

## **EFFECT OF SALINITY AND SALINITY ACCLIMATIZATION ON SURVIVAL AND GROWTH OF THE WILD HATCHED MULLET LARVAE USING DIFFERENT FEEDS IN GLASS AQUARIA**

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*(Received : February 5, 2000)*

**Key words:** Salinity Acclimatization, Mullet larvae, Different feeds, Glass aquaria.

### **ABSTRACT**

**T**wo experiments were carried out to study the effect of salinity and salinity acclimatization on survival and growth of mullet larvae in glass aquaria. In the first experiment, a 6×3 complete randomized blocks design was utilized with six water salinities (0, 5, 10, 15, 20 and 35ppt) in three replicates to define the effect of salinity on survival of striped mullet (*Mugil cephalus*) wild larvae collected from El-Max larval collection center at Alexandria Egypt. In the second experiment, a 5×3 complete randomized blocks design was utilized with five starting points of salinity reduction acclimatization. Starting acclimatization using (3, 6, 9, 12 and 15 ppt salinities) at three replicates was initiated to study the effect of acclimatization from different points on survival and growth of wild grey mullet (*Liza ramada*) (Risso) larvae. Reducing salinity 1 ppt daily using the appropriate amount of freshly stoked tap water did the descending salinity acclimatization for each starting point. Two test diets (40% crude protein) formulated from commercial ingredients, were used.

In the first experiment, the higher water salinity had a marked negative effect on survival rate. The larvae maintained at 35, 20, 15 and 10ppt salinities suffered 100% mortality after 5, 15, 40 and 30 days respectively. Survival rate of the larvae maintained at 0ppt salinity was the best during the period from day 25 to day 30 of the first experiment, but at 5 ppt salinity survival rate was the best from day 35 until the end of the experiment after 60 days. In the second experiment, the best survival rates were achieved at starting points of acclimatization of 3, 6 and 12-ppt salinities. They were the higher significantly ( $P < 0.05$ ) than the other salinities 9 and 15 ppt. On the average, it was found that starting acclimatization at salinity similar to the natural salinity from which the larvae were caught (about 7 ppt) was the best starting point for the wild mullet larvae acclimatization at laboratory conditions.

## INTRODUCTION

Mullet fish (Mugillidae) are considered amongst the cosmopolitan teleosts that represent some of the most promising fish species for commercial aquaculture for both mono and polyculture systems (Thomson, 1963, Nash and Shehadeh 1980; Benetti and Fagundes Netto, 1991). Mulletts inhabit all coastal waters lying between 42° north and 42° south latitudes. They usually move into groups within coastal waters and enter lagoons, estuaries and rivers in order to feed, but spawn in seawater (Nash and Shehadeh, 1980 Whitehead *et al.*, 1986). Mulletts are also good candidate and play an important role in the fisheries and fish farms of tropical and subtropical countries of the world (Nash and Shehadeh 1980). The wild is still the main source of mullet fry, but high mortality of the collected fry during and after transportation from the collecting sites to the fish farms and culture sites is limiting the development of mullet culture. This is due to differences in the physico-chemical

properties and sudden change in salinities between both sites, next to the stress to which the fish is exposed during transportation (Mabrouk 1991).

Few studies have investigated the salinity effects on survival and growth of these larvae and reported that mullet can be cultured in both brackish and fresh water (Sivalingam 1975, Pillai 1975). Also, some authors indicated that mullet fish prefers brackish water and grow faster in such water areas of the subtropics (Gosline and Brock 1965, Nash and Shehadeh, 1980). Pillai (1975) reported that mullet could be stocked in different salinities even in fresh water lakes, but need acclimatization when the salinities are considerably different in capturing and stocking sites. The newly hatched mullet larvae were found to have a limited tolerance for salinity fluctuations, but no data have been reported regarding larval growth in varying salinities during their rearing period (Sylvester *et al.*, 1975; Lee and Menu, 1981; Walsh *et al.*, 1989, Murashige *et al.* 1991). However, Devaneson and Chaco (1943) found that survival rate of mullet fry could be increased by gradual salinity acclimatization.

The objectives of the present study are to investigate the effect of salinity on survival and growth of the wild mullet larvae and to determine the salinity in which the wild larvae should start salinity reduction acclimatization under controlled laboratory conditions.

## **MATERIALS AND METHODS**

### **Facilities and Fish:**

Two experiments were carried out to study the effect of salinity and salinity acclimatization on survival and growth of striped mullet (*Mugil cephalus*) and grey mullet (*Liza ramada*) (Risso) larvae in glass aquaria respectively. Aquaria (100×30×40 cm) at a working volume of 60 liters were supplemented with continuous aeration using salt water. Desired salinities were made in aquaria by dissolving crude salts obtained from El-Nassr Salines Co. Borg El-Arab Salines Sector,

Alexandria Egypt. Larvae, caught from El-Max larval collection center at Alexandria, ranged in weight from 0.083-0.17g with an average of  $0.13 \pm 0.04$ g. The newly transferred larvae were stocked in glass aquaria for 24 h at salinity similar to the wild salinity from which the larvae were caught (about 7 ppt).

#### **Experimental design:**

**First experiment:** A 6×3 complete randomized blocks design was utilized with six different water salinities (0, 5, 10, 15, 20 and 35 ppt). Treatments were replicated in three glass aquaria, each was stocked with 29 larvae. This experiment was administered to define the effect of salinity on survival of striped mullet (*M. cephalus*) larvae.

**Second experiment:** The point of descending salinity acclimatization was started within 3-15 ppt range to study the effect of the starting point of salinity reduction acclimatization on survival and growth of grey mullet larvae, *Liza ramada* (Risso). A 5×3 complete randomized blocks design was utilized with five starting points of salinities (3, 6, 9, 12 and 15 ppt) repeated in three glass aquaria. According to the results of the first experiment, it was decided to reduce salinity of each starting point 1 ppt daily using the appropriate amount of freshly stocked tap water to help the descending salinity acclimatization. After the concentration of salts in glass aquaria has reached 0ppt salinity, water was kept fresh for 3 days. Also, from the results of experiment 1, salinity concentrations were then increased gradually 1 ppt daily in order to reach 5-ppt salinity (the best treatment of the first experiment).

#### **Diets Formulation and Preparation:**

Two test diets, A and B (40 % crude protein), were formulated from commercial ingredients (Table 1), and used to feed larvae in the first experiment and the second experiment respectively. The ingredients of both feeding mixtures were milled through screen (0.6-mm-diameter hole) before mixing into the diets. Mixtures were homogenized in a feed

mixer (model SNFGA, Kitchen Aid St. Joseph, MI, USA). Corn oil, emulsified with equal amount of water using 0.7 % phosphatidyl choline (lecithin) according to El-Dahhar and El-Shazly (1993), was added to the diets. Boiling water was then blended with the mixtures at the rate of 50% for pelting. An autoclave was used to heat the diets for 15 min after adding boiling water using a maximum pressure of 1.2 kg /cm<sup>2</sup> G. Vitamins and minerals mixture was added to each diet after heat treatment. Diets were pelleted using meat grinder of the kitchen Aid with a 1.5mm diameter and kept frozen in deep-freezer until used. Oil, vitamin mixture and ascorbic acid were increased in experiment 2 after EL-Dahhar (1999).

#### **Management:**

Prepared salt water was stored in one cubic meter fiberglass tanks located over the laboratory. Salinities were adjusted in each aquarium separately. Water temperature was maintained constant at 24 °C by means of electric aquarium heaters, one in each glass aquarium. The front side of each aquarium was covered with dusky plastic sheet to prevent fish disturbance. Fish were fed twice daily (9:00 AM and 2:00 PM). Water of each aquarium was siphoned to eliminate dead fish and wastes. Freshly stocked salt water replaced the removed water. Before the experiment, aquaria were rinsed with chlorinated water for 24h then salt water was supplied to each aquarium.

#### **Analytical methods:**

Salinities were confirmed using burette titration for chloride against standard 0.014N silver nitrate, according to the American Public Health Association (1984). A Cole Palmer (Chicago, IL, USA) Oxygen meter (Model 5946-55) was used to determine dissolved oxygen and water temperature; they were 5.9 ± 0.8 ppm and 24 ± 0.5C respectively.

The analyses of variance (ANOVA) was made according to Snedecor and Cochran (1967).

## RESULTS

The effect of water salinity (0, 5, 10, 15, 20 and 35 ppt) of experiment 1 on survival rate percentage of striped mullet larvae is presented in Table 2. It was observed that increasing water salinity had a marked effect on survival rate. After 24h, a high mortality rate was reported in the highest and the lowest salinity concentrations (35 and 0 ppt). The survival rate of the larvae maintained at 35 ppt salinity (24.1 %) was significantly ( $P<0.01$ ) lower than that of the larvae maintained at 0ppt salinity (58.6 %). Both of them were significantly ( $P<0.01$ ) lower than 97.7, 87.7, 100 and 97.7 % of the remaining salinities (5, 10, 15 and 20 ppt) respectively. No detectable differences occurred within the survival rates among the 5, 10, 15 and 20ppt salinities. The higher the salinity, the less was the time spent for the larvae to suffer 100% mortality. With time the rate of mortality of the larvae maintained at both 35 and 20-ppt salinities increased, reaching 100% after 5 and 15 days respectively. More time was spent for the larvae to suffer 100% mortality with decreasing water salinity. After 20 days, the larvae maintained at 5ppt salinity had a higher survival rate (40.2 %). It was significantly ( $P<0.01$ ) higher than the survival rates (32.2, 6.9, and 4.6%) of the larvae maintained at 0, 10 and 15 ppt salinities respectively. But, after 25 days, larvae maintained at 0-ppt salinity had the best survival rate (25.3%), which was significantly higher than (19.5, 3.5 and 2.3%) those observed for larvae maintained at 5, 15 and 10-ppt salinities respectively. However, mortality reached 100% for larvae maintained at 10-ppt water salinity after 30 days. After 35 days, a higher survival rate (11.5%) was observed for larvae maintained at 5 ppt water salinity followed by those maintained at 0 and 15 ppt water salinities (8 and 1.1%) respectively.

After 40 days, the larvae maintained at 15-ppt water salinity suffered 100% mortality, while the survival rate of the larvae maintained at 5-ppt water salinity (8%) was significantly higher ( $P < 0.01$ ) than (1.1%) of those maintained at 0-ppt salinity until the end of the first experiment after 60 days.

Means of survival rate of the grey mullet larvae, which maintained at the five starting points of descending salinity acclimatization (3, 6, 9, 12 and 15 ppt) of experiment 2 are presented in Table 3. After 24 days, survival rates of the larvae that started acclimatization at 3 and 12 ppt salinity were higher (65.6 and 70%) significantly ( $P < 0.05$ ) than all other treatments. They were still the higher until after 30 days. However, the survival rate of the larvae started at 3ppt salinity was the highest until the end of the experiment (after 54 days). Also, on the average, starting points of acclimatization of 3, 6 and 12 ppt salinity had the best significant ( $P < 0.05$ ) effect on survival rate at the end of the second experiment. Their survival rates were 33.3, 24.4 and 24.4 % respectively.

Weight gain (percent weight gain) and final biomass showed a general increase with the larvae that started at 6-ppt salinity. They gained 149.8% on the average that was significantly ( $P < 0.01$ ) higher than 93.1 and 74.7% of the larvae that started acclimatization at 3 and 9 ppt salinity and higher than 65.9 and 48.2% of the larvae started acclimatization at 12 and 15 ppt salinity respectively (Fig. 1).

## DISCUSSION

From the present results, salinity is an effective factor and could be considered as a determinative factor for survival of mullet larvae. High mortality rate was found with the striped mullet maintained at the higher salinities (20 ppt and 35 ppt) and also with the lower salinity

(0ppt). Also, from the high mortality rate of the larvae maintained at the salinity higher than 9ppt and lower than 3ppt, thus the authors recommend keeping the wild mullet larvae in salinity similar to its wild salinity for 24 h in the laboratory conditions and start acclimatization at the second day.

The data presented at the first experiment suggest that the optimum salinity ranges from 0-5 ppt. This may help explain the relative paucity of mullet larvae captured in marine sites of the Mediterranean sea (Nash and Shehadeh, 1980). Red drum, another euryhaline sciaenid, also grows well as larvae at 5 ppt (Crocker *et al.*, 1981). Holt (1990) found that although salinities of 25-30 ppt are required for development of yolk-sac to first feeding of red drum larvae, older larvae were acclimated to wide range of salinities. For striped mullet (*Mugil cephalus*), Murshige *et al.* (1991) also reported that the optimal salinity for hatching ranged from 30-40 ppt. However, improvement in growth was detected at lower salinity (i.e., 22-23) during the first 15 days posthatching.

The mullet larvae at higher and lower salinities (35 and 0 ppt) poorly survive after 1day and all of them died after 5days when maintained at 35 ppt. Meanwhile, some of them recovered after sometimes at 0 ppt and commenced normal feeding and swimming behavior. Similar behavior was observed when European bass *Dicentrarchus labrax* were transferred to freshwater (0.5 ppt), including loss of appetite, heavy mortality within a few days, and like mulloway, exhibited complete recovery when returned to 5 ppt (Dendrinis and Thorpe, 1985). The present results also support those of Greyand McDonall (1993) and Fielder and Bardsley (1999) that juvenile mulloway prefer water with some marine influence, but are able to survive in fresh water for at least short periods of time.

The inability of mullet to survive when suddenly transferred to freshwater differs from that observed for some other euryhaline fishes.



Red drum are found naturally in freshwater (Holt *et al.* 1981; Crocker *et al.*; 1981) and have been successfully stocked into freshwater impoundments in the USA (Lasswell *et al.*, 1977). Juveniles and adult of striped mullet (*Mugil cephalus*) are known to exhibit a remarkable tolerance of salinity fluctuations. They also have the ability to cope with the salinity fluctuation (Sylvester *et al.*, 1975; Lee and Menuy 1981; Walsh *et al.*, 1989). El-Ebiary, (1982) investigated the effects of salinity on survival of striped mullet and pointed out that the lowest mortality rate was recorded in the larvae reared at 5 ppt salinity, while, the highest mortality rate was reported in the larvae reared at 30-ppt salinity followed by those reared at 0.75-ppt salinity. El-Sayed (1991) reported that groups of grey mullet reared in fresh water exhibited poor growth and feed efficiency associated with high mortality rate. Also the striped mullet larvae suffered heavier losses when they were exposed to abrupt change from 20 ppt salinity to freshwater, while the salinity decreasing rate of 5 ppt / 24h resulted in high survival percentages (Mabrouk, 1991).

In experiment 1, salinity had a marked effect on survival rate. The higher the salinity the less was the time spent for the larvae to suffer 100% mortality. Mortality rates decreased with decreasing water salinity from 35 ppt to 5 ppt. At 0-ppt salinity, the larvae exhibited the best survival rate after 25 days and still the best for the day 30. But, the larvae maintained at 5-ppt salinity have got the best survival rate after 35 days until the end of the experiment after 60 days. These results indicate that the mullet larvae may have different salinity requirements for survival at various stages of their development, indicating that the larval need to decrease salinity gradually. In the second experiment, acclimatization of the larvae to reduced salinity of 1 ppt /24h had a marked positive effect on survival and growth. These environmental requirements may force the larvae to migrate from Seawater to fresh water in the rivers. As reported

for euryhaline fish in South Africa, it is thought that adult mullet (*Argyrosomus japonicus*) spawn in inshore waters, and larvae and juveniles recruit to nearshore and estuaries (Beckley, 1990; Fielder and Bardsley, 1999)

The uncovered maintenance requirements known from poor results for the larvae reared in the first experiment of this study may reflect the low ingestion rate of the formulated diet 1. As a result, growth retardation and skeleton abnormalities were observed using the diet 1, can be partly explained by probable dietary unbalanced composition. Increasing energy and vitamins levels in the second experiment could enhance survival rate of the larvae at the period of acclimatization and give better results of survival and growth of mullet larvae using diet 2. Also formulated diets must be fortified by vitamins to overcome the amount shortage due to leaching (Halver, 1988 and El-Dahhar, 1999).

Fish, which display a degree of euryhalinity, may utilize varying levels of energy for osmoregulation as demonstrated by Woo & Kelly, (1995). They reported that the silver sea bream (*Sparus sarba*) adapted to a near isosmotic environment of 15ppt salinity exhibited higher growth rate than that of fish held in seawater or a hyposmotic environment of 7ppt salinity. Also, Brown and Tytler (1993) reported that adaptation to environmental salinity involved changes in gut water absorption in Turbot larvae, where the absolute water absorption increases with the environmental salinity increase, which implies changes in the water permeability of skin/or developing gills.

Mires and Shak (1974) found that, when striped and grey mullet fry were transferred from 24.6 ppt to 8.22 ppt salinity concomitant with sudden changes in temperature, they suffered high mortality. This may explain the high mass mortality that occurred in larvae reared at the higher (35ppt) and the zero salinity in the first experiment.

experiment showed that mortality rate of grey mullet larvae maintained at the lower salinities was lower for a longer period. The best starting point of salinity reduction acclimatization was 3-6 ppt water salinity; e.g. around the same salinity of the natural water from which the larvae were caught. Devaneson and Chaco (1943) reported that gradual lowering of salinity markedly reduced the mortality of mullet fry.

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Table 1. Composition and chemical analyses of the test diets (A and B) used during the study (g / 100 g dry weight).

	Diet A	Diet B
<b>Ingredients:</b>		
Wheat flour	9.7	7.0
Shrimp meal	30.0	30.0
Soybean meal	10.0	6.0
Yellow corn	4.5	5.0
Fish meal	40.0	40.0
Corn oil	3.0	8.0
Bone meal	2.0	2.0
Vitamin & Min. Mix. <sup>1</sup>	0.3	1.2
Vitamin C	0.5	0.8
<b>Nutrients:</b>		
Dry matter	92.51	91.65
Crude protein (N x 6.25)	39.80	39.60
Crude fat	10.87	13.74
Crude fiber	3.39	2.90
Carbohydrate (NFE) <sup>2</sup>	25.45	25.09
Ash	12.91	10.32
<b>Gross energy:</b>		
Kcal / g diet	4.37	4.51

<sup>1</sup> Vitamin and mineral mixture (kg<sup>-1</sup> premix): Vitamin A, 4.8 million IU; vitamin D<sub>3</sub>, 0.8 million IU; vitamin E, 4 g; vitamin K, 0.8 g; vitamin B<sub>1</sub>, 0.4 g; riboflavin, 1.6 g; vitamin B<sub>6</sub>, 0.6 g; vitamin B<sub>12</sub>, 4 mg; Pantothenic acid, 4 g; Nicotinic acid, 8 g; Folic acid, 0.4 g; Biotin, 20 mg; Choline chloride, 200 g; Cu, 4 g; I, 0.4 g; Iron, 12 g; Mn, 22 g; Zn, 22 g; Selenium, 0.4 g.

<sup>2</sup> NFE is nitrogen free extract.



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Table 2. Means of three replicates of the survival rate percentage of striped mullet larvae assigned to the six salinities of the first experiment.

No.	Days					
	Salinity ppt					
	0	5	10	15	20	35
Start	100	100	100	100	100	100
1	58.6 <sup>B</sup>	97.7 <sup>A</sup>	87.7 <sup>A</sup>	100 <sup>A</sup>	97.7 <sup>A</sup>	24.1 <sup>C</sup>
5	46.0 <sup>B</sup>	66.7 <sup>A</sup>	67.8 <sup>A</sup>	74.7 <sup>A</sup>	74.4 <sup>A</sup>	0.0
10	42.5 <sup>B</sup>	64.4 <sup>A</sup>	50.6 <sup>AB</sup>	50.6 <sup>AB</sup>	1.2 <sup>C</sup>	0.0
15	36.8 <sup>B</sup>	62.0 <sup>A</sup>	25.2 <sup>BC</sup>	10.3 <sup>C</sup>	0.0	0.0
20	32.2 <sup>B</sup>	40.2 <sup>A</sup>	6.9 <sup>C</sup>	4.6 <sup>C</sup>	0.0	0.0
25	25.3 <sup>A</sup>	19.5 <sup>A</sup>	2.3 <sup>C</sup>	3.5 <sup>C</sup>	0.0	0.0
30	16.1 <sup>A</sup>	14.9 <sup>A</sup>	0.0	3.5 <sup>C</sup>	0.0	0.0
35	8.0 <sup>B</sup>	11.5 <sup>A</sup>	0.0	1.1 <sup>C</sup>	0.0	0.0
40	4.6 <sup>B</sup>	11.5 <sup>A</sup>	0.0	0.0	0.0	0.0
45	1.1 <sup>B</sup>	8.0 <sup>A</sup>	0.0	0.0	0.0	0.0
50	1.1 <sup>B</sup>	8.0 <sup>A</sup>	0.0	0.0	0.0	0.0
55	1.1 <sup>B</sup>	8.0 <sup>A</sup>	0.0	0.0	0.0	0.0
60	1.1 <sup>B</sup>	8.0 <sup>A</sup>	0.0	0.0	0.0	0.0

Numbers within the same row followed by different superscript letters are significantly different ( $P < 0.05$ ).

Table 3. Means of three replicates of survival rate percentage of grey mullet larvae maintained at the five starting points of salinity acclimation in the second experiment.

No	Days	Starting point salinity acclimation ppt				
		3	6	9	12	15
Start		100	100	100	100	100
6		75.6 <sup>A</sup>	60.0 <sup>AB</sup>	46.7 <sup>B</sup>	72.2 <sup>A</sup>	71.0 <sup>A</sup>
12		67.8 <sup>A</sup>	57.8 <sup>AB</sup>	45.6 <sup>B</sup>	72.2 <sup>A</sup>	55.6 <sup>AB</sup>
18		67.8 <sup>A</sup>	56.7 <sup>AB</sup>	45.6 <sup>B</sup>	71.1 <sup>A</sup>	51.1 <sup>AB</sup>
24		65.6 <sup>A</sup>	54.4 <sup>AB</sup>	42.2 <sup>B</sup>	70.0 <sup>A</sup>	43.3 <sup>B</sup>
30		65.6 <sup>A</sup>	45.6 <sup>B</sup>	33.3 <sup>B</sup>	56.7 <sup>AB</sup>	37.8 <sup>B</sup>
36		57.8 <sup>A</sup>	41.1 <sup>B</sup>	23.3 <sup>C</sup>	41.1 <sup>B</sup>	31.1 <sup>C</sup>
42		53.3 <sup>A</sup>	36.7 <sup>B</sup>	13.3 <sup>C</sup>	33.3 <sup>B</sup>	28.9 <sup>B</sup>
48		44.4 <sup>A</sup>	30.0 <sup>B</sup>	5.6 <sup>C</sup>	26.7 <sup>B</sup>	26.7 <sup>B</sup>
54		33.3 <sup>A</sup>	24.4 <sup>B</sup>	3.3 <sup>D</sup>	24.4 <sup>B</sup>	13.3 <sup>C</sup>

Numbers within the same row followed by different superscript letters are significantly different ( $P < 0.05$ ).

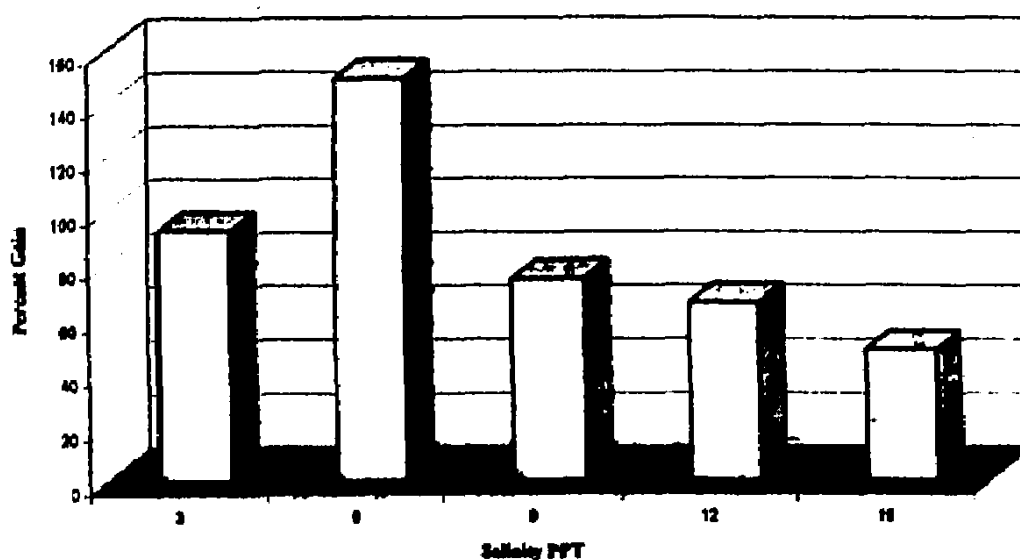


Fig. 1. Percent gain of grey mullet larvae maintained at different water salinities in the second experiment