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Effect of Live Food Enrichment and Temperature on Growth performance Survival and Digestive Tract Development of Grey Mullet, *Liza ramada* Larvae

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ABSTRACT

In aquaculture, larviculture is one of the main bottlenecks in upscaling the production of fish. This is the initial stage whereby fish larvae start feeding. Poor survival rates of the wild mullet larvae is a limiting factor in mullet production and many investigations are needed to determine environmental and nutritional requirements of mullet fish specially in the period of larvae culture. This study was conducted to investigate the effect of different combinations between enriched and unenriched live food (rotifer and artemia), and temperature on grey mullet, *Liza ramada* larvae. Also, the effects of a combination between them on the development of the digestive tract of the larvae is also investigated. In a 2× 2 factorial design study. Mullet larvae, *Liza ramada*, were collected from the Mediterranean Sea at Max bay, Alexandria Egypt. The experiment was carried out in 12 glass aquaria (30 x 40 x 100 cm) containing 30 liters of brackish water (10 ppm) that was prepared by mixing the filtered sea water with fresh water. 40 fish were stocked in each glass aquaria. The 12 glass aquaria was divided into 4 groups. (Three replicates for each treatment). Survival, growth parameters and histological examination of *Liza ramada* were evaluated. The results showed that enriched live food (rotifer and artemia) improved the larvae survival and the growth parameters at temperature 24°c under the experimental conditions of this study. Digestive tract in this stage have few of goblet cells. After feeding with artemia the number of goblet cells and the villi increased more than after feeding with rotifer. The enriched live food improved the histological profiles of osophagus, intestine and liver. Live foods are still the main feed item in commercial larvie culture with enriching them by adding HUFA in order to complete their natural nutritional composition to be suitable for feeding younger larvae. Using the elevated temperature and enriching live feed by HUFA in the present study are useful improving survival,growth and histological structure of digestive tract of *Liza ramada* larvae.

Keywords: live food enrichment, digestive tract, growth parameters, *Liza ramada*.

INTRODUCTION

Mariculture of finfish presents many obstacles, one obstacle is that fish must food regularly or they will die. Fish mortality increase during the onset of exogenous feeding called first feeding, and metamorphosis. During first feeding, food must be small enough for the larval fish to fit its mouth. Also, because larval fish cannot fully digest food, they must take up nutrients, such as free amino acids (FAA), which are usually released when the food is chewed (Sedlacek, 2008). The live food provides the larvae with essential enzymes to digest food, since at this stage, the digestive system of the fish larvae are still under-development. Live foods are, therefore, an

important food source in the early larval stages (Chew and Lim, 2006).

Poor survival rates of the wild mullet larvae is a limiting factor in mullet production and many investigations are needed to determine environmental and nutritional requirements of mullet fish specially in the period of larvae culture (El-Dahhar 1999).

The larvae dietary requirement for essential fatty acids is an important step in the development of marine fish culture for any species (Izquierdo, 1996; Takeuchi, 1997). Eicosapentaenoic acid (20:5 n-3, EPA), docosahexaenoic acid (22:6 n-3, DHA), and arachidonic acid (20:4 n-6, ARA) are all considered essential for marine fish and must be provided in the diet (Bransden *et al.*, 2005).However, the larvae fed on rotifers and artemia containing a low level of n-3 HUFA showed poor growth, high mortality and high rate of deformity. Enrichment of live food for marine fish larvae with n-3 HUFA has been shown to improve growth and survival by many researchers (Rippingale and Payne 2001; Copman *et al*., 2002; Koven 2003 and Mourad 2009).

Several enrichment techniques have been developed, including microalgae, oil-based emulsions and microencapsulated preparations (Barclay and Zeller, 1996 and McEvoy *et al.*, 1996). However, most commercially available enrichment preparations are unable to provide a DHA/EPA ratio > 1 in the enriched *Artimia* (Navarro *et al.*, 1995 and McEvoy *et al.*, 1996); thus, the efficacy of this live prey to deliver sufficient DHA to the developing larvae remains a matter of concern.

The histology of the digestive system and larval development of many fish specimens have been investigated (Hernāndez *et al.*, 2009; Ragi and Norouzi, 2010 and El-Bakary and El-Gammal, 2010). At first-feeding the digestive system is still rudimentary, lacking a stomach, and much of the protein digestion takes place in hindgut epithelial cells (Koven *et al.*, 2001). Such a digestive system is in most cases incapable of processing formulated diets in a manner that allows survival and growth of the larvae comparable to those fed live feeds. In fact, despite recent progress in the development of inert diets for fish larvae (Lazo *et al.*, 2000; Cahu and Infante 2001and Koven *et al.*, 2001), feeding of most species of interest for aquaculture still relies on live feeds during the early life stages. Even the 'artemia replacement' products increasingly used in commercial operations is normally used in co-feeding with live feeds (Curnow *et al.*, 2006; Vega- Orellana *et al.*, 2006; Hamza *et al.*, 2007; Rosenlund *et al.*, 2007).

The present study was conducted to investigate the effect of different combinations between live foods (rotifer and artemia) enrichment, and temperature on grey mullet, *Liza ramada* larvae regarding growth performance parameters ,nutrient,utilization ,fish carcass composition and the development of the digestive tract of the larvae.

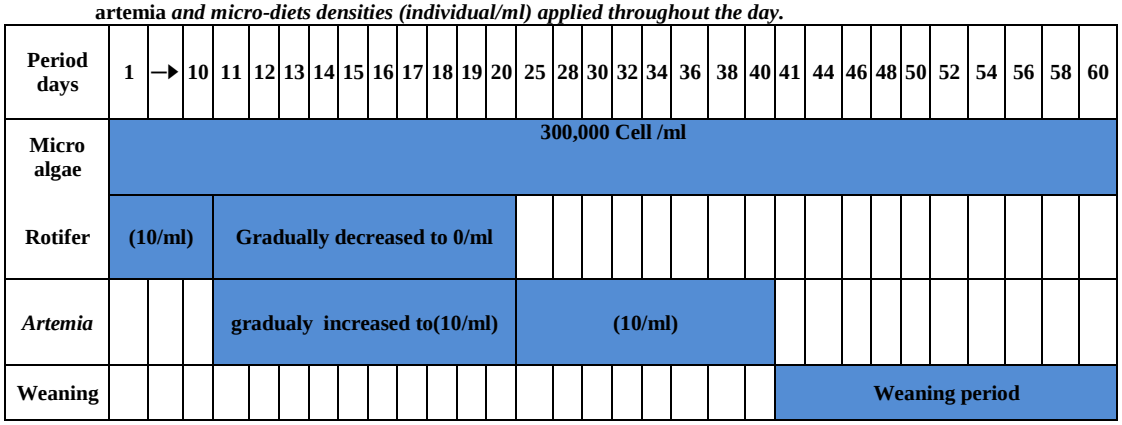
MATERIALS AND METHODS

The present study was carried out in the Marine Fish Laboratory (MFL), Faculty of Agriculture, Saba Bacha, Alexandria University, Egypt. Mullet larvae, *Liza ramada*, (Risso,1826) were collected from the Mediterranean Sea at Max bay, Alexandria Egypt.

Experimental Design

Experiment of the present study was carried out in 12 glass aquaria (30 x 40 x 100 cm) containing 30 liters of brackish water (10 ppm) that was prepared by mixing the filtered sea water with fresh water. Fourty fish were stocked in each glass aquaria. The glass aquaria were divided into 4 groups (three replicates for each treatment). Temperature and feeding were changed according to the following scheme (Figure 1) in a 2× 2 factorial design (Table 1).

Fig 1*: Feeding schedule adopted for larval rearing until 60 days from experiment onset showing microalgae, rotifers,* **artemia** *and micro-diets densities (individual/ml) applied throughout the day.*



Enrichment of Rotifer with Fatty Acid

Rotifer enrichment has been applied in buckets as one litre sea water with the selected medium of each treatment was mixed in feed mixer for about 15 minutes by supplying it by one air stone it has been ready to receive the rotifer. Emulsification of the fish oil was done by adding (100% wet weight) 50% fish oil, 49.3 % warm water and 0.7% emulsifier (Lecithin).

Enrichment of artemia with Fatty Acid

Newly hatched nauplii were transferred to the enrichment tank at a density of 30–50 nauplii / ml. The enrichment medium consists of 1.5 ml fish oil emulsified with 50 ml neutralized seawater maintained at 25 °C using 0.1g phosphatedyl choline (Lecithin). The enrichment emulsion is added in consecutive doses of 0.3 g / l every 12h with strong aeration using air stones to maintain dissolved oxygen levels above 4 ppm. Enriched nauplii were harvested after 24 h HUFAs are obtained after 24-h enrichment with the emulsified concentrates. Nauplii should be transferred or exposed to the enrichment medium as soon as possible before first feeding, so they begin feeding immediately after the opening of the alimentary tract (instar II stage). As a result, the increase of nauplius size during enrichment can be minimized.

Weaning Diet

The ingredients of the weaning diet provided to the fish larvae after 40 days from the onset of the experiment are demonstrated in Table (2).

Table 1 ***Scheme of temperature and feeding regime of the fish larvae.***

treatment	**Temperature °C**	**Feeding**
1	Room temp (19±1°C)	Rotifer (10 R/ml) not enriched
2	Room temp (19±1°C)	Rotifer (10 R/ml) enriched
3	Elevated temp (24±1°C)	Rotifer (10 R/ml) not enriched
4	Elevated temp (24±1°C)	Rotifer (10 R/ml) enriched
After the first 10 days, the rotifer was replaced by artemia gradually in a rate of declining 1 rotifer/day. According to that, the rotifer was completely replaced by artemia after 10 days. The feeding with artemia continued for 20 days as follows:		
1	Room temp (19±1°C)	artemia (10 A/ml) not enriched
2	Room temp (19±1°C)	artemia (10 A/ml) enriched
3	Elevated temp (24±1°C)	artemia (10 A/ml) not enriched
4	Elevated temp (24±1°C)	artemia (10 A/ml) enriched
Weaning diet for 2o days		

Table 2: ***The composition and chemical analysis of the experimental diet used during the study to feed Liza ramada larvae***

Ingredients	Content %
Wheat lour	50.7
Powder milk	12.0
Boiled eggs	17.0
Fish meal	15.0
Fish oil	1.0
Vitamin	0.9
Ascorbic Acid	0.4
CMC	3.0
Chemical analysis	
Moisture	12.0
Crude protein	27.6
Crude lipid	8.76
Crude fiber	00.0
Carbohydrate	44.94
Ash	6.7

The initial body weight recorded at the beginning of the experiment ranged from 0.03 to 0.05 g. At the end of the experiment, samples of 30 fish larvae were randomly collected from each group to be analyzed. Other samples were collected after the completion of each feeding regime period and were preserved in formal saline for histological investigation**.**

Histological studies

After each feeding regime period, three fish larvae were collected from each aquarium for histological investigation to study the histological changes in the digestive tract during development. Fry specimens were collected in the different treatments and were treated by passing through the processes of fixation, dehydration, clearing, embedding and staining as follows:

1. Fixation

The Purpose of this process is to preserve the structures of the tissues and prepare them for the subsequent treatments. Formalin (4%) was used as a good fixative.

1.1. Post fixation and dehydration

Post fixation is necessary for the removal of the fixative to facilitate sectioning. The specimens were washed in 70% ethyl alcohol to remove the formalin, and then were dehydrated by passing through graded series of ethyl alcohol (70%, 80%, 90% and 100%) for 20 to 30 minutes each.

1.2. Clearing and embedding

The dehydrated specimens were cleared in cedar oil overnight or more (cedar oil considered as preservative medium), placed in two changes of benzene (10-15 minute for each change). After that, the process of embedding was applied by passing the specimens through successive series of benzene: Paraffin 2:1, benzene: Paraffin 1:1, then pure paraffin with three changes in oven adjusted at 60°C (melting point of paraffin wax; 58- 65°C). After embedding the specimens were supported by paraffin block and then transversely cut at thickness of 4-5 µ.

1.3. Staining

The sections were stained with Haematoxyline and Eosin as follows:

1.4. Procedures of haematoxyline and Eosin

After the hydration of sections they were placed for 10 minutes or longer in xylene until all the wax is removed or using two changes to insure the removal of paraffin.

1- Absolute alcohol 10 minutes.
2- 95% Ethyl alcohol 10 minutes.
3- 80% Ethyl alcohol 10 minutes.
4- 70% Ethyl alcohol 10 minutes.
5- Wash rapidly in distilled water.
6- Stain in haematoxyline 20 minutes.
7- Differentiation in running water under microscope. At this stage the nuclei are Purple, other parts are grayish.
8- Clean in acid alcohol, (70% eathyl alcohol + drop of hydrochloric acid), one drop.
9- Stopped differentiation by washing off acid alcohol in running water for 5 minutes.
10- Wash rapidly in distilled water.
11- Run slides up to 70% eathyl alcohol 10 minutes for more dibs.

12- Stain in Eosin + 70% eathyl alcohol 10 minutes or more.
13- Differentiation in running water under microscope. At this stage the cytoplasm are pink, other are purple.
14- 95% Ethyl alcohol few dips for 5 minutes.
15- Absolute alcohol for 10 minutes two changes.

Xylene two changes every one for 20 minutes mounting medium keep sections moist with xylene during process; they must not dry add cover glass and Canada Balsam.

Statistical Analysis

Statistical analysis of the experimental results was conducted according to SPSS (version16.00). Duncan's (1955) multiple range tests were carried out to test the significance levels among means of treatments 2× 2 factorial design.

RESULTS

Survival

After feeding with rotifer, temperature increased from19°C to 24°C) significantly (p<0.05) led to increase of the larvae survival rate enriched rotifer combined with elevated temperature increased the survival but the difference was not significant (p>0.05). After feeding with artemia, the normal temperature showed a significant increase survival rate compared to the elevated temperatur (p<0.05). The enriched artemia increased the survival rate significantly difference (p<0.05). Enriched artemia combined with elevated temperature increased the survival rate with significant (p<0.05). After weaning, the survival rate improved with elevated temperature and enriched live food without significantly differences (p>0.05) (Table 3).

After the whole experiment, the survival rate insignificantly improved with normal temperature (p>0.05). Feeding fish on enriched live food until the end of the whole experiment increased the survival rate significantly (p<0.05).

Growth after live food

After feeding with live food, the highest significance (p<0.05) FBW, ADG, WG were recorded in the fry fed enriched live food compared to there fed the enriched. Elevated temperature combined with enrichment diet increased FBW, ADG, WG rate without significantly diffences (p>0.05).

Growth after weaning

After weaning, the highest FBW, WG, ADG were recorded in the fry fed enrichment

Table 3: ***Means ± SE of survival rate (%) of grey mullet, Liza ramada, fry after live food stage, weaning stage and the whole of experiment.***

Treatments		**Survival of *liza ramada* larvae (%)**			
Temperature	**Live Food**	**After feeding Rotifer**	**After feeding artemia**	**After Weaning**	**whole Experiment**
(19±1ºC)	**N**	74.17±2.36	96.67^{a}±2.36	84.91±4.07	60.00±2.70
	E	81.67±2.12	98.93^{a}±0.76	88.48±3.03	72.50±4.68
(24±1 ºC)	**N**	84.17±2.95	59.88^{b}±5.96	87.50±8.84	49.17±8.56
	E	90.83±2.12	100.00^{a}±0.00	87.72±2.60	72.08±2.06
Pooled means					
(19±1ºC)		77.92^{h}±3.70	97.71^{g}±2.33	86.69±4.67	66.25±6.241
(24±1ºC)		87.50^{g}±3.87	79.94^{h}±13.76	87.61±8.24	60.63±10.70
	N	79.17±4.62	78.27^{y}±12.97	86.21±8.74	54.59^{y}±8.73
	E	86.25±3.95	99.46^{x}±0.76	88.09±3.57	72.29^{x}±4.57

Means with different superscript are significantly different (P<0.05).N:normal live food ,E:enriched live food

Table 4: ***Means ± SE of initial body weight (g/fish), final body weight (g/fish), weight gain (g/fish), average daily gain (ADG, g/fish/day) and specific growth rate (SGR, %/day) of grey mullet, Liza ramada fry fed live food (rotifer) normaly or enriched with fish oil.***

Treatment						
Temperature	**Live Food**	**Initial weight**	**Final weight**	**Weight gain**	**ADG**	**SGR**
19±1°C	**N**	0.0420±0.0039	0.173±0.005	0.131±0.004	0.0033±0.0002	3.58±0.19
	E	0.0477±0.0038	0.187±0.019	0.139±0.023	0.0037±0.0005	3.40±0.45
24±1°C	**N**	0.0537±0.0005	0.163±0.002	0.110±0.003	0.0030±0.0000	2.78±0.05
	E	0.0483±0.0040	0.227±0.012	0.178±0.012	0.0047±0.0002	3.89±0.23
Pooled means						
19±1°C		0.0448±0.0052	0.180±0.018	0.135±0.021	0.0035±0.0005	3.49±0.44
24±1°C		0.0510±0.0040	0.195±0.023	0.144±0.024	0.0038±0.0006	3.33±0.41
	N	0.0478±0.0054	0.168^{y}±0.006	0.121^{y}±0.008	0.0032^{y}±0.0002	3.18±0.31
	E	0.0480±0.0051	0.207^{x}±0.024	0.159^{x}±0.026	0.0042^{x}±0.0006	3.64±0.48

Means with different superscript are significantly different (P<0.05) N:normal live food ,E:enriched live food

with rotifer and artimia for 40 day then weaned using the prepared diet for 20 day compared to the larvae that was not enriched with significance (***p***<0.05).. There was an increase significantly (***p***<0.05). recorded in elevated temperature combined with enrichment diet (Table 4).

SGR increased in the fry fed enriched live food and weaning diet without significantly (***p***>0.05), there was significanly increase SGR with higher temperature. There was an increase SGR elevated temperature combined with enrichment (Tables 5 and 6).

FCR improved significantly (p<0.05) with enriched live food and weaning diet. According to the effect of temperature, there was a significant (p<0.05); lower FCR was recorded with elevated temperature combined with enrichment diet (Table 7).

The highest (***p***<0.05) (PER), (PPV %),(EU), were recorded in the fry fed enriched live food and weaning diet.There was an increase (***p***<0.05) recorded with elevated temperature combined with enrichment diet (Table 9).

Table 5: ***Means ± SE of initial body weight (g/fish), final body weight (g/fish), weight gain (g/fish), average daily gain (ADG, g/fish/day) and specific growth rate (SGR, %/day) of grey mullet, Liza ramada, fry fed artimia with or without enrichment for 40 day then weaned using micro diet for 20 days.***

Treatment						
Temperature	**Live Food**	**Initial weight**	**Final weight**	**Weight gain**	**ADG**	**SGR**
19±1°C	**N**	0.173 ±0.005	0.62^{c} ±0.05	0.45^{c} ±0.06	0.014^{c}±0.002	3.97 ±0.33
	E	0.187 ±0.019	0.72^{bc}±0.03	0.54^{bc}±0.01	0.017^{bc}±0.000	4.28 ±0.17
24±1°C	**N**	0.146 ±0.010	0.82^{b} ±0.06	0.68^{b} ±0.07	0.021^{b}±0.002	5.39 ±0.43
	E	0.227 ±0.013	1.10^{a} ±0.08	0.87^{a} ±0.10	0.027^{a}±0.003	4.91 ±0.42
Pooled means						
19±1°C		0.180 ±0.018	0.67^{h} ±0.06	0.49^{h} ±0.06	0.015^{h} ±0.002	4.12^{h} ±0.35
24±1°C		0.186 ±0.029	0.96^{g} ±0.13	0.77^{g} ±0.12	0.024^{g} ±0.004	5.15^{g} ±0.55
	N	0.160 ±0.013	0.72^{y} ±0.10	0.56^{y} ±0.11	0.018^{y} ±0.003	4.68 ±0.66
	E	0.207 ±0.024	0.91^{x} ±0.14	0.70^{x} ±0.14	0.022^{x} ±0.004	4.59 ±0.45

Means with different superscript are significantly different (P<0.05) N:normal live food ,E:enriched live food)

Table 6*: Means ± SE of initial body weight (g/fish), final body weight (g/fish), weight gain (g/fish), average daily gain (ADG, g/fish/day) and specific growth rate (SGR, %/day) of grey mullet, Liza ramada, fry for the whole experiment.*

Treatment		Initial weight	Final weight	Weight gain	ADG	SGR
Temperature	**Live Food**					
19±1°C	**N**	0.042 ±0.004	0.62^{c} ±0.05	0.58 ^{c} ±0.05	0.008 ^{c} ±0.001	3.75 ±0.21
	E	0.048 ±0.004	0.72 ^{bc}±0.03	0.68 ^{bc}±0.04	0.009 ^{bc}±0.000	3.79 ±0.18
24±1°C	**N**	0.054 ±0.000	0.82 ^{b} ±0.06	0.77 ^{b} ±0.06	0.011 ^{b}±0.001	3.77 ±0.11
	E	0.038 ±0.006	1.10 ^{a} ±0.08	1.06 ^{a} ±0.08	0.015 ^{a}±0.001	4.70 ±0.13
Pooled means						
19±1°C		0.045 ±0.005	0.67^{h} ±0.06	0.63^{h} ±0.06	0.009^{h} ±0.001	3.77 ±0.25
24±1°C		0.046 ±0.007	0.96^{g} ±0.13	0.91^{g} ±0.13	0.013^{g} ±0.002	4.24 ±0.33
	N	0.048 ±0.005	0.72 ^{y} ±0.10	0.67 ^{y} ±0.09	0.009 ^{y} ±0.001	3.76 ±0.21
	E	0.043 ±0.007	0.91^{x} ±0.14	0.87 ^{x} ±0.14	0.012 ^{x} ±0.002	4.25 ±0.35

Means with different superscript are significantly different (P<0.05) N:normal live food ,E:enriched live food

Table 7: ***Means ± SE of total feed intake (g), Weight gain (g) and feed conversion ratio (FCR), of grey mullet, Liza ramada, fry after the end of experiment.***

Treatment		Total feed intake	Weight gain	FCR
Temperature	**Live Food**			
19±1°C	**N**	1.65 ±0.10	0.45^{c} ±0.06	3.67 ^{c} ±0.56
	E	1.39 ±0.03	0.54 ^{bc} ±0.01	2.57 ^{bc} ±0.12
24±1°C	**N**	1.78 ±0.25	0.68 ^{b} ±0.07	2.62 ^{b} ±0.12
	E	1.75 ±0.07	0.87 ^{a} ±0.10	2.01 ^{a} ±0.17
Pooled means				
19±1°C		1.52 ±0.12	0.49^{h} ±0.06	3.26 ^{h} ±0.67
24±1°C		1.77 ±0.23	0.77^{g} ±0.12	2.34 ^{g} ±0.24
	N	1.72 ±0.24	0.56 ^{y} ±0.11	3.26 ^{y} ±0.67
	E	1.57 ±0.14	0.70^{x} ±0.14	2.34 ^{x} ±0.25

Means with different superscript are significantly different (P<0.05) N:normal live food ,E:enriched live food)

Table 8: ***Means ± SE of dry matter (%), protein (%), ash (%) and fat content (%) in the carcass of grey mullet, Liza ramada, at the end of the experiment.***

Treatment		Dry matter	Protein	Ash	Fat
Temperature	**Live Feed**				
19±1°C	**N**	34.30 ±0.33	15.10 ±1.02	4.30 ±0.06	14.45 ±0.19
	E	34.91 ±0.90	15.91 ±0.95	4.55 ±0.14	14.90 ±0.74
24±1°C	**N**	34.48 ±0.05	18.19 ±0.60	4.47 ±0.04	11.81 ±0.63
	E	36.77 ±1.97	19.72 ±0.54	4.64 ±0.24	12.42 ±1.53
Pooled means					
19±1°C		34.61 ±0.88	15.50 ±1.27 ^{h}	4.43 ±0.15	14.68 ±0.70
24±1°C		35.62 ±1.91	18.95±0.87 ^{g}	4.55 ±0.22	12.12 ±1.49
	N	34.39 ±0.30	16.65 ±1.44	4.39 ±0.08	13.36 ±1.31
	E	35.84 ±2.03	17.81 ±1.55	4.59 ±0.25	13.44 ±1.52

Means with different superscript are significantly different (P<0.05) N:normal live food ,E:enriched live food)

Table 9: ***Means ± SE of protein efficiency ratio (PER), protein productive value (PPV%) and energy utilization (EU) of grey mullet, Liza ramada, fry after feeding life food with or without enrichment until weaning.***

Treatment		PER	PPV	EU
Temperature	**Live Feed**			
19±1°C	**N**	1.15 ±0.19^{c}	22.42 ±4.75^{c}	17.59 ±2.47^{c}
	E	1.60 ±0.07^{bc}	31.21 ±2.46^{bc}	24.25 ±1.23^{bc}
24±1°C	**N**	1.61 ±0.08^{b}	34.27 ±2.97^{b}	22.10 ±1.26^{b}
	E	2.02 ±0.15^{a}	47.85 ±3.48^{a}	30.41 ±3.51^{a}
Pooled means				
19±1°C		1.38 ±0.23^{h}	26.82 ±5.54^{h}	20.92 ±3.24^{h}
24±1°C		1.81 ±0.20^{g}	41.06 ±5.93^{g}	26.26 ±4.25^{g}
	N	1.38 ±0.23^{y}	28.34 ±6.26^{y}	19.85 ±2.86^{y}
	E	1.81 ±0.20^{x}	39.53 ±6.49^{x}	27.33 ±3.86^{x}

Means with different superscript are significantly different (P<0.05) N:normal live food ,E:enriched live food

Effect of live food on histological characteristic of digestive tract

In cross section of oesophagus of *Liza ramada* larvae fed rotifer for 10 days then fed increasing number of artemia for 10 days after rotifer and constant number of artemia (10/ml) for another 20 days. It is clear that the oesophagus of a group of larvae before feeding *Artemia* were characterized by long sheet of epithelial layer with limited number of goblet cells, the number of villi ranged between 18 and 20 with an average number of about 18±2. (Figure 2A) Wherease the oesophagus of a group of larvae after feeding artemia (Fig.2B) were characterized by large number of goblet cells, the number of villi ranged between 25 and 27 with an average number of about 25±2.

The intestine of a group of larvae before feeding artemia were characterized by few number of goblet and mucous cells (Fig 2C),whereas the intestine of a group of larvae after fed artemia were characterized by large number of goblet and mucous cells (Fig 2 D)

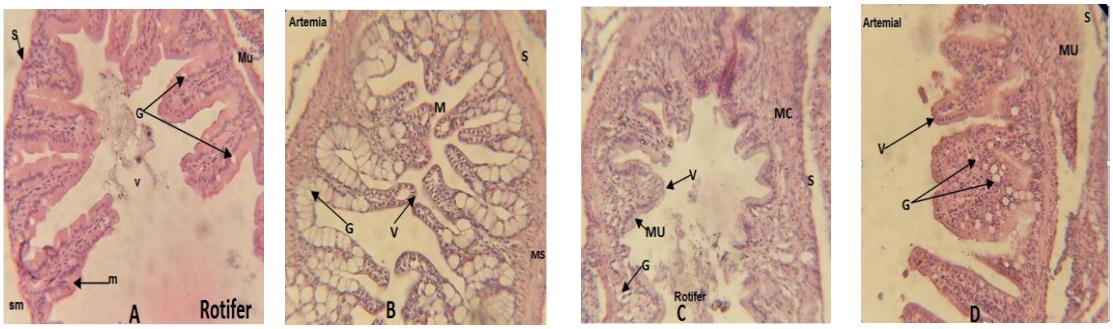


Figure 2: Photo micro graph of cross section in digestive tract of Liza romada larvae fed rotifer for 10 days. Larvae fed increasing number of Artemia for 10 days after rotifer and constant number of Artemia (10/ml) for another 20 days. (A) and (B) Showing effect of live food Photo cross section in oesophagus, (C) and (D) cross section in intestine. M, mucosa; MU, muscularis; S, serosa;V, villi and G, goblet cells staining with haematoxyline and eosin stains× 400.

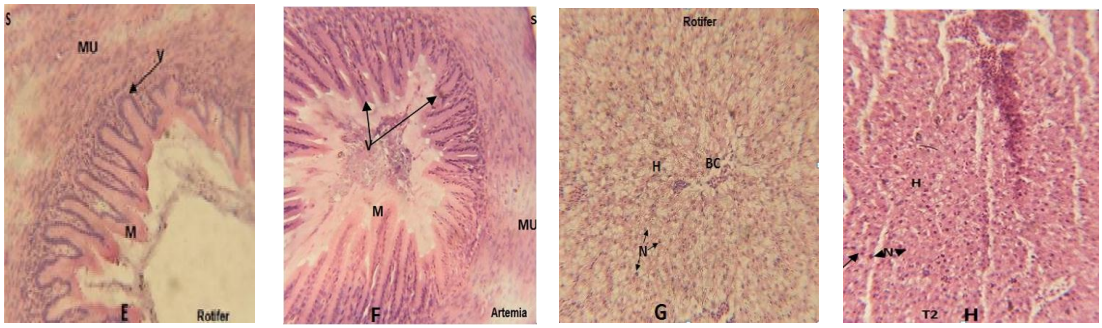


Fig 3: Photo micro graph of cross section in digestive trackt of Liza romada larvae fed Rotifer for 10 days. Larvae fed increasing number of Artemia for 10 days after rotifer and constant number of Artemia (10/ml) for another 20 days. Showing effect of live food Photo (E) and(F) cross section in foregut.M, mucosa; MU, muscularis; S, serosa and V, villi. (G) and (H) cross section in liver showing blood capillaries (BC),hepatic cells (H), Bi nucleated strong cells(arrow head) and N, nuclaus staining with haematoxyline and eosin stains × 400.

The foregut of a group of larvae before feeding artemia were characterized by thick and convoluted villi, the number of villi varied between 24 and 26, with an average number of about 24±2 as indicated in (Fig 3E).While the foregut of a group of larvae after feeding artemia were characterized by long and thin villi varied in number between 33 and 39 with an average number of about 36±3 as indicated in (Fig 3F).

The hepatic cells of larvae before feeding artemia were characterized by faintly staining affinity with large number of senile cells (without nucleus) as indicated in (Fig 3G),while the liver cells of larvae after feeding artemia were characterized by strong staining affinity and large number of binucleated strong cells (Fig 3 H).

Effect of live food and temperature on histological characteristic of digestive tract

In cross section of oesophagus of *Liza ramada* larvae fed increasing number of artemia for 10 days after rotifer and constant number of artemia (10/ml) for another 20 days Showing effect of temperature with enriched artemia It is clear that in (Fig 4) the oesophagus of a group of larvae feeding normal live food + temperature **19±1°C** (t1) was

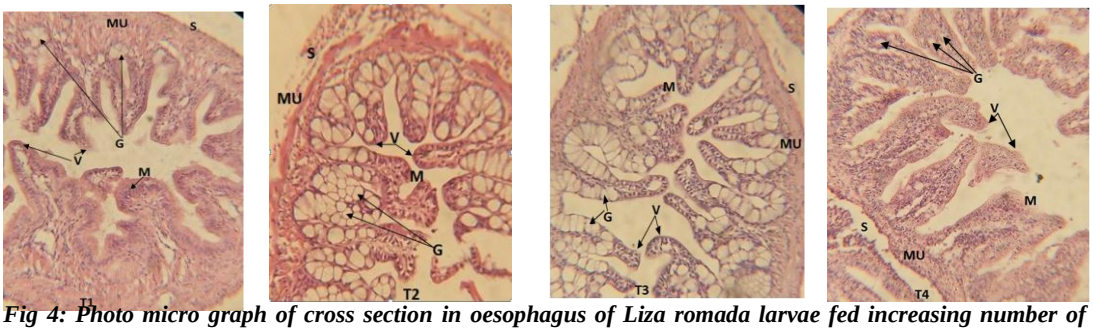


Fig 4: Photo micro graph of cross section in oesophagus of Liza romada larvae fed increasing number of artemia ***for 10 days after rotifer and constant number of Artemia (10/ml) for another 20 days. Showing effect of temperature with enriched*** artemia ***M,mucosa;MU,muscularis;S,serosa;V,the villi and G,goblet cells.(T_1) is room temperature(19) + normal live food, (T_2) room temperature (19) + enriched live food, (T_3) elevated temperature(24) + normal live food, and (T_4) elevated temperature(24) + enriched live food staining with haematoxyline and eosin stains × 400***

characterized by long sheet of epithelial layer with limited number of goblet cells,the number of villi ranged between 20and22 with an average number of about. (20±2) Wherease the oesophagus of agroup of larvae after feeding enriched live food + room temperature **19±1°C** (T2) was characterized by long sheet of epithelial layer with large number of goblet cells, the number of villi ranged between 22and:24 with an average number of about (22±2). The oesophagus of agroup of larvae after feeding normal live food + high temperature **24±1°C**.(T3) was characterized by large number of goblet cells, the number of villi ranged between 18and21 with an average number of about (18±3),and the oesophagus of agroup of larvae after feeding enriched live food + high temperature **24±1°C** (T4) was characterized by moderate number of goblet cells, the number of villi ranged between 22 and 24 with an average number of about (22±2).

The intestine of a group of larvae feeding normal live food + temperature **19±1°C** (T1) were characterized by rarely detected mucous and goblet cells (Fig 5), the intestine of a group of larvae after feeding enriched live food + room temperature **19±1°C** (T2) was characterized by large number of goblet and mucous cells. the intestine of a group of larvae after feeding normal live food + high temperature **24±1°C** (T3) was characterized by small number of goblet cells, and the intestine of a group of larvae after feeding enriched live food + high temperature **24±1°C** (T4) was characterized by moderate number of goblet cells.

The foregut of a group of larvae feeding normal live food + room temperature **19±1°C** (T1) was characterized by thick and convoluted villi.the number of villi ranged between 9 and 12 with an average number of about (9±3) Fig (6), the foregut of a group of larvae feeding enriched live food + room temperature **19±1°C** (T2) were characterized by long and thin villi. the number of villi ranged between 33 and 39 with an average number of about (36±3), the foregut of agroup of larvae after feeding normal live food + high temperature**24±1°C** (T3) the number of villi ranged between 20and25 with an average number of about (23±2) .The foregut of agroup of larvae after feeding enriched live food + high temperature **24±1°C** (T4) the villi was characterized by long and short appearance. The number of villi ranged between (37 and 41) with an average number of about (39±2) .

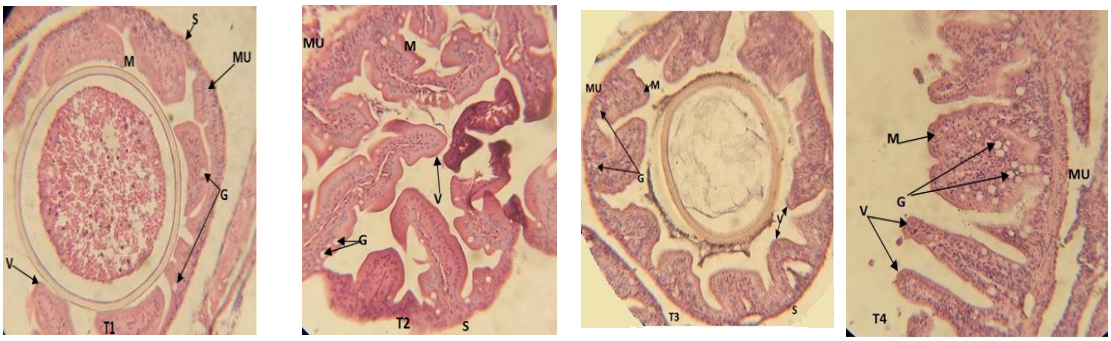


Fig 5: Photo micro graph of cross section in intestine of Liza romada larvae fed increasing number of* artemia *for 10 days after rotifer and constant number of* artemia *(10/ml) for another 20 day

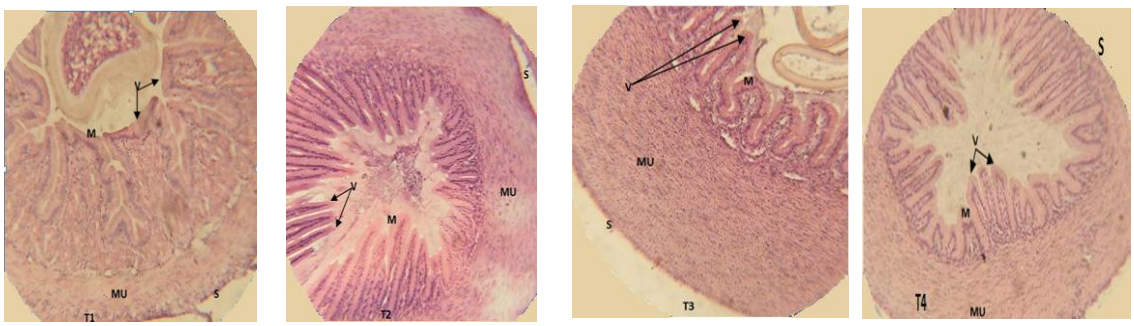


Figure 6: Photo micro graph of cross section in foregut of Liza romada larvae fed increasing number of* artemia *for 10 days after rotifer and constant number of* artemia *(10/ml) for another 20 days. Showing effect of temperature with enriched* artemia*. M,mucosa;MU,muscularis;S,serosa and V,the villi. (T_1) is room temperature(19) + normal live food, (T_2) room temperature(19) + enriched live food, (T_3) elevated temperature(24) + normal live food, and (T_4) elevated temperature(24) + enriched live food staining with haematoxyline and eosin stains × 400

The hepatic cells of larvae feeding normal live food + room temperature 19±1°C (T1) was characterized by faintly staining affinity with large number of senile cells (without nucleus), and oil droplets Fig (7), the liver cells of a group of larvae feeding enriched live food + room temperature 19±1°C (T2) was characterized by large number of bi nucleated strong cells and absence of oil droplets, the liver cells of a group of larvae feeding normal live food + high temperature 24±1°C (T3) was characterized by large number of senile cells, and innervated blood capillaries, and the liver cells of a group of larvae feeding enriched live food + high temperature 24±1°C (T4) was characterized by large number of bi nucleated cells and large number of oil droplets.

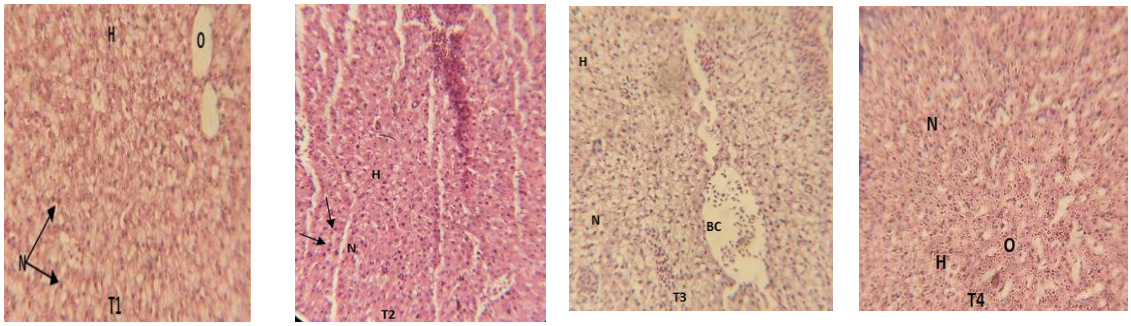


Fig 7: Photo micro graph of cross section in liver of Liza romada larvae fed increasing number of* artemia *for 10 days after rotifer and constant number of* artemia *(10/ml) for another 20 days Showing effect of temperature with enriched* artemia*. blood capillaries (BC),hepatic cells (H), Bi nucleated strong cells (arrow head). (T_1) is room temperature(19) + normal live food, (T_2) room temperature (19) + enriched live food, (T_3) elevated temperature (24) + normal live food, and (T_4) elevated temperature(24) + enriched live food staining with haematoxyline and eosin stains× 400.

DISCUSSION

Live food still in the most intrest for marine fish larvae specialy during the early life stages. Independently, of their nutritional value, live foods are easily detected and captured, due to their swimming movements in the water column, and highly digestible, given their lower nutrient concentration (water content > 80%) (Bengtson, 2003 and Conceiçao *et al*., 2010).

Live preys are able to swim in the water column and are thus constantly available to the larvae. Most formulated diets tend to aggregate on the water surface or, more commonly, sink within a few minutes to the bottom, and are thus normally less available to the larvae than live foods. In addition, since larvae are believed to be 'visual feeders' adapted to attack moving prey in nature, the movement of live food in the water is likely to stimulate larval feeding responses. Finally, live prey, with a thin exoskeleton and a high water content (normally > 80%), have a lower nutrient concentration and may be more palatable to the larvae once taken into the mouth, compared with the hard, dry formulated diet. This last point is rather critical as any feed item must enter the mouth hole, i.e., feed particles have to be smaller than the larva's mouth gape, and are quickly accepted or rejected on the basis of palatability (Bengtson, 2003 and Conceiçao *et al*., 2010).

Several enrichment techniques have been developed, including microalgae, oil-based emulsions and microencapsulated preparations (Barclay and Zeller 1996, Southgate and Lou 1995 and McEvoy *et al.* 1996,). However, most commercially available enrichment preparations are unable to provide a DHA/EPA ratio>1 in the enriched artemia (Navarro *et al.*, 1995, McEvoy *et al*., 1996), thus, the efficacy of this live prey to deliver sufficient DHA to the developing larvae remains as a matter of concern.

The present results showed that after feeding with rotifer, elevated temperature (24˚C) significantly (p<0.05) increased the survival rate, while enriched rotifer increased the survival rate without significant difference (p>0.05). Enriched rotifer combined with elevated temperature insignificant (p>0.05) increased the survival of fish larvae.

After feeding with artemia, the normal temperature showed significant increased of survival rate compared to the elevated temperature.

From an economic point of view, the main variables in larval rearing are survival and growth. The growth rates obtained are probably not the maximum for grey mullet, *liza ramada*. Feeding with (10 rotifers and 10 artemia/ml), using enriched live food (with fish oil) and elevated temperatures resulted higher growth rates of larvae and juveniles.In this respect, Giménez *et al.* (2007) indicated that to maximize common dentex larval survival soon after first feeding under intensive rearing conditions, between 10 and 40 larvae L^{-1}, at least 10 rotifers mL^{-1} should be added to the rearing tanks.

In the present study, the enrichment of artemia (10/ml) with emulsified fish oil showed increment in survival rate of grey mullet larvae. The enriched artemia increased the survival rate significantly to the value of 99.46±0.76 compared to 78.27±12.97 of the normal artemia. The present results are in agreement with thos reported by Abd allah (2009) in the gilthead seabream, *Sparus aurata*, and Mourad (2009) in grey mullet, *Liza ramada*.

In a feeding study by Wilcox and Tracy (2006), the fish survival rate was 30% after 2 weeks post hatch of feeding larval Southern flounder, *Paralichthys lethostigma*, only a DHA enriched rotifer diet. However, larval fish require more than just a nutritive food for survival. Fish need a motile food, which stimulates the development of eyes and nerves.

In the present study, after feeding enriched artemia combined with elevated temperature significantly increased the survival

rate ($p<0.05$) . After weaning, the survival rate improved with elevated temperature and enriched live food . After feeding with live food, the highest ($p<0.05$) FBW, ADG and WG were recorded in the fry fed enriched live food compared to those fed the unenriched live food. Elevated temperature combined with enrichmed live food insignificantly ($p>0.05$) increased FBW, ADG and WG rate . After weaning, the highest ($p<0.05$) FBW, WG and ADG were recorded in the fry fed with enriched rotifer and artimia for 40 days, then weaned using micro diet for 20 days compared to the larvae that fed the unenriched live food . There was a significant increase recorded with elevated, temperature combined with enrichment . This data was in agreement with (and Howell *et al.*, 1995; Morais *et al.* 2004; Villata *et al.*, 2005).

The highest significant (PER), (PPV%),and (Eu),were recorded in the fry fed enriched live food and weaning diet ($p<0.05$). There was a significant increase recorded with higher temperature, combined with live feed enriched using the optimum temperature with marine fish larvae improved feed utilization of seabream and seabass (Sfakianakis *et al.*, 2004; Sfakianakis *et al.*, 2006; Georgkopoulou *et al.*, 2007). Also, protein% of *Liza ramada* was affected by the higher temperature, which increased from 15.50±1.27 to 18.95±0.87 when temperature increased from 19 to 24°C.

In the present study, when the larvae of grey mullet, *Liza ramada*, fed the enriched artemia containing n-3 HUFA, a better correlation was found in growth and survival. Tha same result was found in red sea bream (Izquierdo *et al*., 1989, Abd allah, 2009).

Rodriguez *et al.* (1997) reported that a higher DHA/EPA ratio during the rotifer stage improved the growth and survival of gilthead sea bream. Copeman *et al.* (2002) found that yellowtail flounder fed high DHA/EPA (8:1) had a higher growth and survival than those fed a DHA/EPA ratio of 1.9:1. However, there was no significant difference in the growth of Japanese flounder and turbot larvae when they were fed with different dietary ratios of DHA and EPA (Estevez *et al.*, 1999). Harel *et al.* (2002) investigated the effect of commercial enrichment materials on early development of three larval fish. They reported no significant difference in growth between striped bass (*Morone saxatilis*) and gilthead sea bream larvae fed with artemia enriched with Algamac 2000_ or PL-Cr (DHA-rich phospholipid extract of *Crypthecodinium* sp.). However, the growth of halibut larvae fed artemia enriched with DHA Selco_ was lower than the growth of larvae fed with PL-Cr. Other studies also showed that cod larvae fed high DHA diets showed better growth and survival than those fed low DHA All these studies, suggest the existence of species-specific requirements for the DHA/EPA ratio for growth and survival of marine finfish larvae Harel *et al.* (2002).

Abd allah (2009) studied the effect of live food levels on growth and survival of sea bream larvae using three directions (feeding rates, feeding regime, and enrichment of artemia). From the results of the three directions, it was recommended that using green water system, rotifer rate 2.5/ml from day 0 to day 10 ph (post hatch), 12 artemia/ml enriched with fish oil is the best for gilthead sea bream larvae to give a good growth and best survival.Also mourad (2009) studied the effect of different levels of fish oil, silica and vitamin c used to enrich artemia on survival and growth of the larvae of mullet, *Mugill cephalus*. This study showed that feeding the larvae with artemia treated with fish oil concentration 4 g fish oil and exposed time (6 hr) showed the best survival rate and growth before weaning stage of *Mugill cephalus* larvae and helping in the intraction feeding period. The study also showed that enrichment with fatty acids in artemia nauplii improved its quality and fish food value by increasing significantly the levels of EPA, DHA and ARA highly unsaturated fatty acids using fish oil can be considered as a cheap and

easily adoptable method for commericial hatchery operations.

Several studies suggested that much higher levels of DHA or n-3 highly unsaturated fatty acids (HUFA) could reduce larval survival (Planas and Cunha, 1999). Izquierdo *et al.* (1992) showed that, in larval Japanese flounder (*Paralichthys olivaceus*), lower (or higher) DHA content (1.5%) of artemia did not affect survival, but larvae were significantly larger when fed artemia containing a higher percentage of DHA (up to 3.5%). However, Salhi *et al.* (1994), in their study with gilthead sea bream (*Sparus aurata*), showed that larvae fed with a lower DHA microdiet (>0.5%) had a significantly lower survival than larvae fed with a higher DHA microdiet (1.2 , 1.3%). They suggested that the growth of larvae was affected by a combination of DHA content and total dietary lipid, (Park *et al.*, 2006).

Newly hatched artemia nuaplii enriched with the essential fatty acids (EFA); decosahexaenoic acid (DHA) and ecosapenteanoic acid EPA) improved larval performance in striped bass and palmetto bass (tuncer and Harrell, 1992) cod (Takeuchi *et al* .,1994) red sea bream (furuita et al., 1996a) yellowtail (Furuita *et al*., 1996b) summer flounder (Baker *et al* 1998) milkfish (Gapasin and Duray, 2001) Japanese flounder (kim *et al*., 2002) cobia (Faulk and holt ,2003 and yellowtail snapper (Faulk *et al*., 2005) .

The good growth and feed efficiency of fish fed the diet containing fish oil indicated that grey mullet larvae require n-3 HUFA for good growth and survival like other marine fish such as turbot, striped jack, red sea bream, and gilthead sea bream (Ibeas *et al*., 1996) . Studies on EFA nutrition for Japanese flounder larvae show significant differences in growth between the larvae fed artemia enriched with oleic acid, EPA, or DHA (Furuita *et al*., 1998, 1999) .

The present study described the histological profiles of the digestive tract in grey mullet (*Liza ramada*) fry reared under different combinations of temperature and different feed regimes. The esophagus of *Liza ramada* have longitudinal folds; it was lined by a few layers of stratified squamous epithelium with numerous superficial mucous cells, it changed to simple columnar epithelium at the end of the esophagus. The lamina propria was formed by loose connective tissue. The muscularis mucosa was organized in longitudinal and circular layers of striated muscular fibers. The mucosal surface of the intestine in *Liza ramada* has numerous folds lined by simple tall columnar cells, along with goblet cells, which reacted positive to haematoxyline and eosine staining.

Zouiten *et al.*(2008) indicated that maturation of intestinal tract in *Chelon labrosus* larvae is particularly precocious. They assumed that larvae of *Chelon labrosus* might support early co-feeding and weaning strategies, which could reasonably be initiated since mouth opening.

The histological studies of the alimentary channel across species of fish are becoming more valuable as the interest in fish culture expands and more information is required with regardnto feeding and nutrition. Mucosa is pivotal in digestion, absorpation and metabolic processes .It plays apart in the electrolytic balance,immune response and endocrine functions (El-Bakary and El-Gammal, 2010).

The present study revealed that the longer proximal intestine of grey mullet *liza ramada* has amucosal folds lined by columnar epithelium, but the longest folds and cells were found in grey mullet and, the mucous goblet cells are interspersed between the epithelial absorptive cells .The mucosal folds of intestine that are lined by asingle layer of columnar cells with many mucous goblet cells.The columnar epithelium of the intestinal mucosa may have an absorptive function.

In present study the number of villi increase after feeding artemia which considered as agreat amounte of folds in the intestine agreat amounte of folds were observed in the proximal

intestine,increasing the surface area and enhancing the absorptive activity. In addition to the great amount of folds, thick outer layer of smooth muscle cells as well as the inner non continuous longitudinal smooth muscle were found in *liza ramada*. This may confirm the expectation that higher efficiency of mucosal folds occurs when the motility is in creased. Therefore, this structural arrangement could be considered as apossible adaptation to omnivorous feeding habite . It has been reported that complex folding of the intestinal mucosa with the resultant increase in surface area aids the mixing of food with hepatic and pancreatic digestive juices as well as with mucus secreted by goblet cells.

The final goal of whole experiment presented here was to study the best way to overcome the mortality due to various reasons by changing some of the environmental factors (temperature) and improvement of live feed quality. Although good progress was made concerning the larvae and juvenile grey mullet, *Liza ramada*, in this study, there are a number of areas that need further research, including establishment of optimum stocking density, environmental conditions, different rearing systems and satisfactory diets.

REFERENCES

Abdallah, R.A. (2009). Effect of live feed levels on growth and survival of marine fish larvae. Ph.D. Thesis Faculty of Agriculture (Saba asha), Alexandria University,Egypet.

Baker, E.P; Alves, D.and Bengtson, D.A. (1998). Effects of rotifer and Artemia fatty acid enrichment on survival, growth and pigmentation of summer floun *Paralichthys dentatus* larvae.J.Wor.Aqua.Soc., 29:494-498.

Barclay, W. and Zeller, S. (1996). Nutritional enhancement of n-3 and n-6 fatty acids in rotifers and artemia nauplii by feeding spray-dried Schizochytrium sp. J. World Aquacult. Soc., 27: 314–322.

Bengtson, D.A. (2003). Status of marine aquaculture in relation to live prey : past, present and future.in live feeds in marine Aquacultured by J.G. strottrup and L.A.Mcevoy),pp.1-6.Blacj well publishing , oxford,uk.

Bransden, M.P.; Battaglene, S. C.; Morehead, D. T.; Dunstan, G. A. and Nichols, P. D. (2005). Effect of dietary 22:6n-3 on growth, survival and tissue fatty acid profile of striped trumpeter (*Latris lineate*) larvae fed enriched Artemia. Aquaculture, 243: 331-344.

Cahu, C. and Infante, J.Z. (2001). Substitution of live food by formulated diets in marine fish larvae. Aquaculture, 200: 161-180.

Chew, W.Y.S. and Lim, H.S. (2006). Some improvements to the rotifer (*Brachionus rotundiformis*) mass culture method. Singapore, 32: 52-58.

Conceiçao, L.E.C.; Yufera, M.; Makridis, P.; Morais, S. and Dinis, M.T. (2010). Live feeds for early stages of fish rearing. Aquaculture Research, 41:613-640.

Copeman, L. A., Parrish, C. C., Brown, J. A., and Harel, M. (2002). Effects of docosahexaenoic, eicosapentaenoic and arachidonic acids on the early growth, survival, lipid composition and pigmentation of yellowtail flounder (*Limanda ferruginea*): a live food enrichment experiment. Aquaculture, 210: 285-304.

Curnow J., King J., Bosmans J. and Kolkovski S. (2006). The effect of reduced Artemia and rotifer use facilitated by a new microdiet in the rearing of barramundi Lates calcarifer (BLOCH) larvae. Aquaculture, 257: 204 - 213.

Duncan, D. B. (1955). Multible range and multible F test. Biometric, 11: 1- 42.

El-Bakary and El-Gamma (2010). Comparative Histological, Histochemical and Ultra Structural Studies on the proximal intestine of flath head GreyMullet (*Mugil Cephalus*) and Sea Bream (*Sparus aurata*). World Applied Sciences Journal, 8: 477 – 485

El-Dahhar, A. A. (1999). Effect of heat treated feed and exogenous zymogene on survival and growth of grey mullet larvae Liza ramada (Risso). Egypt. Aqua. Res., 30:165-173.

Estevez A., McEvoy L.A., Bell J.G. and Sargent J.R. (1999). Growth, survival, lipid composition

and pigmentation of turbot (*Scophthalmus maximus*) larvae fed live-prey enriched in arachidonic and eicosapentaenoic acids. Aquaculture, 180:321-343.

Faulk, C. K. and Holt, G. J. (2003). Lipid nutrition and Feeding of cobia Rachyentron canadum larvae. J. Wor. Aqua. Soc., 34(3):368-378.

Faulk C.K. and Holt G.J. (2005). Advances in rearing cobia *Rachycentron canadum* larvae in recirculating aquaculture systems: live prey enrichment and greenwater culture. Aquaculture 249, 231 - 243.

Furuita, H.; Takeuchi, T.; Toyota, M. and Watanabe, T. (1996a). EPA and DHA requirements in early juvenile red sea bream using HUFA enriched Artemia nauplii. Fish. Sci., 62:246-251.

Furuita, H.; Takeuchi, T.; Watanabe, T.; Fujimoto, H.; Sekiya, S. and Imaizumi, K. (1996b). Requirements of larval yellowtail for eicosapentaenoic acid, docosahexaenoic acid and highly unsaturated fatty acid. Fish. Sci., 62:372-379.

Furuita, H.; Takeuchi, T. and Uematsu, K. (1998). Effect of eicosapentaenoic and docosahexaenoic acids on growth, survival and brain development of larval Japanese flounder *(Paralichthys olivaceus*). Aquaculture., 161:269-279.

Furuita, H.; Konishi, K. and Takeuchi, T. (1999). Effects of different levels of eicosapentaenoic acid and docosahexaenoic acid in artemia nauplii on growth, survival and salinity tolerance of larvae of the Japanese flounder, *Paralichthys olivaceus*. Aquaculture., 170:59-69.

Gapasin R. S. J. and Duray, M.N. (2001). Effects of DHA-enriched live food on growth, , survival and incidence of opercular deformities in milkfish, *Chanos chanos*. Aquaculture., 193:49-63.

Georgakopoulou, E., Angelopoulou, A., Kaspiris, P., Divanach, P. and Koumoundouros, G. (2007). Temperature effects on cranial deformities in European sea bass, *DIcentrarchus labrax* (L.). Journal of Applied Ichthyology: 23: 99-103.

Giménez G., KotzamanisY., Hontoria F., Estevez A. and Gisbert E. (2007). Modelling retinoid content in live prey: a tool for evaluating the nutritional requirement and development studies in fish larvae. Aquaculture 267:76 - 82.

Hamza N., Mhetli M. and Kestemont P. (2007) Effects of weaning age and diets on ontogeny of digestive activities and structures of pikeperch (*Sander lucioperca*) larvae. Fish Physiology and Biochemistry 33:121 - 133.

Harel, M., Koven, W., Lein, I., Bar, Y., Behrens, P., Stubblefield, J., Zohar, Y., and Place, A. R. (2002). Advanced DHA, EPA and ArA enrichment materials for marine aquaculture using single cell heterotrophs. Aquaculture, 213: 347-362.

Hernandez D. R.; Perez Gianeselli M. and Domitrovic H. A. Morphology (2009). Histology and histochemistry of the digestive system of South American catfish (*Rhamdia quelen*). Int. J. Morphol., 27(1):105-111.

Howell, B.R.; Beard, T.W. and Hallam, J.D. (1995). The effect of diet quality on the low-temperature tolerance of juvenile sole, *Solea solea* (L.) ICES CM 1995/F, vol. 13. 9 pp.

Ibeas, C.; Cejas, J. Gomez, T.; Jerez, S. and Lorenzo, A. (1996). Influence of dietary n-3 highly unsaturated fatty acids levels on juvenile gilthead seabream (*Sparus aurata*) growth and tissue fatty acid composition. Aquaculture., 142:221-235.

Izquierdo, M. S.; Watanabe, T.; Takeuchi, T.; Arakawa, T. and Kitajima, C., (1989). Requirement of larval red seabream *Pagrus mojor* for essential fatty acids Nipp. Sui. Gakkaishi, 55:859-867.

Izquierdo, M. S.; Arakawa, T.; Takeuchi, T.; Haroun, R. and Watanabe, T. (1992). Effect of n-3 HUFA levels in artemia on growth of larval Japanese flounder (*Paralichthys olivaceus*). Aquaculture., 105:73-82.

Izquierdo M.S., (1996). Essential fatty acid requirements of cultured marine fish larvae. Aquacult. Nutr., 2:183-191.

Kim, K.D.; Lee, S.M.; Park, H.G.; Bai, S.C. and Lee, Y.H. (2002). Essentiality of dietry n-3 highly unsaturated fatty acids in juvenile Japanese flounder *Paralichthys olivaceus*. J. Wor. Aqua. Soc., 33(4): 432-440.

Koven, W., Barr, Y., Lutzky, S., Ben-Atia, I., Weiss, R., Harel, M., Behrens, P., and Tandler, A. (2001). The effect of dietary arachidonic acid (20:4n-6) on growth, survival and resistance to handling stress in gilthead sea bream (*Sparus aurata*) larvae. Aquaculture, 193: 107-122.

Koven, W. (2003). "Key factors influencing juvenile quality in mariculture: a review." The Isreali Journal of Aquaculture-Bamidgeh, 55(4): 283-297.

Lazo J.P., Dinis M.T., Holt J.G., Faulk C. and Arnold C.R. (2000). Co-feeding microparticulate diets with algae: towards eliminating the need of zooplankton at first feeding in red drum *(Sciaenops ocellatus*). Aquaculture 188, 339 - 351.

McEvoy, L. A., Navarro, J. C., Hontoria, F., Amat, F., and Sargent, J. R. (1996). Two novel artemia enrichment diets containing polar lipid. Aquaculture, 144: 339e352.

Morais S., Lacuisse M., Conceicao L.E.C., Dinis M.T. and Ronnestad, I. (2004). Ontogeny of the digestive capacity of (*Solea senegalensis*), with respect to and metabolism of amino acids from Senegalese sole digestion, absorption artemia. Marine Biology145: 243 - 250.

Mourad M. M. (2009). Effect of live food enrichement on survival and growth of Marine larval fish with gonads properties studies. Master Thesis, Faculty of Agricultur Saba Bacha e , Alexandria University,Egypet.

Navarro, J. C., McEvoy, L. A., Amat, F. and Sargent, J. R. (1995). Effects of diet on fatty acid composition of body zones in larvae of the sea bass Dicentrarchus labrax: a chemometric study. Mar. Biol., 124: 177-183.

Park, H. G., Puvanendran, V., Kellett, A., Parrish, C. C., and Brown, J. A. (2006). Effect of enriched rotifers on growth, survival, and composition of larval Atlantic cod (*Gadus morhua*). e ICES Journal of Marine Science, 63: 285-295.

Planas, M. and Cunha, I. (1999). Larvie culture of marina fish: problems and perspectives. Aquacultre, 177:171-190.

Ragi, A.R. and Norouzi, E. (2010). Histological and histochemical study on The alimentary Canalin Walking Cat Fish (*claris batrachus*) and piranha (*Serrasalmus natterori*). Veterinary Research, Vol.11 (3) Ser. No. 32.

Rippingale R. J. and Payne, M. F., (2001). Intensive cultivation of a calanoid copepod for live food in fish culture. Fisheries Research and Development Corporation ISBN:1 74067 069 8

Rodriguez, C., Perez, J. A., Diaz, M., Izquierdo, M. S., Fernandez- Palacios, H., and Lorenzo, A. (1997). Influence of EPA/DHA ratio in rotifers on gilthead sea bream (*Sparus aurata*) larval development. Aquaculture, 150: 77-89.

Rosenlund G. and Halldoorsson O.L. (2007). Cod juvenile production: researchand commercial developments. Aquaculture, 268: 188 - 194.

Salhi, M.; Izquierdo, M. S.; Hernández-Cruz, C. M.; González, M. and Fernendez-Palacios, H., (1994). Effect of lipid and n-3 HUFA levels in microdiets on growth, survival and fatty acid composition of larval gilthead seabream (*Sparus aurata*) Aquaculture., 124:275-282.

Sedlacek, Chris. (2008). The biochemical composition of naupii derived from stored nondiapause and diapause copepod eggs and the biology of diapausing eggs journal of Experimental Marine Biology And Ecology, 318: 183-190.

Sfakianakis, D. G, Koumoundouros, G.f Divanach, P. and Kentouri, M. (2004). Osteological development of the vertebral column and of the fins in Page11us erythrinus (L. 1758), Temperature effect on the developmental plasticity and morpho-anatomicai abnormalities, Aquacuiture, 232:, 407-424.

SfakianakiS; D. G, Georgakopoulou, Ev Papadakis, I. E., Divanach, P., Kentouri, M,and Kou-moundouros, G. (2006). Environmental determinants of haemal lordosis in European sea bass, *Dicentrarchus. labrax* (Linnaeus, 1758), Aquacuiture, 254: 54-64.

Southgate, P.C.and Lou, D.C., (1995). Improving the n-3 HUFA composition of artemia using microcapsules containing marine oils. Aquaculture, 134: 91–99.

Takeuchi, T.; Zheng, K.; Yoseda, K.; Hirokawa, J. and Watanabe, T., (1994). Nutritive value of

DHA-enriched rotifer for larval cod. Nipp. Suisan Gakkaishi, 60:641-652.

Takeuchi, T. (1997). Essential fatty acid requirements of aquatic animals with emphasis on fish larvae and fingerlings. Rev. fish. Sci., 5: 1-25.

Tuncer, H. and Harrell, R. M., (1992). Essential fatty acid nutrition of larval striped bass, Morone saxatilis, and palmetto bass, M. saxatilis. Aquaculture., 101:105-121.

Vega-Orellana O.M., Fracalossi D.M. and Sugai J.K. (2006). Dourado (*Salminus brasiliensis*) larviculture: weaning and ontogenetic development of digestive proteinases. Aquaculture, 252:484 - 493.

Villalta M., Estévez, A., Bransden, M.P. and Bell J.G. (2005). The effect of graded concentrations of dietary DHA on growth, survival and tissue fatty acid profile of Senegal sole (*Solea senegalensis*) larvae during the artemia feeding period. Aquaculture 249:353 - 365.

Wilcox, J. A. and Tracy, P.I., (2006). Improving live Feeds:Effect of a mixed diet of copepod hauplii (Acartia tonsa) And Rotifers on the Sur vival and growth of first feeding Larvae of the Sou thern flounder, paralichthys Lethostigma . Journal of the World Aquaculture Society, 37 (1) :113 -120.

Zouiten, D., khemis, I.B., Raoufbesbes. And Chantal Cahu . (2008). Ontogeny of the digestiveract of thick lipped grey mullet (*chelon labrosus*) larvae reared in mesocosms.Aquaculture,volume279,166-172.r

تأثير إثراء الغذاء الطبيعي ودرجة الحرارة على معدل النمو والإعاشة وتطور القناة الهضمية ليرقات البوري

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تعتبر رعاية اليرقات واحدة من اساسيات زيادة الانتاج السمكي، ونظراً لانخفاض معدلات الإعاشة لليرقات البرية، تحتاج العديد من الأبحاث الي تحديد الاحتياجات البيئية والغذائية لأسماك البوري خاصة في مرحلة رعاية اليرقات. أجريت هذه التجربة لدراسة تأثير تداخلات مختلفة من التغذية الطبيعية ودرجات الحرارة على نمو يرقات البوري وتطور القناة الهضمية لليرقات. تم جمع يرقات اسماك البوري من خليج الماكس في البحر الأبيض المتوسط بالإسكندرية، مصر. أجريت الدراسة في 12 حوض زجاجي بأبعاد (100x40x30 سم) تحتوي على 30 لتر من المياه متوسطة الملوحة (10 جزء في المليون) تم تحضيرها بواسطة خلط ماء البحر مع ماء عذب. تم تخزين 40 سمكة في كل حوض زجاجي. تم تقسيم ال 12 حوض زجاجي الي 4 مجموعات (ثلاث مكررات لكل معاملة والتصميم الإحصائي 2X2 تجربة عامليه). تم تقدير الإعاشة، عوامل النمو والفحص التشريحي لأسماك البوري. أوضحت النتائج أن الغذاء الطبيعي المدعوم (روتيفر وأرتيميا) حسن من إعاشة اليرقات ومعدلات النمو عند درجة حرارة 24° تحت الظروف المعملية. من خلال الدراسة التشريحية لوحظ قلة عدد الخلايا الكأسية بالجهاز الهضمي بعد مرحلة التغذية بالروتيفر. وزاد عدد الخلايا الكأسية والخمائل بعد مرحلة التغذية بالأرتيميا. وتحسن التركيب التشريحي لكلا من المرئ، الأمعاء والكبد بعد التغذية على الغذاء الطبيعي المدعوم. وفي النهاية، مازال الغذاء الطبيعي هو المكون الرئيسي لرعاية اليرقات تجاريا بإضافة الأحماض الدهنية الغير مشبعة طويلة السلسلة (HUFA) وذلك من أجل إكمال تركيب الغذاء الطبيعي ليكون مناسباً لتغذية اليرقات صغيرة الحجم مع درجات الحرارة المرتفعة (24°)، وذلك ادي الي تحسن معدلات الإعاشة، النمو والتركيب التشريحي للجهاز الهضمي في اليرقات.