear in the outermost part of the ooplasm and gradually increased in number and size as the oocyte grows and the yolk layer continues to become thicker in proportion to the adjacent layer of the cytoplasm. Finally, the larger yolk spheres are apparently formed by the fusion of smaller yolk globules. Moreover, numerous lipid droplets remain in the peripheral part of the yolk mass (Fig. 4 C).

The male:

1- Immature stage (Pond III, 15 ‰):

The completely immature or infantile testes consist of small closely packed cysts or nests of spermatogonia with different diameters. The testes were separated into lobules. The spermatogonia were rounded in shape and varied in number (Table 4 & Figure 5 A).

2- Mature stage (Pond II, 25 ‰):

This stage was characterized by the predominance of spermatozoa overall the earlier cell stages (spermatogonia, primary spermatocytes, secondary spermatocytes and spermatids) present in the lobules. The lumina of the lobules contained a fair amount of sperms and the spermatic duct was full of sperms (Table 4 & Fig. 5 B).

3- Ripe stage (Pond IV, 5 ‰ & Pond I, 40 ‰):

At this stage the lobules and ducts were packed with mature spermatozoa, but the earlier germ cells were fewer and embedded in the lobule walls. The tunica albuginea appeared thin and stretched and the lobules distended to their maximum diameter, and their walls also become thin and stretched. In addition, the seminiferous lobules were separated by thin layer of interlobular connective tissue (Table 4 & Fig. 5 C).

Table 4: Maturity stages of reared *Liza carinata* in different salinities at the end of experiment.

	Stage I		**Stage II**		**Stage III**		**Stage IV**	
	♀	♂	♀	♂	♀	♂	♀	♂
5 ‰					+	+		+
15 ‰		+	+					
25 ‰			+		+	+		
40 ‰			+		+		+	+

It can be concluded that, the maximum gonadal maturation at salinity 40‰ (represents the natural environment of this fish) reached to ripe stage for male and female. Gonadal maturation of salinity 5‰ reached to ripe stage for male and yolk vesicle stage for female (may be attributed to save energy in osmoregulation process).

Many authors have reported the influence of water salinity on fish development. In Mullet (*Mugil sp.*) larvae, growth was estimated at 3 to 24 ‰ and the best results in terms of weight increase were recorded 17‰ (Peterson *et al*., 1999).

There is a wide range of results, but no definitive explanation of the reasons leading to higher or lower growth in different salinities. This conclusion coincides with several studies reported the effects of salinity on growth of fishes (Sampaio *et al*., 2001). It has been proposed that high rates of protein turnover can result in reduced growth, which can be detected by higher nitrogen excretion rates (Carter *et al*. 1998).

Thus, in marine fishes like as sea bass larval performances appear to be significantly improved under isotonic conditions, as has also been reported in the rearing of other marine species such as *Sparus aurata* (Tandler *et al*., 1995) and *Mugil cephalus* (Hare *et al*., 1998). The decreased performances recorded at high salinity during early development may be linked to limits in the osmoregulation capacity during early larval development.

The effects of salinity on growth are complex, vary among species and are not readily predicted (Iwama, 1996). While it is widely accepted that rearing of fish near their iso-osmotic point has an energy saving effect (Gaumet *et al*., 1995; Boeuf and Payan, 2001), few studies have addressed the effects of increased salinity on growth in true freshwater species. In marine fish, decreasing the salinity towards iso-osmolality often increases growth, which is commonly explained by a reduction in energy expenditure associated with ion regulation (Brett, 1979; Jobling, 1995).

CONCLUSION

It can be concluded that *Liza carinata* could be reared in low saline conditions (5‰). It performs the maximum gonadal maturation at salinity 40‰ (represents the natural environment of this fish) reached to ripe stage for male and female. At salinity 5‰, gonadal maturation reached to ripe stage for male and yolk vesicle stage for female (may be attributed to the saving of energy in osmoregulation process).

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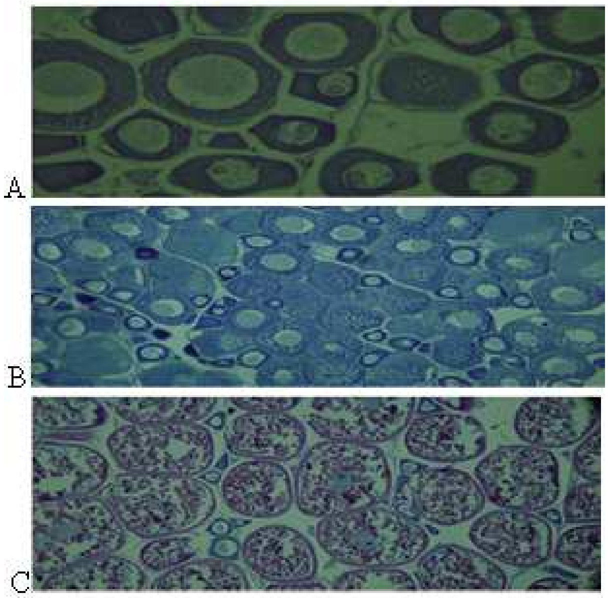

Fig. 4: Photomicrographs of transverse sections of the ovary (at maximum maturation stage) of *Liza carinata* reared in different water salinities:

A- Perinucleolar stage (15%). H. & E., X 80.

B- Yolk vesicle stage (5% & 25 %). H. & E., X 20.

C- Ripe stage (40 %). H. & E., X 40.

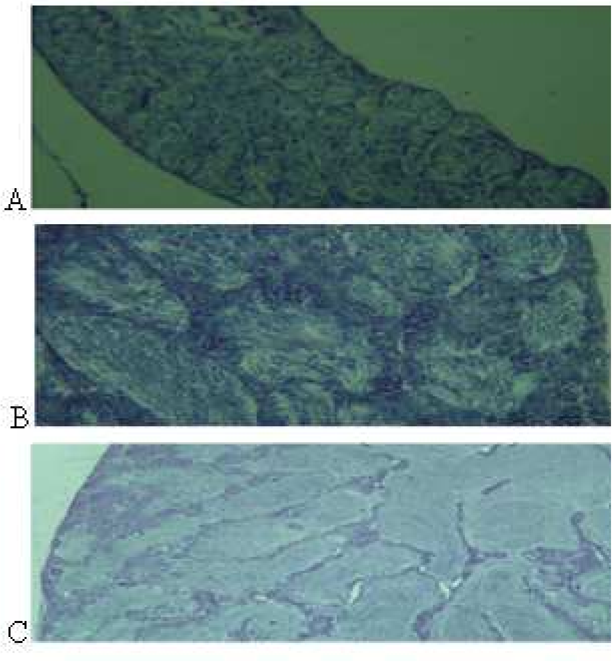

Fig. 5: Photomicrographs of transverse sections of the testis (at maximum maturation stage) of *Liza carinata* reared in different water salinities:

A- Immature stage (15%). H. & E., X 100.

B- Mature stage (25 %). H. & E., X 80.

C- Ripe stage (5% & 40 %). H. & E., X 40.

Arabic Summary

نمو ونضج المناسل لسمكة السهلية المستزرعة فى درجات الملوحة المختلفة

كمال فتحى البرعى - مجدى مصطفى الحلفاوى - وحيد فرج الله محمود - أمل محمد أمين
أمل محمد رمضان - ويسرى عبد العزيز سليمان
المعهد القومى لعلوم البحار والمصايد – فرع خليجى السويس والعقبة

تم أجراء تجربة استزراع سمكة السهلية وتأثير الملوحة عليها فى أحواض أسمنتية حجمها (4 X5 X1,2م) يحتوى كل منها على 20 متر مكعب مياة منزوعة الكلور، وأستمرت التجربة لمدة 210 يوم، حيث يتم يوميا إستبدال حوالى مايقرب من 15 الى 20 % من مياه الاستزراع للمحافظة على جودة المياه لتظل نتائج تحاليلها الكيميائية والطبيعية فى الحدود المسموح بها. تمت التغذية فى جميع أحواض التجربة على علائق أسماك صناعية تحتوى على نسبة 25 % بروتين وبنسبة 5 % من الوزن الكلى للأسماك. كانت درجات الملوحة محل الدراسة هى 5، 15، 25 و 40 ‰ وذلك من خلال 12 حوض بمعدل ثلاثة أحواض لكل درجة ملوحة. وقد سجلت الأسماك المرباة على درجة ملوحة 5 و 40 ‰ أعلى نمو فى الطول (10,77 سم ± 0,097 و 11,39 سم ± 0,088) وكذلك الوزن (12,3 جم ± 0,319 و 15,02 جم ± 0,44) على التوالى بفرق جوهرى (P<0.05). أما الاسماك المرباة فى ملوحة 15 و 25 ‰ فقد حققت أقل نمو فى الطول (9,15 سم ± 0,23 و 9,1 سم ±0,1) بينما سجل الوزن (7,41 جم ± 0,53 و 7,83 جم ± 0,29) على التوالى وبفرق غير جوهرى (P>0.05) ووجد أن معامل الحالة لايتأثر بشكل جوهرى بإختلاف درجات الملوحة حيث تراوحت بين 0,93 و1,03. وبالنسبة لدليل المناسل فقد تأثر بشكل معنوى مع أختلاف درجات الملوحة حيث وجد أنه بالنسبة للذكور والاناث لهم نفس التغيرات حيث وصلت لأعلى مستوى عند ملوحة 40 ‰ 8,9 للإناث و 2,1 للذكور. كما وجد أيضا أن النضج الجنسى عند درجة ملوحة 5 ‰ وصل الى مرحلة إكتمال النضج للذكور ومرحلة حويصلات المح للإناث، بينما عند درجة الملوحة 40 ‰ وصل إلى مرحلة إكتمال النضج لكلا الجنسين. تلك النتائج ترشح إستزراع سمكة السهلية فى المياة العسر قليلة الملوحة وهذا يؤدى الى توفير نفقات إستزراعها.