

# THE EFFECT OF SALINITY AND TEMPERATURE ON THE GROWTH AND SURVIVAL OF PENAEID POSTLARVAE<sup>1</sup>

By

**MINDA C. VALENCIA**

Fishery Biologist, Fisheries Training Division  
Bureau of Fisheries and Aquatic Resources

## ABSTRACT

The effect of salinity on the survival and growth of *Penaeus monodon* (Fabricius), *P. semisulcatus* (de Haan), *P. merguensis* (de Haan) postlarvae was examined. Postlarvae P<sub>15</sub> were gradually acclimated to the desired salinity before starting the experiments. Minced tuna or lizard fish meat given at a rate of 100% body weight was used as feed. High survival of *P. monodon* was obtained at 30 ppt salinity; however, growth was faster at 10 ppt. On the other hand, with *P. semisulcatus*, growth and survival rates were higher at 30 ppt. Survival rates for *P. marchiensis* and *M. ensis* were the same at the salinity ranges of 10 to 25 ppt and 10 to 30 ppt, respectively, although lower salinities apparently favored faster growth. With *P. japonicus*, growth was essentially similar at 10 to 25 ppt; however, survival rates were higher at 20 and 25 ppt.

Tolerance of *P. monodon*, *P. semisulcatus*, *P. merguensis*, *P. japonicus*, *M. ensis* and *M. endeavouri* to sudden changes in salinity was also examined. Postlarvae were held at a given salinity and transferred to salinities higher or lower than the initial salinity. Results showed wide salinity tolerance of all species examined.

Preliminary studies on the effect of temperature-salinity combination on *P. monodon* postlarvae were also conducted. Postlarvae were subjected to three temperatures: 21-23°C, 25-30°C and 33-35°C and three salinity levels: 10, 20 and 30 ppt. Results suggest that both salinity and temperature interact to

---

<sup>1</sup> A Research Paper submitted to SEAFDEC during the First Southeast Asian Regional Training on Aquaculture Research in Tigbauan, Iloilo, Philippines (1976-1977) under the supervision of Dr. F. F. M. Catedral.

affect growth and survival of *P. monodon*. A combination of low to intermediate temperature and intermediate to high salinity gave higher survival; however, low salinity-high temperature combination favored faster growth.<sup>2</sup>

### INTRODUCTION

Temperature and salinity are important ecological factors in determining basic relationships between the organism and its environment (Venkataramiah, *et al.*, 1972). The effect of these factors on the function, structure and adaptation of marine and brackishwater animals had been reviewed by Kinne (1963, 1964). Salinity and temperature were also shown to affect survival, growth and the food conversion efficiency of a few shrimp species (Johnson and Fielding, 1956 and Zein-Eldin and Aldrich, 1965).

Studies on the tolerance of *Penaeus monodon* to salinity and temperature have been undertaken at SEAFDEC, Tigbauan, Iloilo, Philippines (Catedral, *et al.*, 1975). Results indicated that postlarvae from P<sub>10</sub><sup>2</sup> can tolerate salinities between 5 and 39 ppt, from an initial salinity of 36 ppt, with acclimation. However, postlarvae P<sub>5</sub> to P<sub>9</sub> were found to be less tolerant to salinity changes. Growth appeared to be faster at lower salinities. The temperature studies suggested that postlarvae can withstand temperatures between 24°C and 35°C at salinities of 35 to 39 ppt. At 37.5°C, high mortality was obtained.

Although these studies were conducted on *P. monodon*, the effect of temperature on salinity tolerance of postlarvae, and vice versa, is not known. Postlarvae stocked in fishponds are generally subjected to varying combinations of these two factors. A knowledge of the combined effect of salinity and temperature will help in the understanding of how these factors interact to effect the postlarvae.

Studies on salinity and temperature effects on survival and growth of other penaeids have been reported by other investigators. Zein-Eldin and Aldrich (1965) found that postlarvae of *P. aztecus* can survive temperatures of as low as 11°C with almost no growth at salinities of about 15 ppt or above. Zein-Eldin and Griffith (1969) also studied the effects of salinity and temperature on the growth and survival of postlarvae of two penaeids (*P. aztecus* and *P. seti-*

<sup>2</sup> P<sub>10</sub> is 10 days old after molting from the mysis stage.

*ferus*) and obtained results which suggested differences in response between the two species. This work was, however, done with penaeids not common in the Philippines. Knowledge of differences among the penaeids found here could aid in the understanding of species differences and selection of environments for culture. With the increasing demand for prawns, efforts should be extended not only towards the culture of *P. monodon* but to other shrimp species as well.

### MATERIALS AND METHODS

#### Experimental Animals

*Penaeus monodon*, *P. semisulcatus*, *P. merquiensis*, *P. japonicus*, *Metapenaeus ensis* and *M. endeavouri* reared in the Wet Laboratory, SEAFDEC, Tigbauan, Iloilo, were used in the study. At stage P<sub>15</sub>, the postlarvae were acclimated for two days in the laboratory prior to starting the experiments. Only active and healthy postlarvae were chosen.

#### Feeding

Minced tuna or lizard fish meat was fed to the postlarvae at a rate of 100% body weight. Minced tuna meat (white) contained 20.0% protein, minced tuna meat (red), 30.0% protein, and lizard fish meat 15.0% protein (See Appendix A).

#### Measurement of Growth

Growth was determined by measurement of body weight. The postlarvae were first blotted with filter paper and allowed to air-dry for one minute prior to weighing. Initial weights were measured on at least 20 postlarvae at the start of the experiment and the average taken. At the end of the experiment, the average weight of the survivors was again measured.

#### Experimental Design

*Salinity effects on growth and survival.* The experimental species were acclimated to the desired salinity for two days. Salinities of 10, 20, 30 and 40 ppt were used for *P. monodon*; 10, 20 and 30 ppt for *P. semisulcatus* and *M. ensis*; and 10, 20 and 25 ppt for *P. japonicus*. All experiments were conducted in duplicates.

Postlarvae were stocked into 10-liter aquaria at a rate of 10 postlarvae per liter of water. Two-thirds of the total volume of water was changed once a week while wastes were siphoned out daily before feeding. Aerating system was provided for the entire experimental set-up. Experiments were conducted for four weeks or until survival rates of postlarvae in one experimental salinity went below 10%.

*Salinity tolerance.* Postlarvae were acclimated to a given salinity (i.e., 15, 20, 25, 30, 35 and 40 ppt) for a few days prior to using them in the experiments. They were transferred to 4-liter beakers with salinities higher or lower than the initial salinity. Survival rate was then measured after 24 hrs. Percentage survival was observed on at least 10 postlarvae with two or more replicates per experimental run.

*Effect of salinity-temperature combination on growth and survival.* The effect of temperature-salinity combination on growth and survival of *P. monodon* was examined using three different temperatures (21-23°C, 25-30°C and 33-35°C) and three salinity levels (10, 20 and 30 ppt). Postlarvae were acclimated for one day to the corresponding temperature-salinity combination before commencing the experiments. One hundred postlarvae were stocked into 20-liter aquaria at a stocking rate of 10 postlarvae per liter of water. Temperature was maintained using either an electronic thermostat water bath or immersible glass heaters provided with thermostats. Experimental temperatures varied within 2°C throughout the study.

#### *Physico-Chemical Measurements*

Physico-chemical parameters such as nitrite concentration, ammonia concentration, pH, salinity and temperature were measured periodically. Nitrite and ammonia concentrations were determined spectrophotometrically (Strickland and Parsons, 1972). Salinity and pH measurements were made using a refractometer (American Optical) and pH meter, respectively.

## RESULTS AND DISCUSSION

### *Effect of Salinity on Growth and Survival*

Table I shows the effect of salinity on growth and survival of the different penaeid species examined. Growth rates (percent in-

TABLE I. Effect of salinity on the survival and growth of Penaeid post-larvae.

Species	Culture Period (day)	Salinity (ppt)	% Increase in Weight ( fry/day)	% Survival
<i>Penaeus monodon</i>	28	10	35.1	11.6
		20	17.4	42.3
		30	10.8	71.0
		40	1.4	14.3
<i>P. semisulcatus</i>	16	10	35.3	7.4
		20	32.3	20.2
		30	54.2	39.6
<i>P. merguensis</i>	25	10	57.8	4.3
		20	45.7	6.7
		25	48.8	5.9
<i>P. japonicus</i>	25	10	16.0	7.9
		20	16.9	21.1
		25	10.3	25.0
<i>Metapenaeus ensis</i> <sup>1</sup>	23	10	35.4	43.5
		20	28.5	44.5
		30	0.6	45.5

<sup>1</sup> Affected by the fungus, *Lagenidium*.

crease in weight) of 35.1 and 58.8% were obtained at 10 ppt for *P. monodon* and *M. ensis*, respectively. On the contrary, growth rate of *P. semisulcatus* 54.2% was highest at 30 ppt. For *P. merquiensis* and *P. japonicus*, percentage increase in weight of the postlarvae was generally the same at all salinity levels. Detailed information on the initial and final weight measurements of the different species are given in Appendix B.

The effect of salinity on survival rates of the different species also varied (Table I). Highest survival rates were obtained at 30 ppt for *P. monodon* and *P. semisulcatus* with values of 71.0 and 39.6%, respectively. On the other hand, survival rates of *P. japonicus* increases with salinity although the difference at 20 and 25 ppt was small. Survival rates of *P. merquiensis* and *M. ensis* were the same at all salinity levels used.

From the results, it can be seen that under laboratory conditions, *P. monodon* postlarvae grow better at low salinity (10 ppt); however, survival rates are highest at the salinity of seawater (30 ppt). On the other hand, *P. semisulcatus* postlarvae grow and survive better at 30 ppt. Survival rates for *P. merquiensis* and *M. ensis* postlarvae are the same at all salinity levels examined, although lower salinities apparently favor faster growth. With *P. japonicus* postlarvae, growth is essentially similar at 10 to 25 ppt; however, survival rates are higher at 20 and 25 ppt.

Throughout the study, experimental salinities varied within one ppt and temperature fluctuated from 24.5-28.2°C for all the species examined. pH varied from 7.4 to 8.6 while nitrite and ammonia concentrations ranged from 0 to 26.50 and 0 to 12.54 ppm, respectively. It has been reported that no significant effects on growth and survival of *P. monodon* postlarvae were observed at these nitrite and ammonia concentration levels (Catedral, *et. al.*, Progress Report, 1975). Detailed ranges of nitrite and ammonia concentrations, pH and temperature are shown in Appendix C.

#### Salinity Tolerance

Figs. 1-6 show the tolerance of *P. monodon*, *P. semisulcatus*, *P. merquiensis*, *P. japonicus*, *M. ensis* and *M. endeavouri* to sudden changes in salinity. The high mean percentage survival at a wide range of salinity (i.e., 10 to 40 ppt) suggests wide salinity tolerance of all species examined. Above 45 ppt, however, higher mortalities were

obtained, even at an initial salinity of 35 ppt. Salinities of 55 ppt and above were found to be lethal to all the species.

Table II shows the salinities to which postlarvae could be brought from given initial salinities without causing undue stress to the species. The values given are those which gave at least 60% survival (Figs. 1-6). It can be seen that increasing the initial salinity increases the tolerance towards higher salinities up to 45 ppt; likewise, decreasing the initial salinity increases the tolerance towards lower salinities.

#### Salinity-Temperature Combination

Preliminary results on the effects of combination of salinity and temperature on growth and survival of *P. monodon* postlarvae obtained from two experimental runs are shown in Table III. High survival rates of *P. monodon* were obtained at salinity-temperature combinations of 20 ppt and 21-23°C, 30 ppt and 21-23°C, 20 ppt and 25-30°C, 30 ppt and 25-30°C and 20 ppt and 33-35°C. At salinity-temperature combinations of 10 ppt and 25-30°C and 10 ppt and 33-35°C, low survival rates were obtained, although faster growth was apparently favored. It may be noted that at salinities of 10 and 20 ppt, growth is directly dependent on temperature up to 35°C. At 30 ppt, maximum growth occurs at 25-30°C. Survival rates at 10 ppt are much lower than at 20 and 30 ppt, however, with temperatures of 33-35°C, survival rates at 30 ppt salinity is much reduced. In the study, problems were encountered due to occurrence of the fungus, *Lagenidium* and equipment malfunction. Thus, some experimental runs were discarded.

#### Ecological Implications

The role of salinity and temperature in the growth and development of shrimp and other species has been a subject of much investigation (Kinne, 1963, 1964). Zein-Eldin and Aldrich (1965) reported that wide ranges of salinity and temperature were well tolerated by *P. aztecus* postlarvae. Zein-Eldin and Griffith (1966) also reported that the growth of *P. aztecus* is directly dependent on temperature up to 32.5°C. However, maximal increase in growth per unit temperature was observed in the temperature range of 17.5-25°C. Work on *P. aztecus* and *P. setiferus* also showed that both species tolerate wide ranges of temperature and salinity, but some differences between species existed (Zein-Eldin and Griffith, 1969). The present study using penaeids found locally also show species differences with

TABLE II. Tolerance of Penaeid postlarvae toward sudden changes in salinity.

Initial Salinities (ppt)	Salinity Units, ppt											
	P. monodon		P. semisulcatus		P. merguensis		P. japonicus		M. endeavouri		M. ensis	
	Lowest	Highest	Lowest	Highest	Lowest	Highest	Lowest	Highest	Lowest	Highest	Lowest	Highest
15	3	40	-	-	-	-	-	-	-	-	-	-
20	3	40	-	-	-	-	-	-	-	-	-	-
25	-	-	10	40	5	35	10	40	-	-	-	-
30	5	40	10	45	10	35	-	-	10	45	5	45
35	10	45	10	45	-	-	-	-	15	45	5	45
40	-	-	-	-	-	-	-	-	-	-	-	45

TABLE III. Effect of salinity-temperature combination on the survival and growth of *P. monodon*.

Salinity, ppt Temperature °C	% Survival			% Increase in weight/fry/day		
	10	20	30	10	20	30
21-23	15.0	48.7	42.0	12.5	9.3	12.7
25-30	8.7	36.3	63.9	37.9	14.6	12.7
33-35	5.7	35.1	6.3	58.9	18.0	6.1

respect to tolerance to salinity and its effect on growth and survival of the postlarvae. In general, however, the major effect of higher salinity is higher survival but slower growth, whereas, low salinity allows faster growth (*i.e.*, *P. monodon*, *P. merquiensis*, and *M. ensis*). The growth rates obtained in this study agree with the observations made by Gunther (1945, 1950), and Pearse and Gunther (1957) that penaeid postlarvae require lower salinities for growth. This study also shows that with *P. monodon*, salinity effects on growth and survival are influenced by temperature.

#### CONCLUSIONS AND RECOMMENDATIONS

The following conclusions can be drawn from this study:

1. Of the three salinity levels tested, 10 and 20 ppt appear to provide better growth for all species examined except *P. semisulcatus*. With *P. semisulcatus*, both survival and growth rates were higher at 30 ppt. However, high survival was obtained at 30 ppt for all species examined.
2. Postlarvae ( $P_{15}$  and up) can tolerate wide changes in salinity. However, above 45 ppt, higher mortality was obtained. Salinities of 55 ppt and above appear to be lethal to all species examined.
3. Preliminary results obtained on the combined effects of salinity and temperature upon growth and survival of *P. monodon* postlarvae suggest that salinity and temperature interact to affect growth and survival of the species. A combination of low to intermediate temperature and intermediate to high salinity gave higher survival; however, low salinity-high temperature combination favored faster growth.

Further studies should be made to determine the combined effect of salinity and temperature upon growth and survival of the penaeid species examined. Other natural factors such as food and light should also be considered.

#### ACKNOWLEDGMENT

I extend my heartfelt gratitude to my adviser Dr. Francis Fred M. Catedral for his unselfish guidance, assistance and valuable criticisms throughout the study. I am also grateful to Dr. Cesar T. Villegas and Mr. Hiroshi Motoh for their comments and suggestions; to the Wet Laboratory staff for supplying the postlarvae; to the Pathology Laboratory staff for identifying the fungus, *Lagenidium*; to the Phycology Laboratory staff for supplying the diatoms; and to the Training and Extension staff for helping secure facilities and equipment for the experiments.

Appreciable amount of my gratitude is also due to the personnel in the Chemistry Laboratory for helping finish this work; to all my friends for offering good suggestions and encouragement; and to all those who in one way or another helped complete this work.

Special thanks are due to Director Felix R. Gonzales, Bureau of Fisheries and Aquatic Resources, and Mr. Pablo T. Tamesis, Officer-in-Charge, Fisheries Training Division, BFAR, for giving me the privilege to attend this training in Aquaculture Research.

#### REFERENCES

- CATEDRAL, F.F., N. VALERA, C.M. CASALMIR, and A.J. QUIBUYEN. 1975. Establishment of optimal water conditions for mass seed production of *Penaeus monodon*. SEAFDEC, Tigbauan, Iloilo. pp. 1-4.
- GUNTHER, G. 1945. Studies on marine fishes of Texas. Publications of the Institute of Marine Science. University of Texas. 1:1-90.
- . 1950. Seasonal population changes and distributions as related to salinity of certain invertebrates of the Texas coast, including the commercial shrimp. Publications of the Institute of Marine Sciences. University of Texas. 2:7-51.
- JOHNSON, M.C. and J.R. FIELDING. 1956. Propagation of the white shrimp, *Penaeus setiferus* (Linn.) in captivity. Tulane Stud. Zool. 4:175-190.

KINNE, O. 1963. The effect of temperature and salinity on marine and brackishwater animals I. Temperature. *Oceanog. Mar. Biol. Ann. Rev.* 1:301-340.

——— 1964. The effect of temperature and salinity on marine and brackishwater animals II. Salinity and temperature combinations. *Oceanog. Mar. Biol. Ann. Rev.* 2:281-339.

PEARSE, A.S. and G. GUNTER. 1957. Salinity. In: *Treatise on Marine Ecology and Paleontology*. Vol. I. J.W. Hedgpeth, ed. Geological Society of America, Memoir 67. New York. pp. 129-158.

STRICKLAND, J.D.H. and T.R. PARSONS. 1972. A practical Handbook of Seawater Analysis. Bulletin 167 (2nd ed.) Fisheries Research Board of Canada. Ottawa. 310 pp.

VENKATARAMIAH, A.G.J. LAKSHMI and G. GUNTHER. 1972. The effects of salinity, temperature and feeding levels on the food conversion, growth and survival rates of the shrimp *Penaeus aztecus*. Marine Technology Society. Food-Drugs from the Sea. pp. 1-11.

ZEIN-ELDIN, Z.P. and D.V. ALDRICH. 1965. Growth and survival of postlarval *Penaeus aztecus* under controlled conditions of temperature and salinity. *Biol. Bull.* 129:212-213.

——— and G.W. GRIFFITH. 1966. The effect of temperature upon the growth of laboratory held postlarval *Penaeus aztecus*. *Biol. Bull.* 131-186-196.

——— 1969. An appraisal of the effects of salinity and temperature on growth and survival of postlarval penaeids. *FAO Fish Rep.* 3:1015-1025.

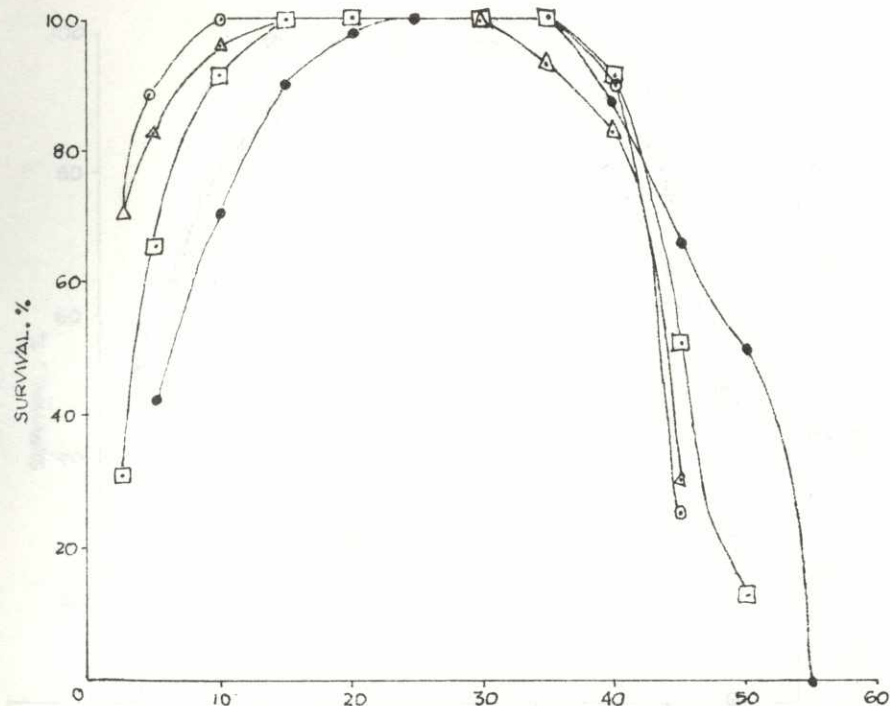


FIGURE 1. Salinity tolerance of *P. monodon* postlarvae ( $P_{15}$ - $P_{34}$ ). Postlarvae were acclimated to initial salinities of 35 ppt (●), 30 ppt (□), 20 ppt (△) and 15 ppt (○) for at least 2 days and transferred to salinities higher or lower than the initial salinity. Survival was observed after 24 hours.

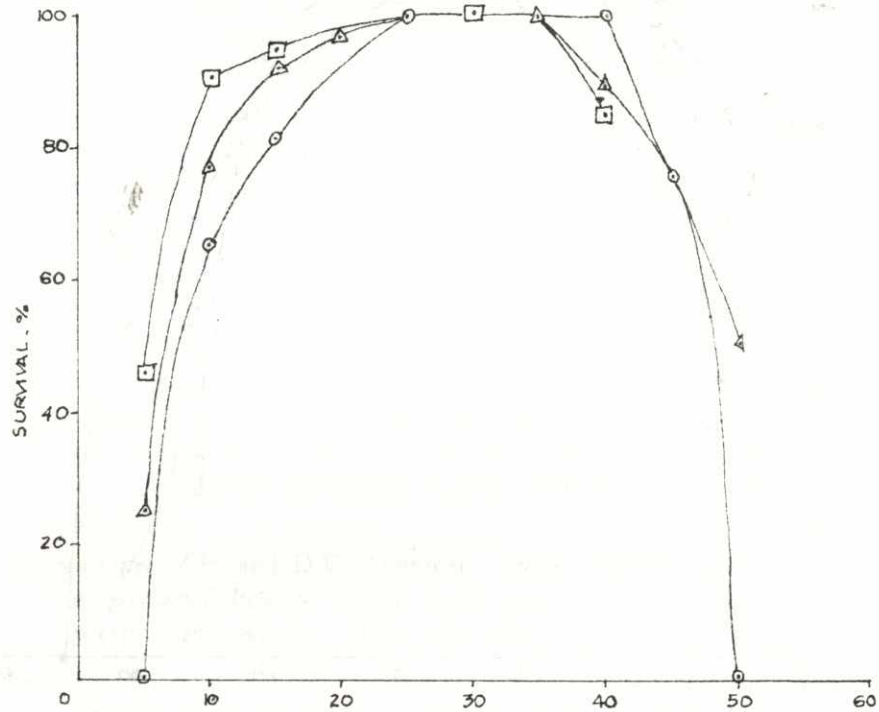


FIGURE 2. Salinity tolerance of *P. semisulcatus* postlarvae ( $P_{21}$ - $P_{34}$ ). Postlarvae were acclimated to initial salinities of 35 ppt (o—o), 30 ppt (A—A) and 25 ppt (□—□) for at least 2 days and transferred to salinities higher or lower than the initial salinity. Survival was observed after 24 hours.

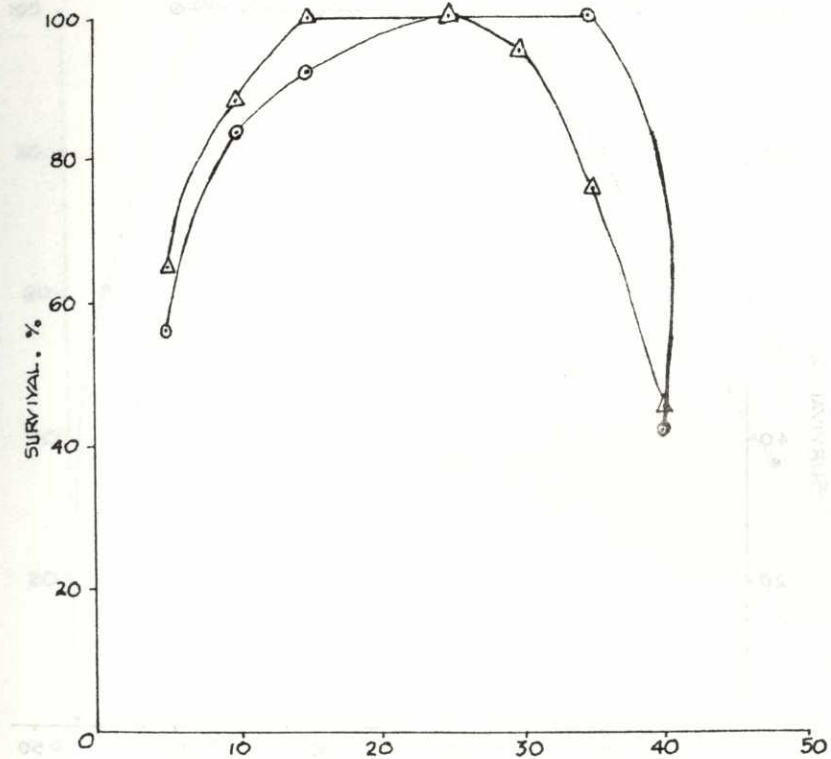


FIGURE 3. Salinity tolerance of *P. merquiensis* postlarvae ( $P_{21}$ - $P_{33}$ ). Postlarvae were acclimated to initial salinities of 30 ppt (o—o) and 25 ppt (A—A) for at least 2 days and transferred to salinities higher or lower than the initial salinity. Survival was observed after 24 hours.



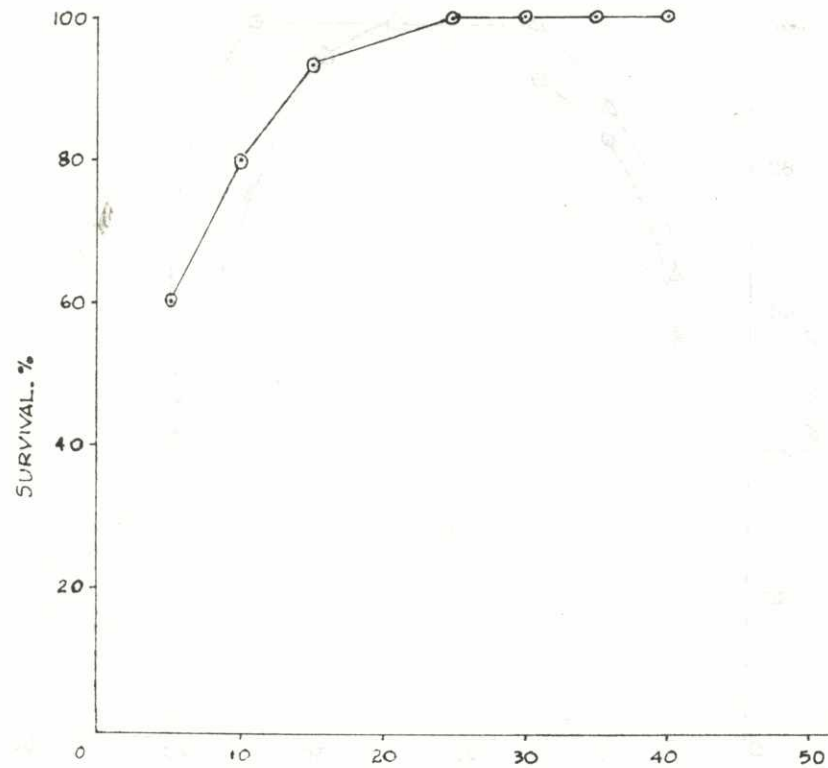


FIGURE 4. Salinity tolerance of *P. japonicus* postlarvae ( $P_{29}$ - $P_{36}$ ). Postlarvae were acclimated to initial salinity of 25 ppt (o—o) for at least 2 days and transferred to salinities higher or lower than the initial salinity. Survival was observed after 24 hours.

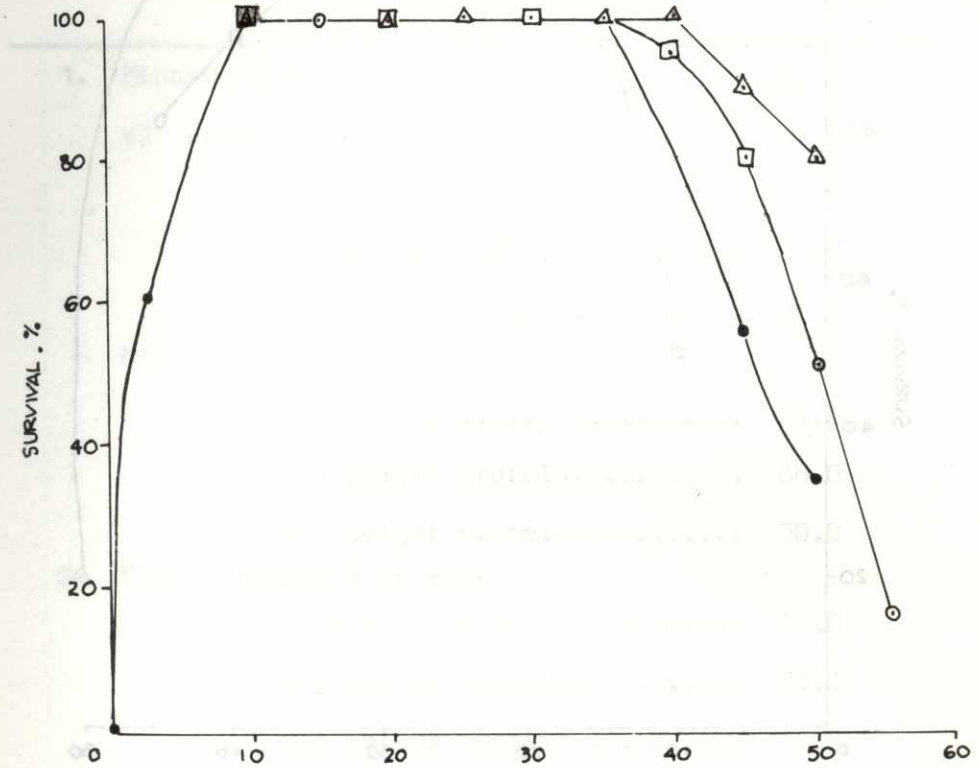


FIGURE 5. Salinity tolerance of *M. ensis* postlarvae ( $P_{16}$ - $P_{38}$ ). Postlarvae were acclimated to initial salinities of 40 ppt (o—o), 35 ppt (A—A), 30 ppt (□—□) and 25 ppt (●—●) for at least 2 days and transferred to salinities higher or lower than the initial salinity. Survival was observed after 24 hours.

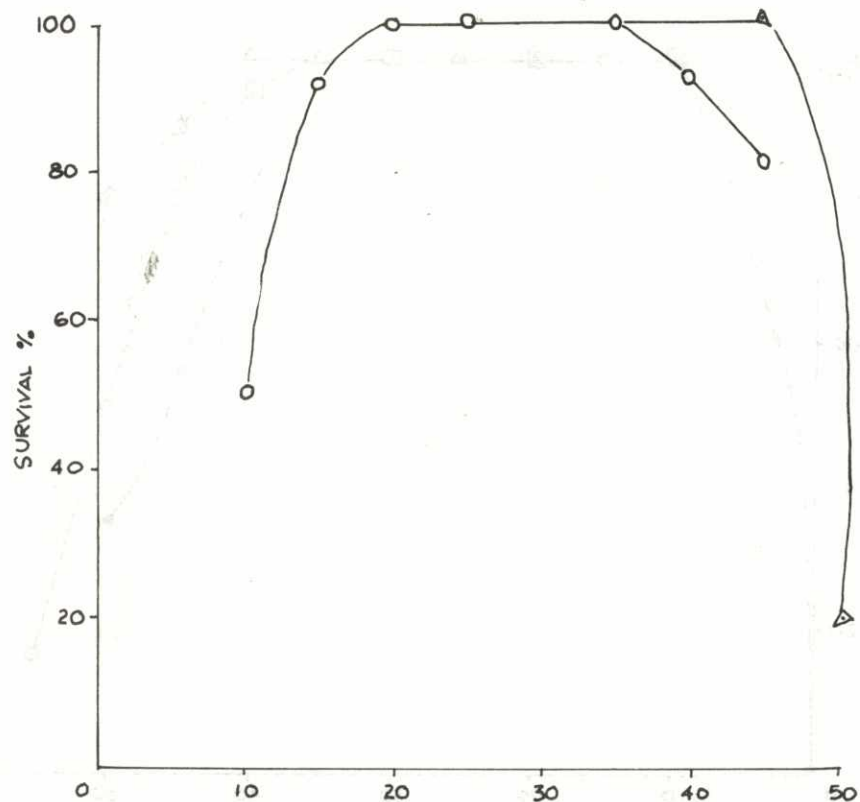


FIGURE 6. Salinity tolerance of *M. endeavouri* postlarvae ( $P_{18}$ - $P_{40}$ ). Postlarvae were acclimated to initial salinities of 35 ppt ( $\Delta$ ) and 30 ppt ( $\circ$ ) for at least 2 days and transferred to salinities higher or lower than the initial salinity. Survival was observed after 24 hours.

APPENDIX A. Protein analysis of feeds<sup>1</sup>

1. Minced tuna meat	%
a) white flesh	
moisture .....	79.0
dry weight protein.....	96.9
wet weight protein.....	20.0
b) red flesh	
moisture.....	66.0
dry weight protein.....	88.0
wet weight protein.....	30.0
2. Minced lizard fish meat	
moisture.....	81.0
dry weight protein.....	77.0
wet weight protein.....	15.0

<sup>1</sup>Analysis done by Veronica Dy, Chemist, SEAFDEC, Tigbauan, Iloilo.

## APPENDIX B. Initial and final weight measurements of Penaeid postlarvae.

SPECIES	Salinity (ppt)	% Survival	Initial Weight (mg/fry)	Final Weight (mg/fry)
<u>P. monodon</u> (1st run) 28 days <sup>1/</sup>	10	12.76	3.72	53.75
		19.15		30.54
	20	50.00	3.72	24.15
		54.17		25.47
	30	65.22	3.72	22.35
		64.58		18.31
<u>P. monodon</u> (2nd run) 16 days	10	2.85	1.33	9.80
	20	22.85	1.33	2.23
	30	83.33	1.33	1.90
	40	14.28	1.33	1.60
<u>P. semisulcatus</u> (1st run) 16 days	10	12.50	2.52	10.40
		6.66		3.50
	20	7.14	2.52	22.75
		8.00		22.25
	30	54.29	2.52	30.74
		55.55		24.18

<sup>1</sup> Culture period.

## APPENDIX B. (Continued)

SPECIES	Salinity (ppt)	% Survival	Initial Weight (mg/fry)	Final Weight (mg/fry)
<u>P. semisulcatus</u> 16 days (2nd run)	10	5.00	1.8	10.00
		5.26		5.25
	20	23.91	1.8	11.95
		41.66		8.79
	25	28.57	1.8	12.52
		20.00		12.52
<u>P. merquiensis</u> 25 days	10	4.16	1.5	25.75
	20	4.44	1.5	15.75
		6.66		16.00
	25	8.89	1.5	15.25
<u>P. japonicus</u> 25 days	10	2.50	8.5	28.50
		13.33		27.33
	20	17.17	8.5	27.33
		24.44		29.57
	25	24.44	8.5	20.91
		25.53		20.62

SPECIES	Salinity (ppt)	% Survival	Initial Weight (mg/fry)	Final Weight (mg/fry)
<u>M. ensis</u> <sup>1/</sup> 23 days	10	43.50	1.05	9.60
	20	44.50	1.05	7.90
	30	45.50	1.05	2.50

<sup>1</sup> Replicated but affected by the fungus, *Lagenidium*.

APPENDIX C. Range of nitrite, ammonia, pH and temperature.

SPECIES	Salinity (ppt)	pH	Temperature (°C)	Nitrite (ppm)	Ammonia (ppm)
<u>P. monodon</u> (28 days) 1/	10	7.5-8.4	24.5-27.5	0.07-15.99	0-4.82
	20	7.7-8.3	-do-	0.05-16.45	0-7.49
	30	7.8-8.2	-do-	0.04-17.16	0-7.97
	40	8.2-8.4	-do-	0.09-11.15	0.05-8.02
<u>P. semisulcatus</u> (16 days)	10	7.9-8.4	26.0-27.5	0.06-23.85	0-11.97
	20	7.8-8.5	-do-	0.04-26.50	0.05-11.40
	30	7.6-8.4	-do-	0.02-23.85	0-11.68
<u>P. merquiensis</u> (25 days)	10	8.1-8.4	26.0-28.0	0.12-13.12	0.06-11.97
	20	7.6-8.1	-do-	0.72-25.18	0.06-7.69
	25	7.4-8.1	-do-	0.68-23.85	0.04-11.40
	10	7.7-8.6	25.0-28.2	0.86-26.50	0.13-11.97
<u>P. japonicus</u> (25 days)	20	8.0-8.4	-do-	0 -14.57	0.01-12.54
	25	7.6-8.3	-do-	0.08-26.50	0.01-11.97
	10	8.0-8.3	25.8-27.0	0 -0.01	0 -3.79
<u>M. ensis</u> (23 days)	20	8.0-8.4	-do-	-do-	0 -6.47
	30	7.8-8.5	-do-	-do-	0 -8.32