

## RELATION OF NUTRITION AND SALINITY TO GROWTH IN BLACK MOLLIES

M.B. MINBATTIWALLA AND G.S. GAZDAR

*Wilson College, Chowpatty, Mumbai - 400 007.*

### ABSTRACT

Optimum growth, impaired growth and mortality in black molly are studied in diluted sea water, sea water and also by introducing laboratory-prepared diets. Rapid acclimation in conjunction with enhanced growth capacity was attained at 2‰ concentration of common salt in water, and 2‰ and 5‰ dilutions of sea water. 22‰ protein content in the diet was found to be adequate for growth and fertility of fishes acclimated at a salinity of 2‰. Higher protein content and other nutritive elements were necessary for fishes acclimated at 5‰ and 14‰. Fertility was increased in sea-water dilutions rather than in common salt-water dilutions.

### INTRODUCTION

The ability to survive at different salinity levels varies in different species of fish, depending on the degree to which their osmoregulatory processes can be adapted. In contrast to birds and mammals, most fishes under natural conditions do not cease growth after reaching sexual maturity (Lagler *et al.*, 1962). Food being a major environmental factor often determines fecundity (Wootton, 1979). Hence, nutrition becomes a vital factor for good growth and active breeding behaviour. Thus, diets were formulated and experimented in order to find an ideal food supplement to yield maximum growth. Also, effect of salinity on growth rate was studied when fishes were fed on these diets.

Progressive dilutions of common salt and sea water were used to examine the effect on growth in juvenile mollies,

within the first three months after birth, keeping all other parameters constant.

### MATERIAL AND METHODS

Fry of mollies were reared in the laboratory from inbred stock and maintained at ambient temperature in freshwater. They were fed daily with commercially dried fish food, supplemented once a week with the same amount of live *Tubifex* worms. A thin layer of algae was allowed to grow on the glass at the sides of the aquaria, which served as a supplementary vegetarian diet, and it also provided a shield from excess light. Gravid mollies from this stock were isolated to await release of fry. First day siblings were used in batches of 10 and transferred to various salinities at ambient temperature.

A special flake diet was prepared in the laboratory. Each group was fed a fixed quantity of diet, which was determined after experimentation (approx. 6% of their body weight), twice a day. The same amount of live chironomid larvae (when available) or boiled beef liver were substituted for flake diet once a week. All the wet ingredients (nos. 12-19) in each Food formula (Table 1) were blended together. The dry ingredients (nos. 1-11) were added to the former and blended with lukewarm water to the required consistency. Vitamins, antibiotics and antioxidants were then added to the above mixture and blended. This was spread on clean plastic sheets and dried at 60°C (for formula A, B) and at 27°C (for formula C) and drawn out into flakes of approximately 1mm thickness. The flakes were packed in air tight containers and stored at room temperature till required.

A weekly record of the total weight and length of each fish from each group was kept from Day 1 for a period of three months. The average weight and length for each group of fish as well as their maturation time and survival were recorded. Fry were maintained at ambient temperature for the pilot set and for subsequent three sets.

For the pilot set, 50 fry were divided and acclimated to 2‰, 5‰, 9‰ and 14‰ concentration of common salt in water and a control group (Chart 1). They were fed food formula I-A twice a day. Set no.2 had five experimental groups of salinities 2‰, 5‰, 14‰ and 20‰ concentration of common salt in water and a control group. Fifty siblings were divided and acclimated progressively as in the pilot set and fed food formula I-B twice a day. Set no. 3 had four experimental groups, 2‰ and 5‰

29th Jan	2nd Feb	6th Feb	10th Feb	14th Feb	18th Feb	
Control (F/W)	50	40	30	20	10	
2‰ common salt		10	10	10	10	D
5‰ common salt			10	10	10	A
9‰ common salt				10	10	Y
14‰ common salt					10	1

Chart 1 : No. of siblings (10) acclimated at each salinity of common salt dilutions made with F/W, allowing five days for acclimation in each salinity.

Table 1 : Gram (g) weight composition of 25 ingredient combinations prepared into flakes for desirable yield in growth of black mollies.

Ingredients	Food formula	Food formula	Food formula
	A	B	C
	wt in g	wt in g	wt in g
1. Powdered Alfalfa leaves	250	-	-
2. Sun-dried fish & shrimp meal	93	50	100
3. Wheat flour	-	30	40
4. Wheat germ (Weikfields)	-	2	-
5. De-oiled groundnut oil-cake	-	-	50
6. Sodium bicarbonate (NaHCO <sub>3</sub> )	-	-	2
7. Calcium carbonate (MgSO <sub>4</sub> )	-	-	2
8. Magnesium sulphate (MgSO <sub>4</sub> )	-	-	2
9. Dried bovine blood	-	-	10
10. Common salt	-	-	5
11. Bread	250	-	-
12. Whole egg, raw	2 eggs	-	1 egg
13. Boiled carrots	-	-	25
14. Scalded lettuce	-	-	25
15. Boiled peas	-	-	20
16. Scalded spinach	-	-	5
17. Raw beef heart muscles	-	-	50
18. Raw beef mince	250	-	-
19. Boiled beef liver	-	-	10
20. B.H.A. <sup>1</sup>	-	0.03	0.03
21. Codliver oil <sup>2</sup>	-	4ml	10ml
22. Becadexamin <sup>3</sup>	-	1 capsule	2 capsules
23. Terramycin <sup>4</sup>	-	1 capsule	2 capsules
24. Evion <sup>5</sup>	-	1 capsule	2 capsules
25. Cecon <sup>6</sup>	-	1 ml	2 ml

<sup>1</sup>B.H.A. - Butylated Hydroxy Anisole - Amrut Industrial Product.

<sup>2</sup>Seven Seas codliver oil - Universal Generics Pvt. Ltd.

<sup>3</sup>Becadexamin - Multivitamin Multimineral capsule - Glaxo India Ltd.

<sup>4</sup>R<sup>x</sup>Terramycin - Antibiotic capsule - Pfizer Ltd.

<sup>5</sup>Evion - Vitamin E-Merck (India) ltd.

<sup>6</sup>Cecon - Vitamin C drops - Abbott Laboratories (India) Ltd.

Table 2 : *Percentage survival of black mollies in the four experimental sets.*

Sets	Medium					
	Fresh water (control)	2‰	5‰	9‰	14‰	20‰
Pilot set	70	70	70	40	90	-
	----- Acclimations in common salt -----					
Set no. 2	70	100	30	-	40	20
	----- Acclimations in common salt -----					
Set no. 3	77.7	88.8	66.6	-	88.8	-
	----- Acc. in common salt -----				Acc. in seawater	
Set no. 4	100	100	100	-	100	
	----- Acclimations in sea water -----					

Table 3 : *Fertility and number of fry produced within the experimental time, in the four experimental sets.*

Sets	Medium					
	Fresh water (control)	2‰	5‰	9‰	14‰	20‰
Pilot set	nil	nil	nil	nil	nil	-
	Acclimations in common salt					
Set no. 2	nil	6	nil	-	nil	nil
	Acclimations in common salt					
Set no. 3	nil	nil	nil	-	nil	-
	Acc. in common salt				Acc. in seawater	
Set no. 4	nil	4	nil	-	5	-
	Acclimations in sea water					

concentration of common salt in water, 14‰ dilution of sea water and a control group. Thirty-six siblings were divided in batches of nine and acclimated in each group. Food formula I-C was fed to the siblings in this set as well as in the final set. For the forth and final set, 40 siblings were divided and acclimated to 2‰, 5‰ and 14‰ dilutions of sea water and a control group.

## RESULTS AND DISCUSSION

The pilot set showed an anomaly in overall growth pattern (Figs.1a,1b). There was a marginal increase in weight and length upto the end of 4th week, after which a steady rise in weight and length was noticed. At the end of the 13th week, best overall growth was obtained by 2‰ and 14‰ followed by 9‰, fresh water control and 5‰. The general level of metabolism might have been higher in 2‰ and 14‰ concentrations of common salt in water, resulting in increased appetite and quick assimilation of nutrients, finally leading to beneficial growth. Fish acclimated in 9‰ concentration of common salt in water attained a better overall growth. The medium exerted stress not enabling the mollies to survive the experimental period (Table 2). No fry was produced during the experimental period in this set (Table 3).

Food formula A was modified, adding wheat flour and wheat germ instead of alfalfa leaves and bread (Table 1-B) to attain good textural quality and to provide a rich source of crude proteins.

100% survival as well as maximum average weight and length gain were obtained in 2‰ salinity (Fig 2a, 2b) compared to salinities in Set no. 2; this was the only dilution in which six fry were obtained by the end of the 14th week of acclimation (Table 2). Acclimation of fish in 14‰ dilution of common salt compared well with 2‰ dilution in growth measurements, but not for survival and fertility; hence, acclimation in 14‰ dilution of sea water was tried in set no. 3. Poor growth and survival were noted in 5‰ and 20‰ salinity at the end of the experimental period.

The results derived collectively from the experiments of the pilot set and set no. 2 indicate that : (1) A higher protein content in fish food required for growth particularly when acclimated to salinities higher than 2‰ dilutions of common salt. (2) 2‰ concentration of common salt in water was required for favourable growth, survival and fertility; however, survival for newly born fry was poor in the medium (all the six fry died within a week).

Growth maxima as defined by Zeitoun *et al.* (1976), may be in a range of dietary protein concentration from 40% to 50%. Hence, the best diet formulation must incorporate the best balance of protein and energy sources. In view of this, food formula B was further modified to contribute towards optimum growth and improvement in survival and fertility with respect to salinity alone within the experimental time. Consequently, in food formula C (Table 1-C) quantities of fish

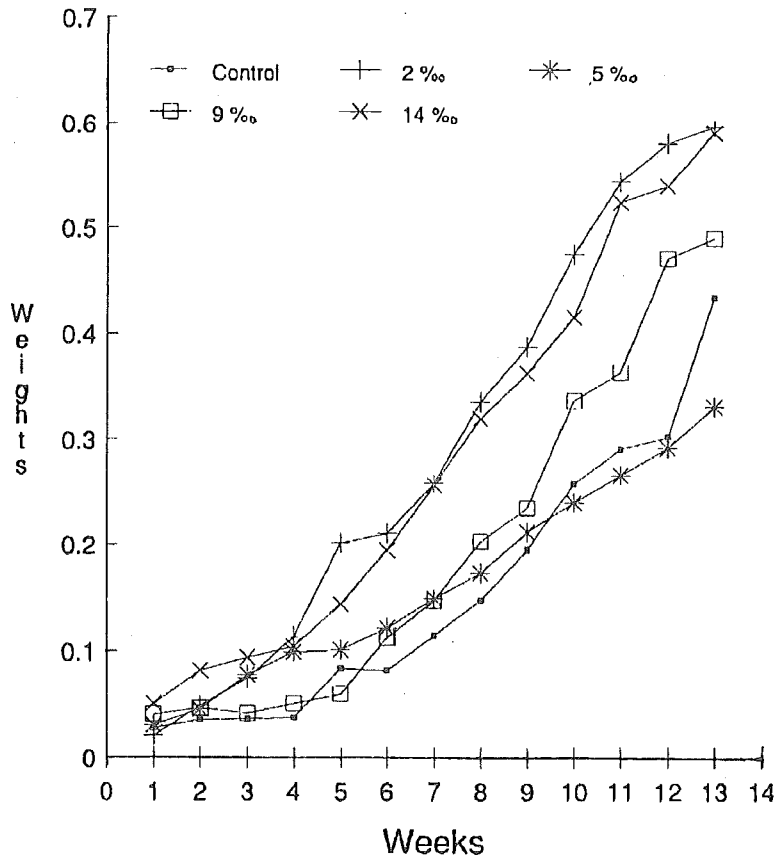


Fig. 1a : Average weight of mollies (pilot set) at different salinities.

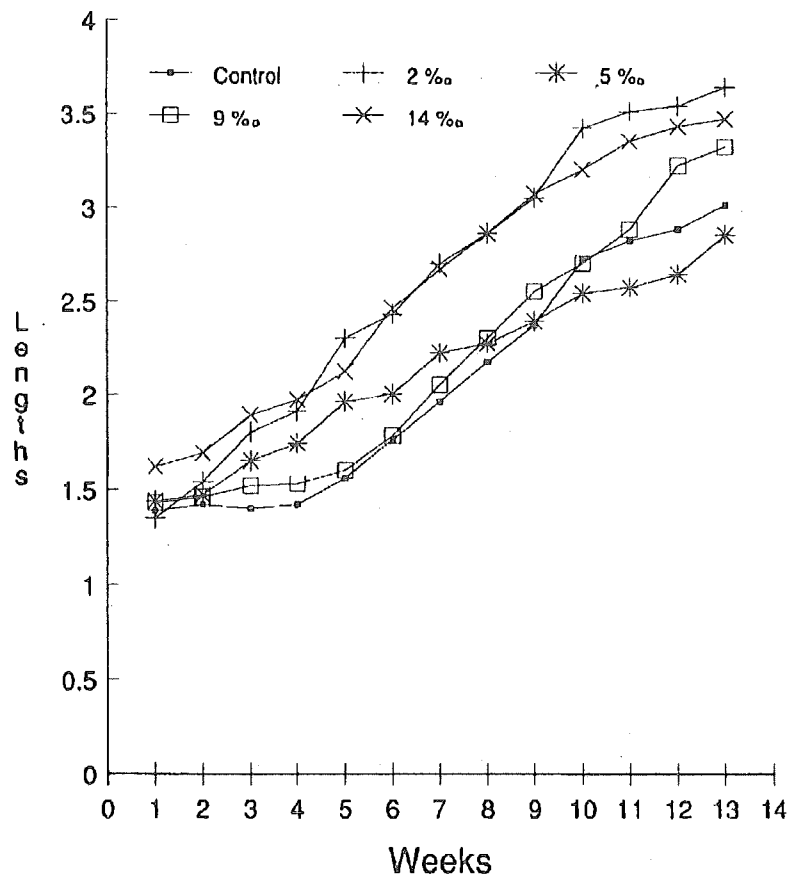


Fig. 1b : Average length of mollies (pilot set) in different concentrations of common salt in water.

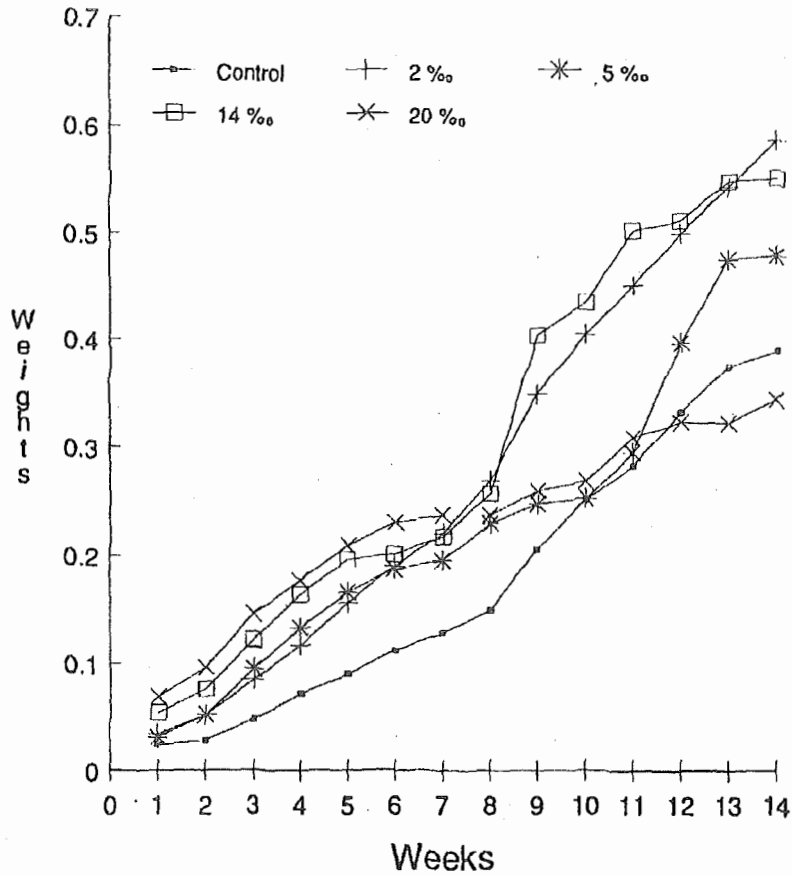


Fig. 2a : Average weight of mollies (set no.2) at different salinities

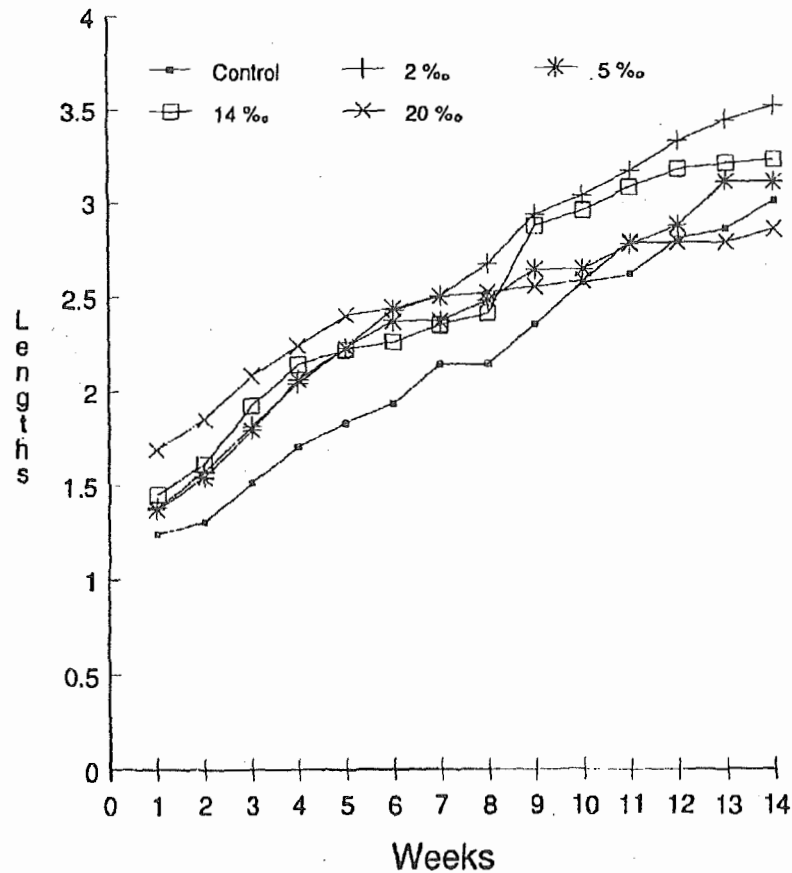


Fig. 2b : Average length of mollies (set no.2) in different concentrations of common salt in water.

and shrimp meal were increased, while raw beef heart muscles, boiled liver, eggs and dried bovine blood were added to enhance the Animal Protein Factor, besides preventing loss of essential amino acids during processing (Bender, 1978).

De-oiled groundnut oil cake provided additional proteins while peas, carrots, lettuce and spinach supplied beta-carotene and minerals to the diet. Mineral mixtures of  $\text{NaHCO}_3$ ,  $\text{CaCO}_3$ ,  $\text{MgSO}_4$  and common salt were incorporated, so that growth might not be affected due to mineral deficiencies. The qualitative analysis by conventional methods showed the contents of ash to be 10.58%, moisture 11.75%, lipids 11.49%, protein 52.03% and carbohydrates 14.15%.

In set no.3 a dramatic overall increase was seen in the growth of fishes acclimated to 14‰ sea water as compared to the other salinities (Figs. 3a, 3b). Higher fertility within the experimental time was observed in fishes acclimated in 14‰ sea water, and the newly born fry were healthy and survived well without showing any signs of lethargic behaviour. Thus, diluted sea water was a better medium for the growth, survival and fertility within the shortest possible period.

In order to ascertain effectiveness of sea water in lower saline dilutions of 2‰ and 5‰ a last and final set no. 4 was undertaken. The fish acclimated in 5‰ salinity showed maximum weight gain followed by fish in 2‰ salinity, but was the reverse for gain in length (Figs. 4a,

4b). Fish acclimated in 14‰ salinity showed better gain in weight compared to those in fresh water control; their growth in length was less than that of the latter. A noticeable feature in this set was a 100% survival in all the salinities (Table 3). These results confirmed 2‰ and 5‰ sea water dilutions to be the best for survival.

Kinne (1962) showed that the adjustments of salinity tolerance in *Cyprinodon macularis* persisted throughout the lives of the fish hatched from eggs that were exposed during the first 3-6h after oviposition, in water in which the parent fish lived and also that these adjustments were not transmitted to the next generation. The present study, however, does not fully support Kinne's conclusion as mollies bred till F3 generation showed adjustments of salinity tolerance in 2‰, 5‰ and 14‰ dilutions of sea water. However, for these adjustments to be termed genetic would require acclimations to be continued for several generations.

Weight gain as a factor for measure of growth, has been criticized to be inaccurate since gain in weight may result from deposition of fat rather than from true growth (Maynard and Loosli, 1969). However, in the present study, true growth has been observed as weight gain and increase in size have resulted from acclimations in different salinities rather than food, which was kept constant throughout the four experimental sets.

The present experimental findings thus support the hypothesis that several



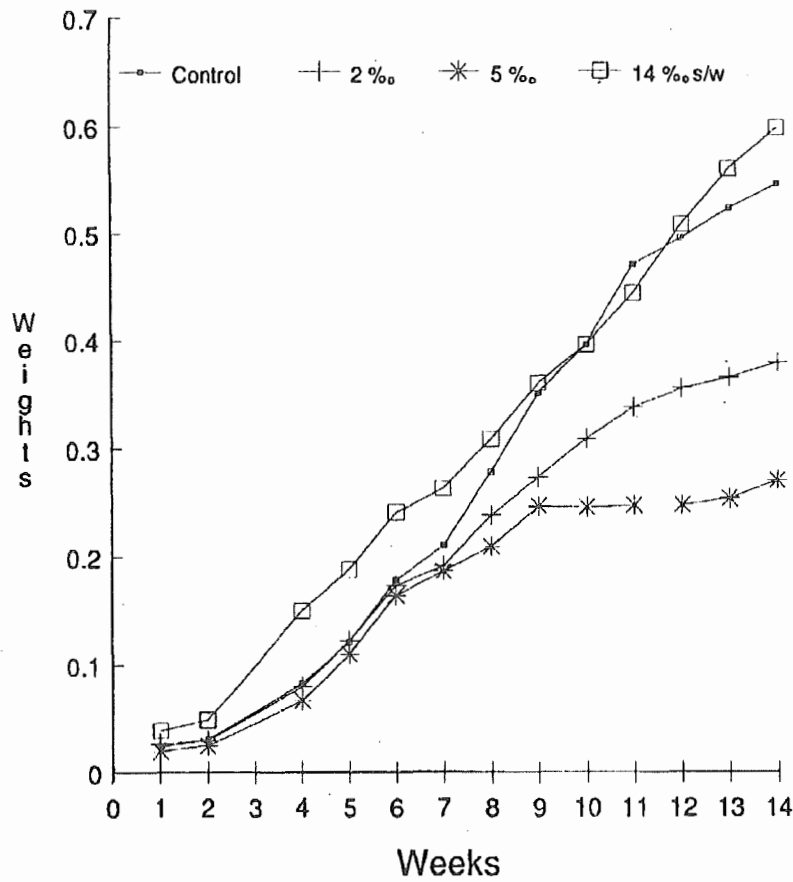


Fig. 3a : Average weight of mollies (set no.3) in different concentrations of common salt in water and in different dilutions of seawater

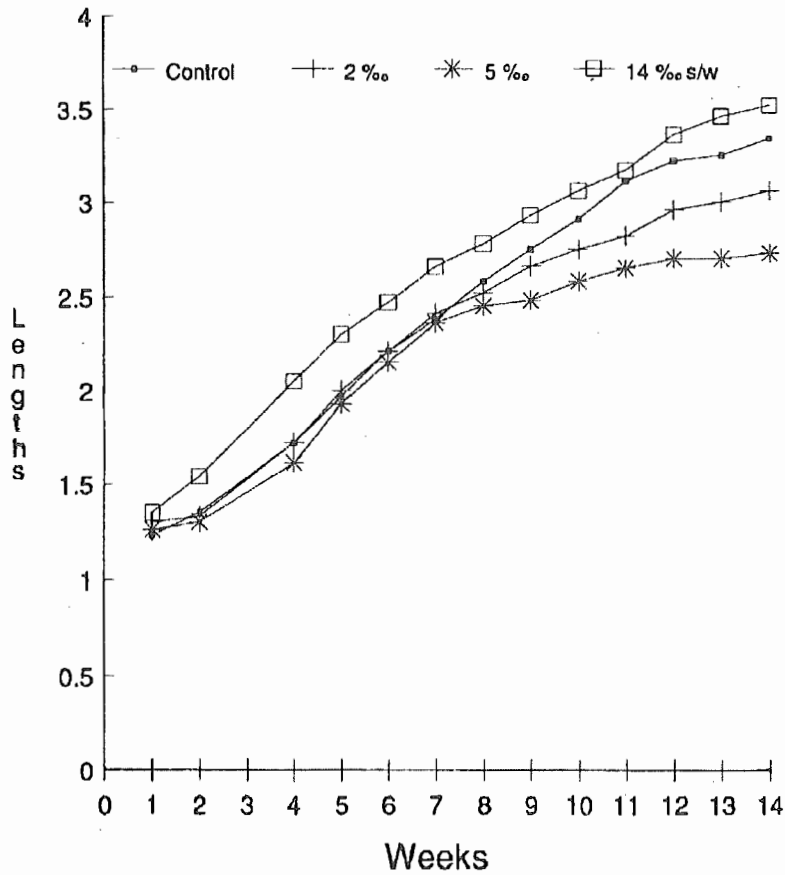


Fig. 3b : Average length of mollies (set no.3) in different concentrations of common salt in water and in different concentrations of seawater.

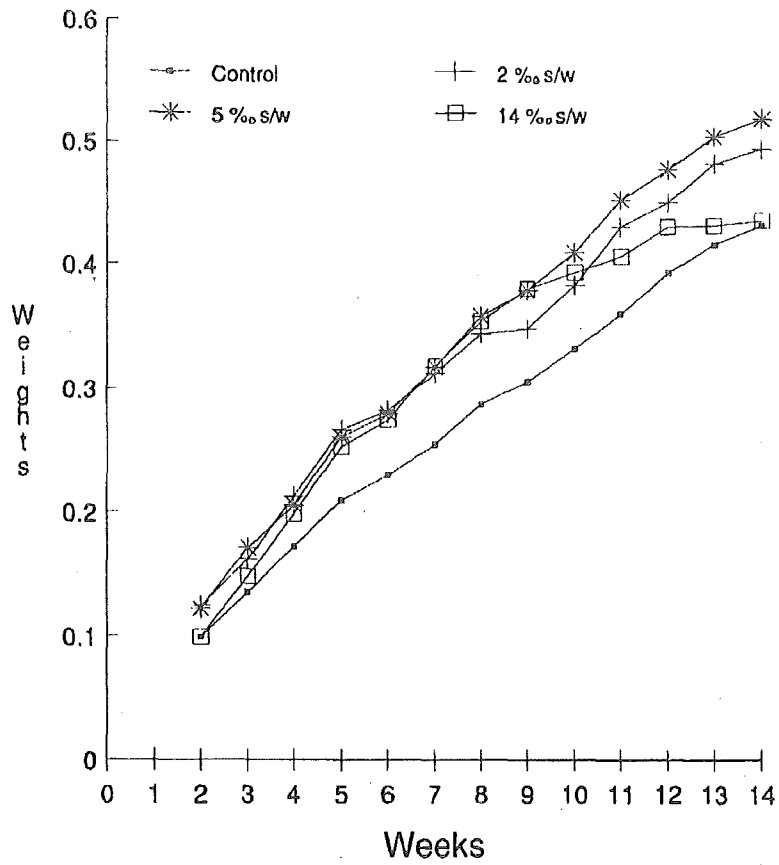


Fig. 4a : Average weight of mollies (set no.4) in different dilutions of sea water

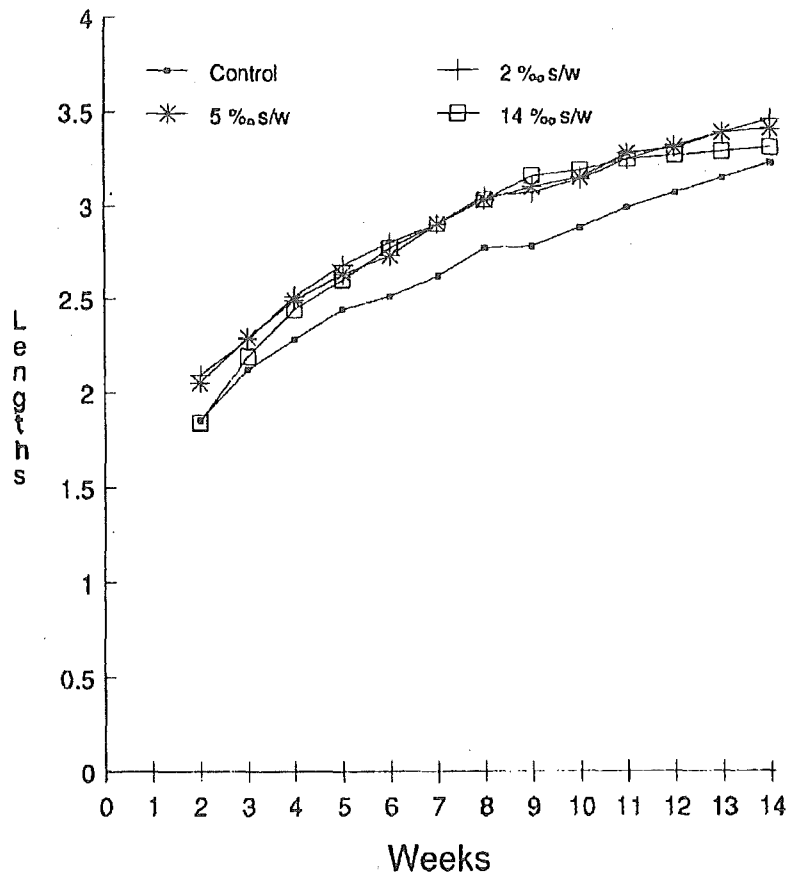


Fig. 4b : Average length of mollies (set no.4) in different concentrations of seawater.

euryhaline species of fish thrive well in waters of different salinity. In conjunction with this hypothesis, the theory, that "the larger size of the marine forms is due to higher osmotic content of the medium" (Canagaratnam, 1959), also holds true.

#### ACKNOWLEDGMENTS

We are indebted to Drs. S. P. Karmarkar and B. F. Chappgar for helpful discussions and suggestions and for reviewing the manuscript. We also thank Mr. N. Behramfram and Mr. R. Chinoy for their assistance in Harvard Graphics.

#### REFERENCES

- Bender, A. E.** 1978. In : *Food Processing and Nutrition*. Academic Press, New York, London. pp. 59-79.
- Canagaratnam, P.** 1959. Growth of fishes in different salinities. *J. Fish. Res. Bd. Canada*. **16**(1) : 121-130.
- Kinne, O.** 1962. Irreversible non-genetic adaptation. *Comp. Biochem. Physiol.* **5** : 265-282.
- Lagler, K. F. Bardach, J. E. and Miller, R.R.,** 1962. *Ichthyology*. John Wiley & Sons Inc., New York.
- Maynard, L. A. and Loosli, J. K.** 1969. *Animal Nutrition*. McGraw-Hill Book Co., New York.
- Wootton, R. J.** 1979. Energy costs of egg production and environmental determinants of fecundity in teleost fishes. pp. 133-159. In: P. J. Millar (Ed.) *Fish Phenology. A Symposia of the Zool. Soc. London*. No.44.
- Zeitoun, I.H., Ullrey, D. E., Magee, W. T., Gill, J.L. and Bergen, W.G.** 1976. Quantifying nutrient requirements of fish. *J. Fish Res. Bd. Canada*. **33** : 167-172.