

Instantaneous Mortalities and Multivariate Models: Applications to Tilapia Culture in Saline Water*

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Hopkins, K.D. and D. Pauly. 1993. Instantaneous mortalities and multivariate models: applications to tilapia culture in saline water, p. 105-111. In M. Prein, G. Hulata and D. Pauly (eds.) *Multivariate methods in aquaculture research: case studies of tilapias in experimental and commercial systems*. ICLARM Stud. Rev. 20, 221 p.

Abstract

The "per cent mortalities" commonly used by aquaculturists do not allow separation of the different components of fish mortality between stocking and harvesting in aquaculture experiments. It is shown that "instantaneous" or exponential mortalities, as used in fish population dynamics, have the properties required for such separation, especially when used in conjunction with a multiple regression model. Examples drawn from tilapia experiments conducted in seawater tanks in Kuwait and brackishwater ponds in the Philippines are presented.

Introduction

Survival and mortality are of great importance to aquaculturists because, in combination with growth, they determine net yield. However, this importance is not reflected in the amount of effort expended by aquaculturists to analyze survival and mortality. Aquaculturists typically analyze survival by computing the per cent survival at the end of each experiment and test for significant differences between treatments using ANOVA. Further analyses of mortality are usually not conducted. This situation is in distinct contrast to fisheries biology where substantial portions of textbooks deal with methods for analyzing survival and mortality (Pauly 1984; Sparre et al. 1989).

Aquaculturists have a strange dual perspective on fish mortality. First, at a visceral level, aquaculturists feel that mortality occurs only when something goes wrong (e.g., anoxia or a disease outbreak). One hundred per cent survival is a goal to strive for. At the same time, production models

use standard survival rates for particular life stages (e.g., Clifford 1985 uses a 75% survival rate from postlarvae to juvenile shrimp in semi-intensive culture). How can this dual perspective exist? A possible reason is that mortality is usually hidden from view unless a major catastrophe occurs. Therefore, the aquaculturist feels mortalities are caused by the catastrophes and the analysis of mortality is complete if the catastrophe has been identified. However, the number of fish harvested is almost always less than the number stocked even when no catastrophes have occurred. There is usually no indication of when or how the fish disappeared. The missing fish are a mystery which remains hidden by the standard expressions of survival rate.

This paper presents a methodology which can be used in our attempts to solve the mystery of the missing fish. It reviews the computation and attributes of two numerical representations of mortality, percentage mortalities and instantaneous rates of mortality. A multiple regression method to quantify the effects of external factors on instantaneous mortality is then presented. Examples using data from tilapia culture in seawater tanks and brackishwater ponds are given.

Mortality Rates

Aquaculturists typically use percentage mortalities (%M) which are computed as follows:

$$\%M = (N_0 - N_t) / N_0 \times 100 \quad \dots 1)$$

where N_0 is the number at the start of the culture period and N_t is the number present at time t . Percentage mortalities have the following characteristics:

1. They are restricted to time periods for which they were computed. For example, 20% over 100 days is not the same as 10% over 50 days. Thus, %M from experiments with different durations cannot be directly compared;
2. Percentage mortalities are not additive. A 10% rate attributed to one cause plus 10% from a second cause does not give a total relative mortality rate of 20%; and
3. Percentage mortalities are binomially distributed. Thus, depending on the range of values, square root or arcsin

transformations may be needed before statistical analyses which require normal distributions can be used (Gomez and Gomez 1976).

Fisheries biologists, on the other hand, typically use instantaneous rates of mortality. These are also known as exponential rates of mortality. They are based on the following relationship.

$$N_t = N_0 e^{-Zt} \quad \dots 2)$$

where N_t = the number at time t , N_0 = the initial number, and Z is the instantaneous rate of total mortality (Pauly 1984). Instantaneous rates of mortality have the following characteristics:

1. Instantaneous rates of mortality assume that the number of fish in a particular cohort decline at a rate proportional to the number of fish alive at any point in time (Everhart and Youngs 1981). This exponential decline (Fig. 1) has been shown in many situations and species of fish;
2. As long as the unit of time used in the computation of Z is the same, instantaneous rates from experiments with different durations can be directly compared. Also, if the time units are different, the units for one Z can be converted into the units of

another by simply multiplying with the appropriate time conversion factor (e.g., multiply $Z_{\text{day}^{-1}}$ by 30 to equal $Z_{\text{month}^{-1}}$);

3. Instantaneous rates are additive; and
4. Instantaneous rates of mortality usually are normally distributed.

Based on the characteristics listed above, instantaneous rates are considerably superior to percentage mortalities when conducting comparisons.

Similarly to the growth analyses discussed in much of the rest of this volume, analyzing mortality data from several experiments simultaneously has the potential for furthering our understanding of the factors affecting mortality. To quantify the effects of external factors on mortality, we propose a multiple linear regression of the form:

$$Z = a + b_1 X_1 + b_2 X_2 + \dots + b_n X_n \quad \dots 3)$$

where $X_1 \dots X_n$ are environmental and treatment variables recorded during the culture period.

Example 1 - *Oreochromis niloticus* in Brackishwater Ponds

This example is representative of a typical pond experiment in which the only available mortality data are the difference between numbers stocked and harvested. The experiments were conducted at the Brackishwater Aquaculture Center of the University of the Philippines in the Visayas as part of the USAID-supported Pond Dynamics/Aquaculture Collaborative Research Program (PD/A CRSP). Descriptions of the pond facilities can be found in Egna et al. (1987).

Data from experiments were pooled for the analyses for this paper. The experiments were designed to determine the effects of various nutrient input regimes (e.g., inorganic fertilizer, manure, feed and various combinations of these inputs) and season on fish yields and water quality (Table 1). The first two experiments, labeled cycle 1, used "impure" *Oreochromis niloticus* stocks which were felt to be contaminated with *O. mossambicus*. The other three experiments used a "purer" strain of *O. niloticus*. Details of the experimental protocols can be found in the program's work plans (PD/A CRSP, undated; PD/A CRSP 1984; PD/A CRSP 1985).

The data were extracted from the CRSP database, a large computerized database containing information from CRSP experiments throughout the world (Hopkins et al. 1988a). RBase for DOS was used for data extraction and computation of

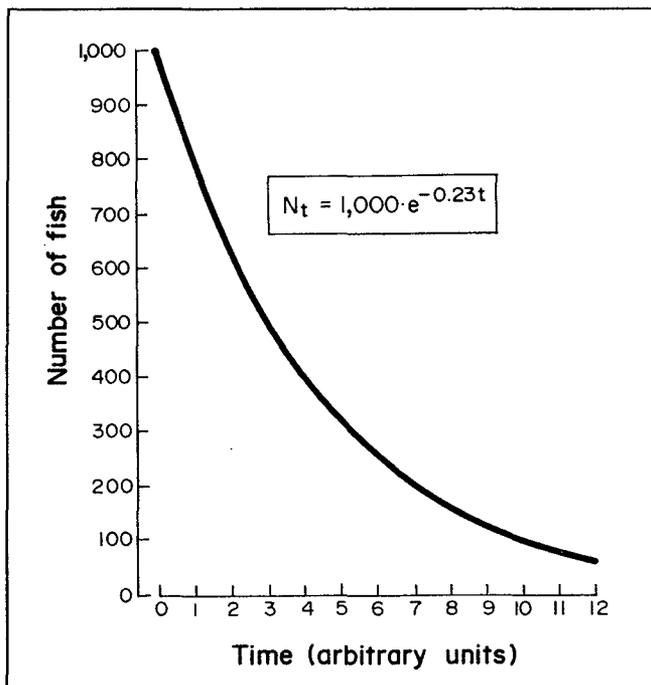


Fig. 1. Negative exponential decline of an initial population of fish ($N_0 = 1000$) exposed to a total mortality ($Z = 0.23$) per time unit.

average salinities. Regression analyses, including residual analyses, were conducted using SPSS/PC+.

A cursory view of the data indicated a positive relationship between salinity and mortality (Fig. 2) which appears to be approximately linear over the range 23 to 36 ppt salinity. However, two problems are apparent with such a simple linear relationship. First, it would have a negative y-intercept. As mortality cannot be negative, this is impossible. This effect can be overcome by taking the logarithm of Z. Second, stock "purity" seems to affect the response. We took accounts of both of these problems via the more general model:

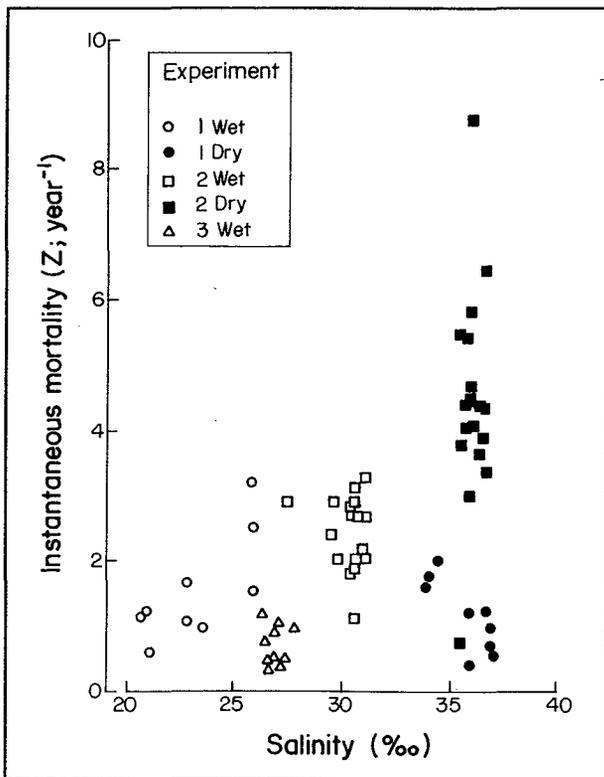


Fig. 2. Scatterplot of instantaneous mortalities as a function of salinity in brackishwater ponds.

$$\ln Z = a + b_1 S + b_2 P + b_3 (S \times P) \quad \dots 4)$$

where S is the average salinity, P is a dummy variable indicating relative stock "purity" (1 = "im-pure" and 0 = "pure"), and S x P is an interaction term. Taking the natural logarithm of Z ensures that predicted values of Z cannot be less than zero.

The results of the regression are shown in Table 2. All three terms are significant. The resulting equation is

$$\ln Z = -4.976 + 0.181 S + 5.692 P - 0.200 (S \times P) \quad \dots 5)$$

A graphical representation of the results is presented in Fig. 3. The salinities encountered during these experiments were extremely deleterious to the "purer" *O. niloticus*. The fish contaminated with *O. mossambicus* actually showed a decrease in mortality as the salinity increased. More detailed analysis to examine the effects of other water quality parameters and growth rates on mortality could be performed by simply expanding the model (the data to perform such analyses are presented in Appendix II).

Example 2 - Red Tilapia in Seawater Tanks

Data for this example were drawn from a set of experiments conducted at the Mariculture and Fisheries Department of the Kuwait Institute for Scientific Research. In these experiments, several tilapia species and hybrids were screened for their culture potential in seawater. As the experiments

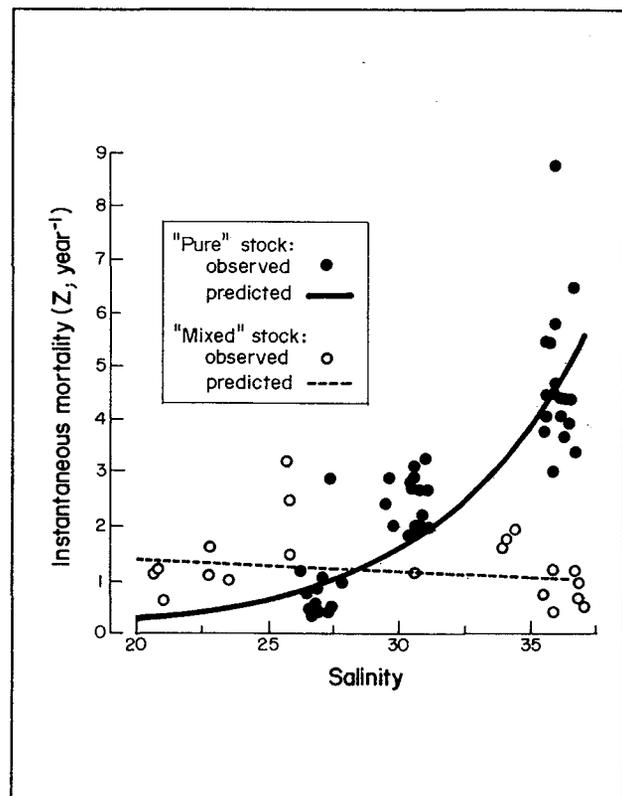


Fig. 3. Observed and predicted rates of instantaneous mortality in brackishwater tilapia ponds.

Table 1. Summary of brackishwater tilapia culture experiments (CRSP, Philippines).

Experiment	Stocking date	Harvest date	Number of ponds	Treatments	Average water temperature (°C)	Average salinity (ppt)	Average mortality Z-year ⁻¹
Cycle 1 Wet season	22 Jul 83	12 Dec 83	9	Inorganic fertilizer	25.1	23.3	1.53
Cycle 1 Dry season	1 Feb 84	3 Jul 84	9	Inorganic fertilizer Manure only	23.8	35.8	1.15
Cycle 2 Dry season	24 Nov 84	25 Apr 85	21	Inorganic fertilizer only Manure & inorganic fertilizer Manure & feed Manure & inorganic fertilizer & feed No inputs	25.9	36.0	4.55
Cycle 2 Wet season	3 Oct 85	28 Feb 86	21	Manure only Inorganic fertilizer only Manure & inorganic fertilizer Manure & feed Manure & inorganic fertilizer & feed No inputs	25.0	30.4	2.48
Cycle 3 Dry season	3 Sep 86	13 Nov 86	12	Chicken manure at 125, 250, 500 or 1,000 kg dry matter·ha ⁻¹ ·week ⁻¹	28.7	26.9	0.66

Table 2. Statistics of multiple regression model (equation 5) for predicting Z as a function of species "purity"^a and salinity^b.

Independent Variables	Estimate	Stand. Error	Beta Coefficients ^c	t
Intercept	-4.976	0.6047	-	-8.23
Salinity (S)	0.181	0.0189	1.044	9.6
Species purity ^c (P)	5.692	0.8221	3.066	6.92
Interaction (SxP)	-0.200	0.0264	-3.283	-7.58

^aSpecies purity is a dummy variable with 0 = pure *O. niloticus* and 1 = *O. niloticus* contaminated with *O. mossambicus* genes.

^bOverall model statistics are: multiple R: 0.79; R²: 0.63; adjusted R²: 0.61; standard error: 0.51; degrees of freedom due to regression: 3; due to residuals: 68.

^cSee Prein and Pauly (this vol.) for a definition.

were conducted in tanks, observations of daily mortalities were possible. The experimental methodology and some preliminary analyses are presented in Hopkins et al. (1989). The growth data were also used in the detailed presentation of the derivation, computation and attributes of the expanded Gulland and Holt plot (Hopkins et al. 1988b).

Data from a single experiment using a red tilapia from Taiwan are used in this example. These data were selected because they appeared to meet most of the assumptions for regression (see Hopkins et al. 1988b). Testosterone-treated red tilapia were stocked as 1-g fingerlings into 2-m³ tanks in August 1983. They were harvested in March 1984 at an average weight of 132 g. Salinity was 38 to 41 ppt and temperatures ranged from 20 to 28°C. Mortalities were noted daily.

Observations during the experiments indicated a substantial increase in mortality immediately following sampling for fish size and when the temperatures decreased, in winter. To quantify the influences of handling and temperature on mortality, each sampling period was divided into two parts: a) the week immediately following sampling, and b) the remainder of the period. The number of mortalities during each part of the sampling period was determined from the daily mortality records. Instantaneous rates were computed for each subperiod and compared.

The method described in the previous paragraph is quite straightforward when all of the mortalities are accounted for. However, a few fish were unaccounted for at the end of the experiment. Thus, two instantaneous rates of mortality were required to account for all of deaths. Z_u , the rate of unrecorded mortality is defined as the mortality rate which accounts for deaths not recorded during the culture period. Z_u is assumed to be constant over the whole experiment. Z_r is the rate of recorded mortality. The total instantaneous rate of mortality during a particular time period, Z_t is the sum of the unrecorded and recorded mortality rates:

$$Z_t = Z_u + Z_r \quad \dots 6)$$

where Z_r is the instantaneous rate of recorded mortality during the period t .

The procedure used for computing Z_t and Z_u was:

1. Compute total mortality for the entire culture period on a daily basis:

$$Z = -\ln(N_h/N_s)\Sigma d \quad \dots 7)$$

where N_h is the number of fish at harvest, N_s is the number of fish stocked, and Σd is the total duration of the experiment in days.

2. Compute Z_u by using the additive property of instantaneous rates. To do this, multiply Z_t by the per cent of total mortalities which were unrecorded:

$$Z_u = Z[1 - \Sigma N_r / (N_s - N_h)] \quad \dots 8)$$

where ΣN_r is the total number of mortalities recorded during the entire experiment.

After computing Z and Z_u , Z_r was computed for each part of the sampling periods. As the time periods were relatively short, the computations were done in a sequential fashion. For longer periods, the computational method for concurrent mortalities should be used (Ricker 1975).

3. Compute the number of fish included in unrecorded mortality (N_u) during the first week after sampling:

$$N_u = N_i - N_i \cdot e^{-Z_u t} \quad \dots 9)$$

where N_i is the number of fish at the start of the period, Z_u is the rate of unrecorded mortality from equation 8 and $t = 7$ days.

4. Compute the number of fish remaining at the end of the first week after sampling:

$$N_t = N_i - N_u - N_r \quad \dots 10)$$

where N_t is the number of fish at the end of period and N_r is the number of deaths during the period.

5. Compute the total rate of daily mortality (Z_t) during the first week after stocking:

$$Z_t = -\ln(N_t/N_i)/t \quad \dots 11)$$

6. Obtain Z_r by subtraction:

$$Z_r = Z_t - Z_u \quad \dots 12)$$

7. The process is then repeated for the second part of the sample period with N_t from the

preceding part of the period becoming the N_i for the subsequent part. The value of t changes depending upon the length of the period being examined. These sequential computations can be easily accomplished with a spreadsheet program.

All of the Z_r were converted to an annual basis and the effects of handling, temperature and fish weight on these rates were examined by computing the following multiple regression:

$$\ln(Z_{r+1}) = a + b_1H + b_2T \quad \dots 13)$$

where H is a dummy variable indicating the presence or absence of handling and T is the average daily water temperature ($^{\circ}\text{C}$) during each period. As in the first example, the natural logarithm of Z was used to cause Z to level out near zero. The addition of 1 to Z_r was required because some of the values of Z were zero and the logarithm of zero is undefined. The resulting regression equation was:

$$\ln(Z_{r+1}) = 4.157 + 0.265H - 0.159T \quad \dots 14)$$

The regression output is presented in Table 3. The greater variability with corresponding decrease in the R^2 exhibited during this experiment compared with the pond experiment is probably a result of the shorter time periods. Also, preliminary analyses of the screening trials with other species indicated that mortality rates from short time periods may not always be normally distributed.

The analysis of the red tilapia data allowed the quantification of the effects of temperature and handling on mortality (Fig. 4). This information can be extremely useful when determining if the

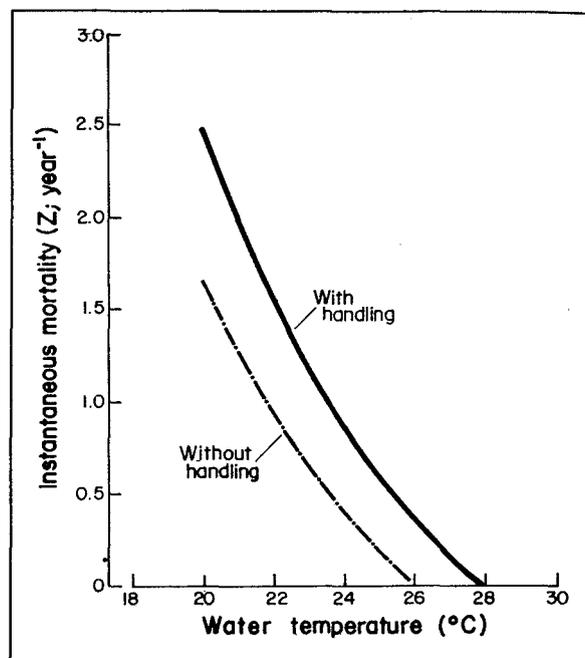


Fig. 4. Estimated effects of temperature and handling on the mortality of red tilapia in seawater.

value of information from additional samples outweighs the resulting increase in mortality. Also, the positive effects of heating the water on mortality could be compared to the cost of heating.

Conclusion

Instantaneous rates of mortality can be used with multiple regression procedures to quantify the effects of external factors on mortality. These quantified effects can then be used in decisionmaking and for bioeconomic modelling.

Data collected over long periods (i.e., several months) is much easier to use than data collected over very short periods (i.e., 1 to 3 weeks). When working with short period data, care must be taken to ensure that the data meet the assumptions of linear regression.

Acknowledgements

The assistance of Carole Miura and Margarita Hopkins in reviewing the data and preliminary analyses is gratefully acknowledged. This paper is

Table 3. Statistics of multiple regression model [equation (14)] for predicting Z as a function of handling^a and temperature^b.

Independent variables	Estimate	Standard error b	Standardized beta	T	Significance level of T
Constant	4.157	0.653	-	6.36	<0.0001
Temperature	-0.159	0.028	-0.657	-5.80	<0.0001
Handling	0.265	0.145	0.207	1.83	<0.0750

^aHandling is a dummy variable with 0 = no handling at the start of the period and 1 = some handling.

^bOverall model statistics are: multiple R: 0.69; R^2 : 0.47; adjusted R^2 : 0.45; standard error: 0.48; degrees of freedom due to regression: 2; due to residuals: 41.

accession number 1054 of the Collaborative Research Support Program in Pond Dynamics/Aquaculture. Its preparation was supported in part by Grant No. DAN 4023-G-00-0031 from the US Agency for International Development.

References

- Clifford, H.C. 1985. Semi-intensive shrimp farming, p. IV-15 to IV-42. *In* G. Chamberlain, M. Haby and R. Miget (eds.). Texas shrimp farming manual. An update on current technology. Texas Agricultural Extension Service, Corpus Christi, Texas.
- Egna, H., N. Brown and M. Leslie. 1987. General reference: site descriptions, materials and methods for the global experiment. Pond Dynamics/Aquaculture Collaborative Research Data Reports. Vol. 1. PD/A CRSP, Oregon State University, Corvallis, Oregon.
- Everhart, W.H. and W.D. Youngs. 1981. Principles of fishery science. Second edition. Cornell University Press, Ithaca, New York. 349 p.
- Gomez, K.A. and A.A. Gomez. 1976. Statistical procedures for agricultural research, with emphasis on rice. International Rice Research Institute, Los Baños, Philippines. 294 p.
- Hopkins, K.D., J. Lannan and J. Bowman. 1988a. Managing a database for pond research data - the CRSP experience. *Aquabyte* 1(1):3-4.
- Hopkins, K.D., M.L. Hopkins and D. Pauly. 1988b. A multivariate model of tilapia growth, applied to seawater tilapia culture in Kuwait, p. 29-39. *In* R.S.V. Pullin, T. Bhukaswan, K. Tonguthai and J.L. Maclean (eds.) The Second International Symposium on Tilapia in Aquaculture. ICLARM Conf. Proc. 15, 623 p.
- Hopkins, K.D., M.Ridha, D. Leclercq, A.A. Al-Ameeri and T. Al-Ahmad. 1989. Screening tilapia for culture in seawater in Kuwait. *Aquacult. Fish. Manage.* 20:389-397.
- Pauly, D. 1984. Fish population dynamics in tropical waters: a manual for use with programmable calculators. *ICLARM Stud. Rev.* 8, 325 p.
- PD/A CRSP. undated. CRSP work plan: first experimental cycle. Vol. 1. experimental protocol and methods. Oregon State University, Marine Science Center, Newport, Oregon.
- PD/A CRSP. 1984. CRSP work plan: second experimental cycle. Oregon State University, Marine Science Center, Newport, Oregon.
- PD/A CRSP. 1985. CRSP work plan: third experimental cycle. Oregon State University, Marine Science Center, Newport, Oregon.
- Ricker, W.E. 1975. Computation and interpretation of biological statistics of fish populations. *Bull. Fish. Res. Board Can.* 191. 382 p.
- Sparre, P., E. Ursin and S. Venema. 1989. Introduction to tropical fish stock assessment. Part 1 - Manual. *FAO Fish. Tech. Pap.* 306/1. FAO, Rome. 337 p.

Multiple Regression Analysis of Relationships Between Management Inputs and Fish Yield or Profit in Fish Polyculture Experimental Ponds*

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Abstract

The multiple regression analysis presented in this paper was applied to data from experiments carried out during 1974-1985 at the Fish and Aquaculture Research Station, Dor, Israel. The objective of these experiments was to test the possibility of complete or partial substitution of supplemented feed by manure. Due to the large number of variables affecting fish yield, or profit, multivariate statistical methods were used.

Nonlinear relationships existed between fish yield (or profit) and each of the management variables (amounts of feed and manure, density and weight at stocking of common carp and tilapia). Multiple regression analysis identified stocking density of common carp and an interactive combination of amounts of feed and manure as the major factors affecting yield and profit. Manure had a stronger effect on profit than did the feeds. The highest profit was obtained in treatments in which a low ration of supplemental feed was added to a high amount of manure and not in the treatment in which only supplemented feed was applied.

The analysis presented here indicates the potential of multiple regression for the purpose of extracting information from fish culture data and identifying predictors of increased production and profit.

*ARO Contribution No. 40-H. Translation of an article published originally in Hebrew, in *Israel Agresearch* 4(1): 73-84, 1990.

Introduction

Polyculture of fish occurs in a complex ecological system, in which species with different feeding habits are stocked together in the same pond, with the aim of increasing fish production through optimal utilization of the various components of the natural food produced in the pond (Tang 1970). Herein, the amount of available natural food and the growth rate of the different fish species depend on environmental and management factors. Only the latter, such as stocking time, stocking density and size at stocking of the different fish species, quality and quantity of supplemented feed, and activities such as fertilization and manuring which increase natural food production (Wohlfarth and Hulata 1987), are controlled by the fish farmer. The large number of variables affecting fish production suggest the use of multivariate statistical methods. Such techniques were suggested or applied by, for example, Hopkins and Cruz (1982), Pauly and Hopkins (1983) for aquaculture data, by Winnans (1984) and by Milstein et al. (1985a, 1985b) for other areas of aquatic ecology, and by Ziv and Goldman (1987) for wheat culture data, among others.

In the analysis presented here, multiple regression was applied to data collected over 10 years of pond experiments at the Fish and Aquaculture Research Station, Dor. These data stem from experiments not specifically designed for the present study; rather they were generated in the frame of a study dealing with (partial) substitution of supplemental feeds by manure (Wohlfarth and Hulata 1987). Milstein et al. (1988) applied canonical correlation analysis to the dataset and reached the following conclusions: (a) the yield of each fish species was affected mainly by its own stocking density followed by interactions with other species; (b) the best yield and growth of tilapia were obtained with individual stocking weights of over 13 g; and (c) common carp (*Cyprinus carpio*) was affected negatively by silver carp (*Hypophthalmichthys molitrix*) density, and positively by nutrient inputs; its best performance was obtained at silver carp density below 1,000-ha⁻¹.

The multiple regression treatment of the same dataset, presented in this paper, aimed at extracting the quantitative relations between the target variables (yield or profit) and the management