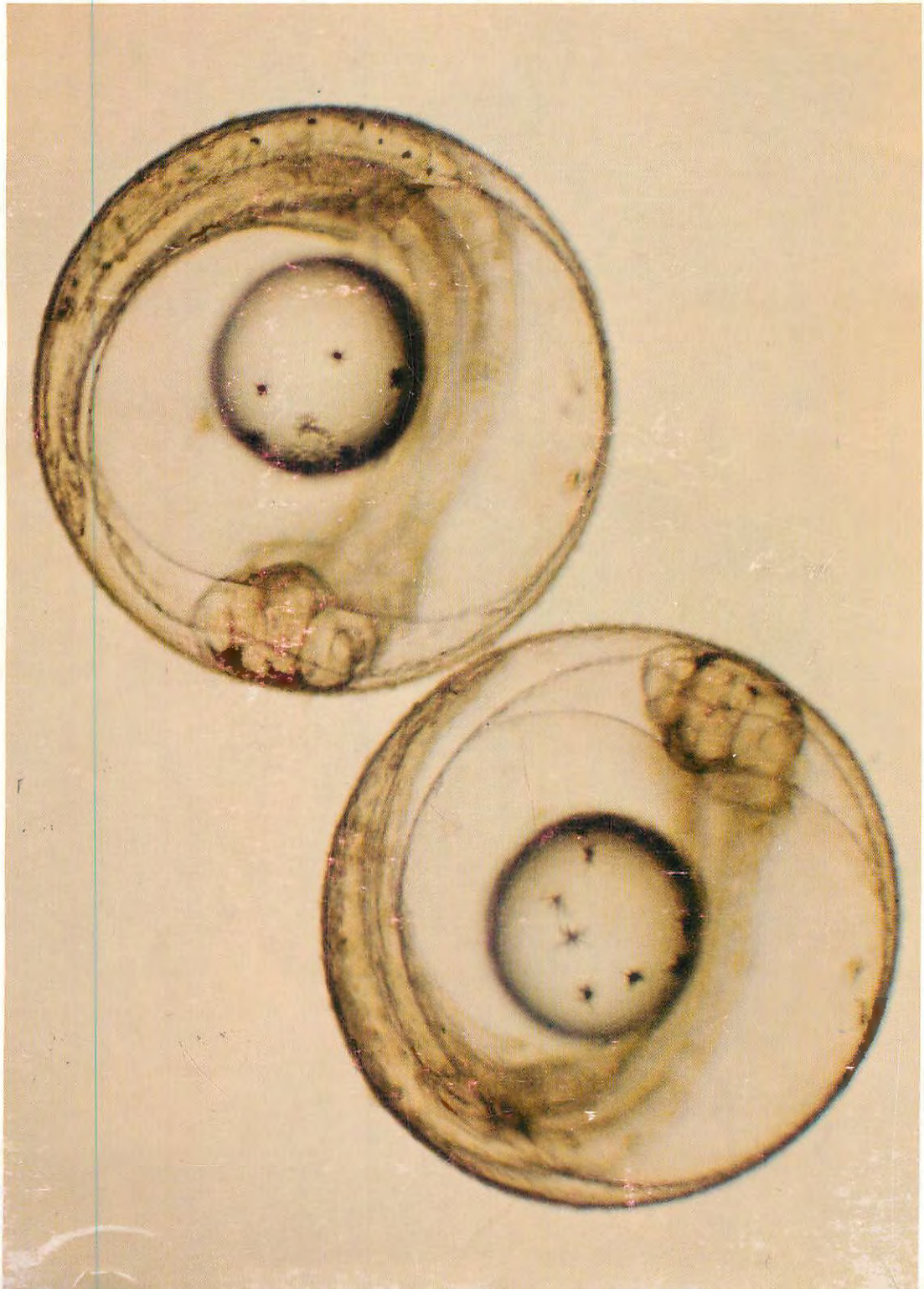
# THE GREY MULLET

## Induced Breeding and Larval Rearing

Oceanic Institute · Waimanalo, Hawaii



Report No. OI-72-76-1

# THE GREY MULLET (Mugil cephalus L.):

# INDUCED BREEDING AND LARVAL REARING

# RESEARCH 1970-1972

by

OCEANIC INSTITUTE
Waimanalo, Hawaii 96795

for

National Sea Grant Program
National Oceanic and Atmospheric Administration
U.S. Department of Commerce
Rockville, Maryland 20852

Principal Investigator - Ziad H. Shehadeh, Ph.D.

Co-Investigator - Kenneth S. Norris, Ph.D.

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This report is the result of the combined efforts of the following staff and colleagues:

Ziad H. Shehadeh, Ph.D. - Principal Investigator
Kenneth S. Norris, Ph.D. - Co-Investigator
Ching-Ming Kuo, Ph.D.
Colin E. Nash, Ph.D.
Lawrence P. Raymond, Ph.D.
Edward M. Donaldson, Ph.D. - Visiting investigator
Gary L. Peterson, M.S.
William D. Madden, B.S.
Kathy Milisen, B.S.
Marvin Ching, B.S.
James E. Ellis, B.S.
Thomas P. Dohl, B.S.
Marjorie Harvey, B.S.
Wayne A. Harvey, M.S.
Diane Henderson, B.A.
Herb Knudsen
Chuck Peterson and Roger Coryell - Photography
Rose Collins - Division Secretary
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Mr. Howard Butcher, III

Front cover: Laboratory spawned mullet eggs just prior to hatching. Photo by Chuck Peterson

## Preface

The grey or striped mullet, Mugil cephalus L., is a prized food fish in Southeast Asia, Japan, the Pacific island nations and the Mediterranean basin. In the United States, it is, at present, a major food fish only in the State of Hawaii, where it fetches an average wholesale price of $1/pound. Mullet juveniles also represent an untapped resource of significant commercial value as live bait in the tuna fishing industry.

The expansion of the mullet pond culture industry, as well as the realization of the bait potential, has been hampered, and in most cases precluded, by the limited availability of wild juveniles. Due to the capricious nature of this supply, long term efforts aimed at the control of the reproductive cycle of this and other species of mullet were initiated in Taiwan, Israel and at the Oceanic Institute in Hawaii.

Research at this laboratory centered around two major goals: (1) the achievement of complete control over the reproductive cycle of the grey mullet, and (2) the development of hatchery techniques for the mass propagation of the larvae of this species. The first of these goals required realization of the following specific objectives:

- To develop a statistically valid in vivo method for the assessment of ovarian development in female breeders
- To induce spawning in the laboratory by means of hormone injection according to exact and repeatable techniques
- To induce spontaneous spawning and natural fertilization in laboratory tanks in order to replace manual methods
- To establish a captive brood stock population of adult mullet to permit selective breeding and subsequent genetic selection
- To bring about out-of-season spawning by manipulation of environmental parameters

These objectives have been realized and are described in this report. Out-of-season spawning data will be published in a second report which will include results of research currently in progress. The present report also includes preliminary results of initial larval rearing tests, an investigation into the possible use of soluble eye-lens proteins as genetic markers, and a preliminary analysis of the mullet market in Hawaii.

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University of Hawaii
Honolulu, Hawaii 96822

Dr. E.M. Donaldson 1
Fisheries Research Board
of Canada
Pacific Environmental Institute
4160 Marine Drive
West Vancouver, B.C.

Mr. Takuji Fujimura 1
Keehi Fisheries Station,
Area 4
Hawaii Division of Fish & Game
Sand Island Road
Honolulu, Hawaii 96818

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Professor of Oceanography
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College Park, Maryland 20742

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Div. of Technical &
Vocational Education
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Corpus Christi, Texas 78404

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Lehigh University
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Virginia Institute of
Marine Science
Gloucester Point, Virgina 23062

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Institute
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Bay Campus
St. Petersburg, Florida 33701

Dr. John Noakes 1
Department of Geology
University of Georgia
Athens, Georgia 30601

Mr. James Andrews 1
Skidaway Institute of
Oceanography
55 West Bluff Road
Savannah, Georgia 31406

Mr. Paul Venno 1
Dept. of Sea & Shore Fisheries
State House
Augusta, Maine 04330

Dr. George Fulton 1
Department of Biology
Boston University
Boston, Massachusetts 02215

Dr. Alva Harris & Curt Rose 1
Dept. of Biological Sciences
Nicholls State College
Box 2021
Thibodaux, Louisiana 70301

Dr. Robert Corell 1
Chairman, Mechanical
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University of Texas
School of Medicine
Galveston, Texas 77550

Dr. Warren R. Healey 1
Director, Marine Resource
Division
New England Council
Statler Office Building
Boston, Massachusetts 02116

Dr. Frank Mather, III 1
Woods Hole Oceanographic
Institution
Woods Hole, Massachusetts 02543

Dr. David Hickok 1
Director, Sea Grant Program
University of Alaska
108 East Third Ave.
Anchorage, Alaska 95501

Professor Charles Stephan 1
Chairman, Dept. of Ocean Eng.
Florida Atlantic University
Boca Raton, Florida 33432

Dr. Russell Riese 1
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St. Thomas, Virgin Islands 00801

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Massachusetts Institute of
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Cambridge, Massachusetts 02139

Dr. Richard Tibby 1
Catalina Marine Science Center
University of Southern California
University Park
Los Angeles, California 90007

Dr. Rita Colwell 1
Department of Biology
Georgetown University
Washington, D. C. 20007

Dr. Wheeler North 1
California Institute of Technology
Pasadena, California 91109

Dr. Jack Davidson 1
Director, Sea Grant Program
University of Hawaii
2540 Maile Way
Honolulu, Hawaii 96822

Dr. John P. Harville 1
Director, Moss Landing
Marine Laboratory
Moss Landing, California 90350

Dr. Robert W. Holmes 1
Assoc. Prof. of Marine Biology
University of California
Santa Barbara, California 93106

Dr. Herbert Frolander 1
Director of Sea Grant Program
Oregon State University
Corvallis, Oregon 97331

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Sea Grant Director
University of Michigan
Ann Arbor, Michigan 48104

Dr. John Knauss 1
Graduate School of Oceanography
University of Rhode Island
Kingston, Rhode Island 02881

Dr. Richard C. Bader 1
Associate Director
Institute of Marine Science
University of Miami
Miami, Florida 33149

Dr. John C. Calhoun, Jr. 1
Director, Sea Grant Program
Texas A&M University
College Station, Texas 77843

Dr. Robert Ragotzkie 1
Coordinator, Sea Grant Program
University of Wisconsin
Madison, Wisconsin 53706

Dr. John Lyman 1
Sea Grant Program Coordinator
University of North Carolina
Chapel Hill, North Carolina 27514

Mr. Scott Daubin 1
Woods Hole Oceanographic
Institution
Woods Hole, Massachusetts 02543

Dr. Ramsey Parks 1
Santa Barbara City College
Cliff Drive & Leadbetter Drive
Santa Barbara, California 93105

Mr. William H. Diment 1
Department of Geological Science
University of Rochester
Rochester, New York 14627

Dr. John P. Breslin 1
Director, Davidson Laboratory
Stevens Institute of Technology
Castle Point Station
Hoboken, New Jersey 07030

Dr. Richard Ridenhour 1
Sea Grant Program Coordinator
Humboldt State College
Arcata, California 95521

Dr. William S. Gaither 1
Dean, College of Marine Studies
University of Delaware
Newark, Delaware 19711

Dr. Jack Van Lopik 1
Sea Grant Coordinator
Louisiana State University
Baton Rouge, Louisiana 70803

Dr. George Shor, Jr. 1
Associate Director
Scripps Institutions of Oceanography
La Jolla, California 28401

Captain Arthur Jordon 1
Sea Grant Coordinator
Cape Fear Technical Institute
Wilmington, North Carolina 28401

Dr. Donald F. Mulvihill 1
Department of Marketing
Kent State University
Kent, Ohio 44240

Dr. Martin F. Stempien, Jr. 1
Osborn Laboratories of
Marine Science
New York Zoological Society
Seaside Park, Coney Island
Brooklyn, New York 11224

Mr. Nelson Marshall 1
Graduate School of Oceanography
University of Rhode Island
Kingston, Rhode Island 02881

Alfred J. Weinheimer 1
Professor of Chemistry
University of Oklahoma
660 Parrington Oval
Norman, Oklahoma 73069

Dr. Stanley Murphy 1
Director, Sea Grant Program
University of Washington
Seattle, Washington 98105

Mr. Glen Wade 1
Professor, Dept. of
Electrical Engineering
University of California,
Santa Barbara
Santa Barbara, California 93106

Mr. Ajay K. Bose 1
Department of Chemistry
and Engineering
Stevens Institute of Technology
Hoboken, New Jersey 07030

Mr. Donald W. Melvin 1
Assoc. Professor of
Electrical Engineering
College of Technology
University of New Hampshire
Durham, New Hampshire 03824

Mr. John H. Lech 1
Department of Pharmacology
Medical College of Wisconsin
Milwaukee, Wisconsin 53233

Mr. Clarke Williams 1
Nassau-Suffolk Regional
Planning Board
Hauppauge, Long Island,
New York 11787

Mr. Robert W. Brody 1
College of the Virgin Islands
Caribbean Research Institute
St. Thomas, Virgin Islands 00801

Mr. James F. Wright 1
Delaware River Basin Commission
25 Scotch Road
Trenton, New Jersey 08628

Mr. Clinton Dawes 1
University of South Florida
Tampa, Florida 33620

Dr. Sidney Upham, Director 1
Universities Marine Center
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Ocean Springs, Mississippi 39564

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Dr. Philip Helfrich 1
Hawaii Institute of
Marine Biology
University of Hawaii
P.O. Box 1067
Kaneohe, Hawaii 96744

Dr. Frank J. Hester 1
Area Director, National
Marine Fisheries Service
2750 Dole Street
Honolulu, Hawaii 96822

Dr. C. F. Hickling 1
95, Greenway
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Carl N. Hodges 1
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Buzz Holling 1
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Dr. John Pino 2
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111 West 50th Street
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James E. Ellis 1
Lummi Tribal Center
2099 Lummi Shore Road
Bellingham, Washington 98225

Gary L. Peterson 1
Department of Zoology
Oregon State University
Corvallis, Oregon 97331

Chuck Peterson 1
610 Kumukahi Place
Honolulu, Hawaii 96825

Wayne Harvey 1
1822 Punahou Street
Honolulu, Hawaii 96822

Dr. Karl F. Lagler 1
Dept. of Fisheries
School of Natural Resources
University of Michigan
Ann Arbor, Michigan 49904

Tony Novotney 1
Port Orchard, Washington 98366

Dr. Carl Bond 1
Professor of Fisheries
Dept. of Fisheries and Wildlife
Oregon State University
Corvallis, Oregon 97331

Dr. Theo Raftopoulus 1
Ecole Polytechnique D'Athenes
Athenes (104)
Rue Trois Septembre 84
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Dr. Ch. Meske 1
Max-Planck-Institut fur
Kulturpflanzenzuchtung
2000 Hamburg-Volksdorf
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Luis Alvarez-Lajonchere 1
Centro de Investigaciones Marinas
Universidad de Ra Habana
Avenida 1A, No. 2808
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Mr. Andreas Demetropoulos 1
Fisheries Department
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Tungkang Shrimp Culture Center
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Prof. R. Doumenge 1
Director, South Pacific Islands
Fisheries Development
Agencies
South Pacific Commission
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Noumea, New Caledonia

The Richard K. Mellon 1
Foundation
525 William Penn Place
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111 West 50th Street
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Dr. T. V. R. Pillay 1
Fisheries Resources Section
Food & Agriculture Organization
of the United Nations
Via delle Terme de Caracalla
00100 - Rome, Italy

Dr. V. R. Pantutu 1
Fishery Adviser
Mekong Office/United Nations Ecafe
Bangkok, Thailand 817422

Dr. L. Johnson 1
Freshwater Institute
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Winnipeg 19, Manitoba
Canada

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FAO Regional Office for
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Department of Oceanography
University of Hawaii
Honolulu, Hawaii 96822

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United Nations Development
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39, Avenue D'Iena
75783 Paris Cedex 16

Frank H. Hoff 1
State of Florida, Dept. of
Natural Resources
Marine Research Laboratory
P. O. Drawer F
St. Petersburg, Florida 33731

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I.

# INDUCED SPAWNING OF THE STRIPED MULLET *Mugil cephalus* L.


## Abstract

The response of adult mullet to artifical hypophysation, immediately before the onset of the spawning migration was studied. Both males and females received intraperitoneal injections of mullet or salmon pituitary homogenates with ACTH or Synahorin (APE + HCG) three times per week for 12 days. At the outset, control females were in the tertiary yolk stage with a low mean GSI of 7.6, while males gave thick, non-dispersing milt. Two of four females receiving one salmon pituitary plus 35 RU Synahorin per injection spawned spontaneously in laboratory aquaria. Released eggs were buoyant, well formed and had a mean diameter of 570 $\mu$. Egg fertility could not be ascertained due to protracted contact with sea water. Spawned females extruded a membranous tissue "plug" from the cloacal region which was subsequently retracted. The remaining experimental females were refractory to treatment and histological examination of the ovaries revealed a predominance of atretic oocytes. All males were refractory to treatment and milt hydration was not achieved. It is suggested that induction of early spawning is possible especially with higher doses of pituitary homogenates and Synahorin.



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## INTRODUCTION

The culture of mullet Mugil cephalus L. in artificial impoundments remains contingent on the availability of wild fry. Hatched at sea, these latter make their way to the warmer brackish shallows of estuaries and tidal creeks where they are collected for stocking into ponds. The capricious nature of this supply and the need to breed more resistent and faster growing strains, has prompted investigations into the artifical spawning of this fish.

Successful spawning has since been accomplished in Taiwan (Tang, 1964; Liao, 1969), Israel (Abraham et al., 1967) and Hawaii (Frogner and Hendrickson, unpublished). In the estuaries, bays and coastal waters of Oahu Island, Hawaii, gravid fish are rare and spawning schools are yet to be located. Pond culture of mullet has all but disappeared and the limited supply is usually marketed before the fish attain sexual maturity. The oocytes of female mullet collected in Oahu waters during the spawning season are almost invariably in the tertiary yolk stage while the males usually exude limited amounts of thick, non-flowing milt.

The paucity of ripe spawners led us to investigate the feasibility of inducing ovulation, oviposition and milt hydration in fish collected immediately before the start of the seasonal spawning migration.

## MATERIALS AND METHODS

Adult mullet of both sexes were collected from Kaneohe Bay (Oahu, Hawaii) on 11 February 1968, and transported to the laboratory in aerated tanks. The fish were separated according to sex and maintained in 50 gallon fiberglass aquaria in running sea water (32 $^{o}/_{oo}$). Water temperature and pH were 26$^{o}$ C and 7.60, respectively.

None of the females exhibited the abdominal enlargement characteristic of mature spawners and gentle pressure on the abdomen failed to release eggs. All males, on the other hand, yielded milt on stripping. The milt, however, was of tooth paste consistency and did not disperse in sea water. In order to determine the state of ovarian development in the females, five fish were randomly selected and ovary samples removed and fixed in Bouin's fluid. Seven micron sections were prepared and stained with hematoxylin and eosin. Body measurements were recorded and the gonadosomatic and ponderal indices calculated.

The experimental fish were divided into five groups; three groups of females and two groups of males. Each group was maintained in a separate 50 gallon aquarium in running sea water and each aquarium held a maximum of six fish. Body weights and lengths were recorded and a laboratory acclimation period of 48 h was allowed before injection was initiated.

Salmon and mullet pituitaries, collected from mature spawners and preserved in 95% ethyl alcohol, were used in the study. Pituitaries were homogenized in 0.5 ml of normal mullet saline (0.9% NaCl) and the suspension injected intraperitoneally with ACTH (Parke, Davis & Co.) or Synahorin (Teikoku Hormone Mfg. Co., Ltd.). Injections were carried out three times per week on alternate days; the individual doses for the various groups are listed in Table 3.

## RESULTS

Body measurements of female controls and the experimental groups are shown in Tables 1 and 2 respectively. The gonadosomatic indices of the controls were relatively low, values of 14.0 having been recorded from premigratory adults in previous seasons (Ellis and Madden, unpublished). Without exception, oocytes of this group were found to be in the tertiary yolk stage (Stage IV) (Fig. 1).

Table 1. Body measurements, gonadosomatic and ponderal indices of control females

| Fish no. | Body wt (g) | Fork length (cm) | Gonad wt (g) | GSI | PI* | Oocyte stage |
|---|---|---|---|---|---|---|
| 1 | 412.0 | 30.5 | 32.0 | 7.80 | 0.015 | IV |
| 2 | 411.0 | 32.0 | 27.0 | 6.60 | 0.013 | IV |
| 3 | 466.0 | 32.5 | 26.0 | 5.60 | 0.014 | IV |
| 4 | 424.0 | 32.0 | 52.0 | 12.30 | 0.013 | IV |
| 5 | 357.0 | 31.0 | 20.0 | 5.60 | 0.018 | IV |

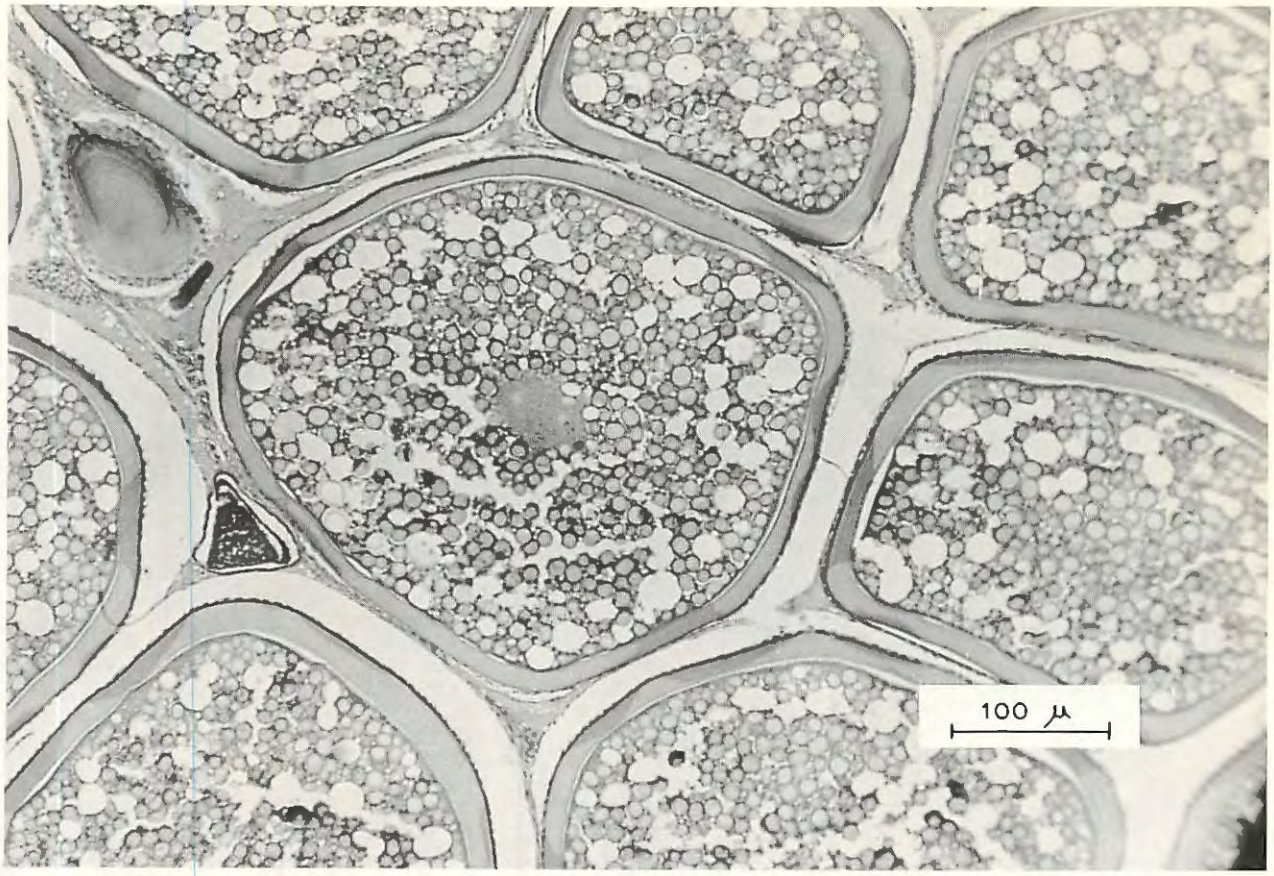


Fig. 1. Representative section through female control ovary. Hemotoxylin and eosin stain.

Table 3 demonstrates that, within the limits of dosage and duration of this study, males were refractory to treatment with mullet pituitary and Synahorin. Milt remained thick and scant. Females, with the exception of two fish in Group IV, also failed to ovulate. Histological examination of ovary sections from non-responsive females (Groups III to V) revealed a predominance of attretic oocytes, indicating that regression was already in progress (Fig. 2).

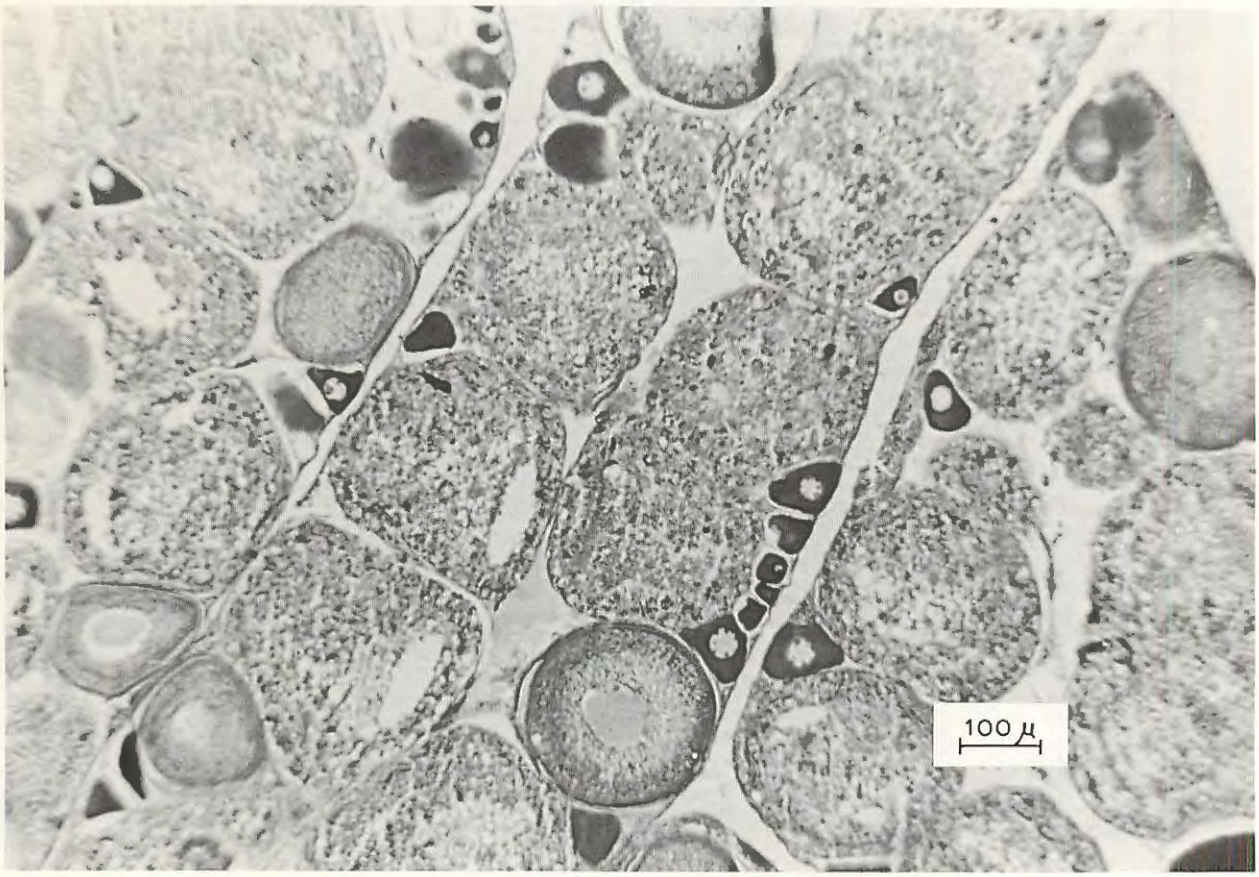


Fig. 2. Representative section from the ovary of a refractory female. Hematoxylin and eosin stain.

One female in Group IV developed an enlarged abdomen following the fifth injection. Intraperitoneal injection was consequently discontinued to avoid damage to the enlarged ovaries. Abdominal enlargement continued during the ensuing 48 h, at the end of which period a tissue "plug" was extruded through the cloaca (Fig. 3) and several thousand eggs were discharged. The dislodged eggs averaged 560 μ in diameter and exhibited well-formed oil droplets measuring approximately one half the diameter of the yolk mass. The extruded "plug" was retracted and the abdomen began to show signs of deflation 24 h later. The fish was therefore stripped manually at that time. Stripping was successful but the majority of the eggs were misshapen and clumped in masses.

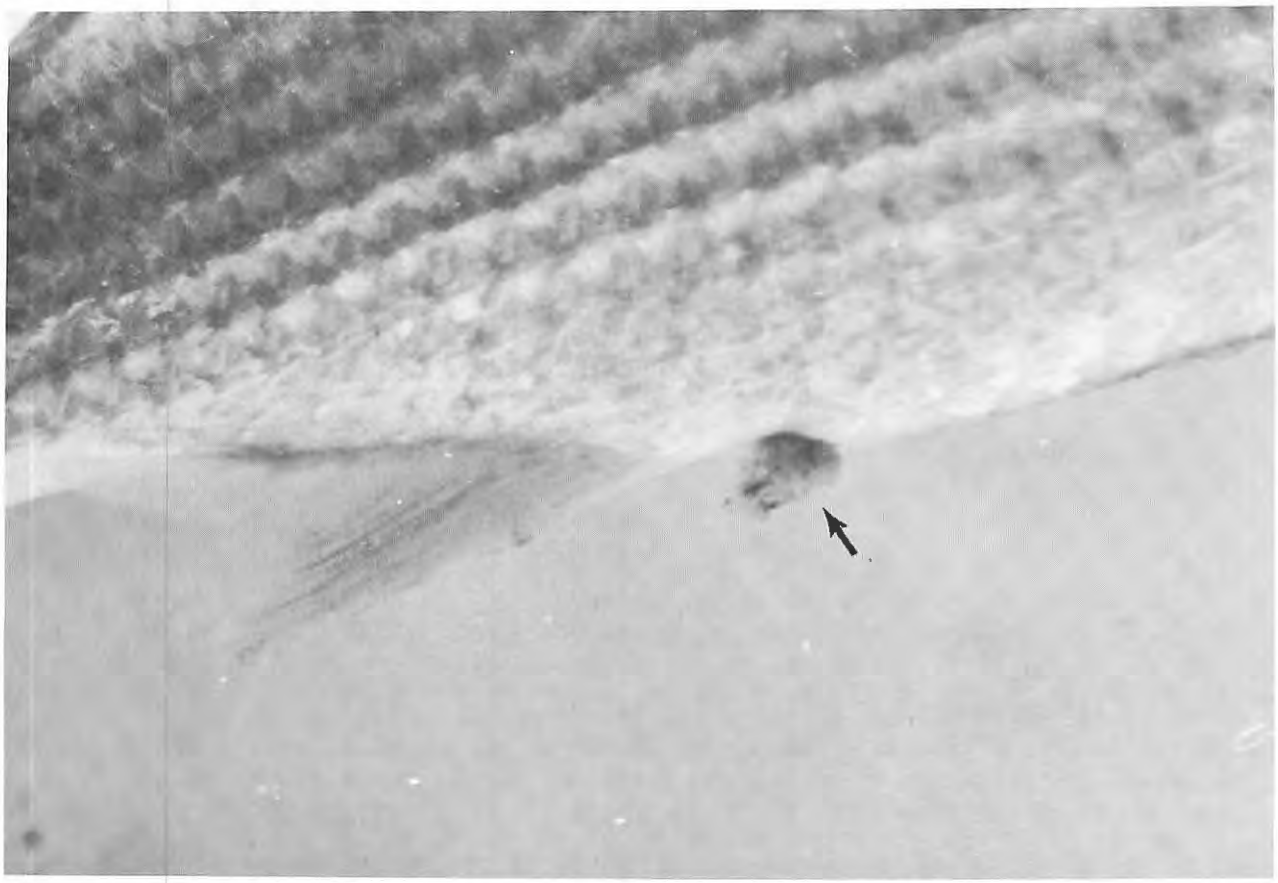

Fig. 3. Spawned female, arrow indicates extruded "plug".

A second female in the same group developed a similar enlargement overnight following the eighth injection. Injection was discontinued at this time also and the fish spawned the following night, 24 h after the onset of abdominal enlargement. The eggs were discovered in the morning, evenly distributed in the aquarium and were similar to those released earlier by the first female; egg diameter averaged 580 μ and a single, well-formed oil droplet was present. A few egg clumps were noted but the majority of the eggs were separate and buoyant. Attempted fertilization of eggs from both females failed. The eggs, however, had been in the water for an indeterminate length of time before they were discovered.

Table 2. Body measurements and ponderal indices of experimental fish

| Group | Fish no. | Body wt (g) | Fork length (cm) | P.I. |
|---|---|---|---|---|
| I | | | | |
| (Males) | 1 | 413.0 | 31.5 | 0.013 |
| | 2 | 390.0 | 31.5 | 0.012 |
| | 3 | 346.0 | 30.0 | 0.013 |
| | 4 | 338.0 | 30.0 | 0.013 |
| | 5 | 390.0 | 31.0 | 0.013 |
| II | | | | |
| (Males) | 1 | 321.0 | 29.5 | 0.013 |
| | 2 | 452.0 | 33.0 | 0.013 |
| | 3 | 386.0 | 32.5 | 0.011 |
| | 4 | 440.0 | 30.0 | 0.016 |
| | 5 | 326.0 | 30.0 | 0.012 |
| | 6 | 415.0 | 33.0 | 0.012 |
| III | | | | |
| (Females) | 1 | 447.0 | 32.5 | 0.013 |
| | 2 | 395.0 | 31.5 | 0.013 |
| | 3 | 376.0 | 30.5 | 0.013 |
| | 4 | 424.0 | 31.5 | 0.014 |
| | 5 | 340.0 | 30.0 | 0.013 |
| IV | | | | |
| (Females) | 1 | 411.0 | 32.0 | 0.013 |
| | 2 | 445.0 | 32.0 | 0.014 |
| | 3 | 423.0 | 32.0 | 0.013 |
| | 4 | 472.0 | 32.0 | 0.014 |
| V | | | | |
| (Females) | 1 | 492.0 | 33.0 | 0.014 |
| | 2 | 471.0 | 33.0 | 0.013 |
| | 3 | 553.0 | 33.5 | 0.015 |
| | 4 | 629.0 | 35.0 | 0.015 |

Table 3. Effects of various doses of hormones on ovulation and spermiation in mullet

| Group | Fish no. and sex | Dosage | Cumulative treatment Pituitary | Synahorin (RU) | ACTH (I.U.) | Results |
|---|---|---|---|---|---|---|
| I | 1-5 ♂ | 2 pituitary (M♂)* + 35 RU Synahorin | 24 | 420 | 0 | - |
| II | 1-6 ♂ | 2 pituitary (M♀)** + 35 RU Synahorin | 24 | 420 | 0 | - |
| III | 1-5 ♀ | 1 pituitary (S)*** + 25 I.U. ACTH | 12 | 0 | 30 | - |
| IV | 1 ♀ | 1 pituitary (S) + 35 RU Synahorin | 5 | 175 | 0 | Spawned |
| | 2 ♀ | 1 pituitary (S) + 35 RU Synahorin | 8 | 280 | 0 | Spawned |
| | 3-4 ♀ | 1 pituitary (S) + 35 RU Synahorin | 12 | 420 | 0 | - |
| V | 1-4 ♀ | 2 pituitary (M) + 35 RU Synahorin | 12 | 420 | 0 | - |

*(M) = mullet pituitary from spawning male.

**(M) = mullet pituitary from spawning female.

***(S) = salmon pituitary, unsexed.

## DISCUSSION

The limited success achieved in this study demonstrates the feasibility of inducing ovulation and oviposition in tertiary yolk stage-females with relatively low gonadosomatic indices. The fertility of the released eggs, however, remains in doubt.

The refractoriness of the majority of males and females in the five groups could be due to: (1) inadequate dosage, (2) the onset of oocyte degeneration prior to the initiation of injection, in the case of the females, or (3) a combination of both. Simultaneous oocyte regression in all females is highly unlikely, especially since follicular atresia was not detected in any of the killed control females. Furthermore, gonad degeneration cannot account for the lack of the milt hydration response in the males. Low dosage, either in individual injections or cumulative treatment, is more likely to be involved. Assessment of the efficacy of the various hypophysation protocols used in this study is possible only if it is assumed that all experimental fish were initially at the same stage of oocyte development. If such an assumption is made, then it appears that spawning was achieved with the lower pituitary doses when applied in conjunction with Synahorin (one pituitary plus 35 RU Synahorin/ 500 g body weight). Tang (1964), on the other hand, reported a threshold dose of a single injection of two mullet pituitaries with 40 RU Synahorin for migratory mullet, while Liao (1969) used doses of 2.5 to 3.5 mullet pituitaries plus 10 to 35 RU Synahorin on the same migratory stock with equal success. This variability in response probably relates to variations in the state of ovarian development. In this study, only two of four females spawned with the lower dose. This invalidates the assumption that all oocytes were at the same developmental stage in Group IV fish. The authors are consequently of the opinion that comparison of dose effects are meaningless, even in the same species, unless oocyte development is ascertained before initiation of treatment.

Mullet used by Tang (1964) and Liao (1969) were collected from natural schools of fish in the course of their spawning migration along the west coast of Taiwan and were probably in an advanced stage of ovarian development. Hypophysation of such fish is aimed at the induction of ovulation and oviposition. Fish used in this study were in the pre-migratory feeding stage in inshore waters and were probably relatively immature by comparison. Hormone treatment in this case would be expected to have a range of effects depending on the degree of ovarian maturation. Those fish in the later stages

of development would probably respond while less mature individuals would most likely develop higher GSI values but may not spawn. Depending on the type of hormone and the dose applied, some of these latter might possibly undergo premature ovulation. This is probably the case with females 1 and 2 of Group IV since, although the released eggs were well formed, with an intact oil droplet, egg diameter was much below that observed in mature spawners (0.930 mm; Shehadeh, unpublished results). Since non-responsive females exhibited a predominance of attretic oocytes, it is very likely that these fish were relatively immature to begin with and the applied dose was too low to induce further development. Our data is more comparable to those of Yashouv (1969) where one of six pond reared females spawned following three injections of carp pituitaries applied at the rate of 1.6, 22 and 2 pituitaries/kg body weight respectively. One of the non-responsive fish was found to have a GSI of 1.4, while a number of others exhibited significant increases in GSI values but did not spawn.

In an effort to standardize conditions in experimental fish, our laboratory is testing the feasibility of removing ovarian eggs by suction through a flared, sterile, polyethylene tubing. Such sampling has been so far practised repeatedly on the same fish with no visible damage. Egg diameter can be measured and the state of oil droplet formation readily determined. Since this study, it has been possible to follow oocyte growth and development in fish under hormone treatment for as long as 60 days. The method and results will be published elsewhere.

The eversion of the tissue "plug" from the cloaca is not clearly understood but the same phenomenon was subsequently noted with handstripped females. The "plug" consists of highly vascularized membranous tissue and appears to block the oviduct. Appreciable pressure had to be applied to the abdomens of handstripped females to obtain egg release via the cloaca. The "plug" was invariably dislodged prior to egg release. It is possible that, in natural spawning, increased abdominal pressure precipitated by the rather sudden hydration of the eggs and subsequent enlargement of the ovaries, is responsible for the dislodgement of the "plug".

Both females 1 and 2 (Group IV) spawned within 48 h following abdominal enlargement; the enlargement probably reflects egg hydration and ovulation. Failure to strip the fish manually at that time resulted in abnormal spawning and "over-ripe" eggs in the case of female 1 and "water hardening" from prolonged contact with sea water in the case of female 2. It is advisable, therefore, to handstrip females 12 to 24 h following abdominal swelling or to place ripe males in the same aquarium.

Further experiments involving more detailed studies of the effects of salmon gonadotropin on vitellogenesis, ovulation and spermiation are presently in progress.

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II.

# VALIDATION OF AN *in vivo* METHOD FOR MONITORING OVARIAN DEVELOPMENT IN THE GREY MULLET (*Mugil cephalus* L.)


## Abstract

The validity of using extruded intra-ovarian oocytes for *in vivo* assessment of ovarian maturity in the grey mullet was established. The diameter of sampled, unfixed oocytes was used as a reference point for comparative purposes. Analysis of variations in oocyte diameters among samples removed from seven different ovarian locations indicated that mullet oocytes develop in synchrony and that *in vivo* samples taken from any area in the ovary would be representative of the entire ovary. Statistical analyses of oocyte diameters and diameter-frequency distributions data from duplicate *in vivo* samples removed from the same ovarian site in each of 17 females showed no significant differences and validated the accuracy of the method. Similar comparison of data from *in vivo* and *in vitro* samples revealed no statistically significant differences.



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## INTRODUCTION

A major problem in the artificial inducement of ovulation and spawning of fish is the inability of the investigator to determine accurately the state of ovarian maturity of individual females. As a result spawning successes have been reported without an adequate frame of reference that permits repetition and replication.

The problem is complicated further by the varying gonadotropic potency of the crude pituitary extracts utilized in hypophysation procedures. This influences the interpretation of dose rate effects.

Without a proper frame of reference for standardization it has been the practice to use gonadotropins in graded series around dose rates recommended by other investigators. When no data is available for some species continuous daily injection over a wide range of dose rates is practiced until spawning is achieved (Clemens, 1968).

In consequence much caution is necessary when interpreting published data in the literature and in the extensive and thorough reviews on the induced breeding of fish (Pickford and Atz, 1957; Clemens, 1968; Shehadeh, 1970).

The present work was undertaken to develop a practical in vivo method to assess ovarian maturity, i.e. the stage of development of intra-ovarian oocytes. Use was made of the existing technique of removing intra-ovarian oocytes from live females with glass or polyethylene cannulas. This method has been used subjectively to assess egg clarity, shape, color, etc., by many workers (Stevens, 1964; Chen et al., 1969: Shehadeh, 1970; Liao, personal communication). The study attempts to apply the technique for in vivo determination of ovarian development of the grey mullet, Mugil cephalus L., and to establish the statistical validity of data obtained with this procedure.

## METHODS AND MATERIALS

The study was conducted during the natural breeding season of the grey mullet, Mugil cephalus L., in Hawaii, namely January and February. The females were taken from captive broodstock held in seawater (32 $^{o}/_{oo}$) and maintained on a diet of Purina Trout Chow. The fish ranged in weight from 596 - 1371 g and measured 33 - 40 cm in length (fork length).

In order to establish the validity of using a limited number of exteriorized intra-ovarian oocytes as reliable indicators of ovarian development, three tests were conducted:

1. To determine whether grey mullet oocytes develop in synchrony such that samples from one location in the ovary can be considered representative of all other areas. This is a critical consideration since it is difficult to determine accurately the in vivo position of the inserted cannula. Three females were sacrificed and one ovary excised from each. The ovaries were then quartered and samples removed from the central portion of each section. In addition, samples were removed from the exact center and two extremities of the ovary making a total of seven samples from each ovary. Sampling sites are represented schematically in Fig. 1.

2. To determine quantitative variation among replicate in vivo samples removed from the same site in single ovaries. Duplicate samples were taken from each of 17 females for this purpose.

3. To compare in vivo and in vitro samples taken from the same female. Nine females were used in this study. In vivo samples were removed first with a cannula after which fish were sacrificed and the sampled ovaries excised. The in vitro samples were taken from the center of the ovary, care being taken to avoid peripheral areas.

In all tests, intra-ovarian oocytes were removed from unanaesthetized females through a polyethylene cannula. The cannula was inserted into the oviduct to a distance of 6.5 cm from the cloacal opening and oocytes withdrawn into the tube by suction. The oocytes were washed and preserved in a solution of 1% formalin in 0.6% NaCl. Previous tests had demonstrated that this solution acted as an adequate preservative without inducing significant changes in volume of the oocytes. The oocytes were placed on a plexiglass slide and measured through a microscope fitted with an ocular micrometer. Fine grooves in the slide aligned the oocytes and facilitated measurements. Diameters were measured along the horizontal axis and values groups in 50 $\mu$ class intervals. Sample size varied between 100 and 200 oocytes. Mean oocyte diameter for each sample was calculated and compared by t-test. Statistical differences between diameter frequency distributions were calculated by chi-square determinations.

## RESULTS

Most of the females utilized in this study were at the tertiary yolk stage of oocyte development. The appearance of an exteriorized intra-ovarian oocyte at this stage, under transmitted light, and its corresponding histological section are shown in Figs. 2 and 3 respectively. The dense black appearance

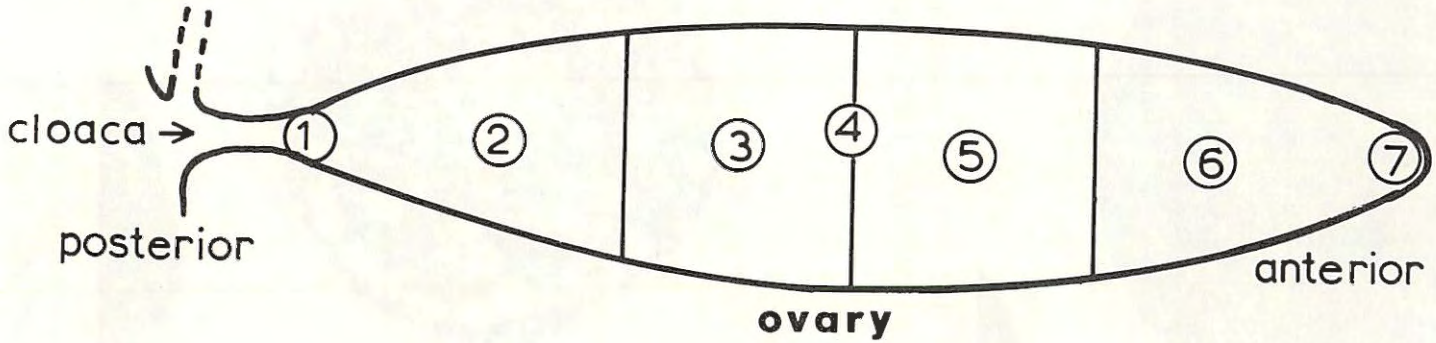


Fig. 1. A schematic representation of in vitro sampling sites in the mullet ovary.

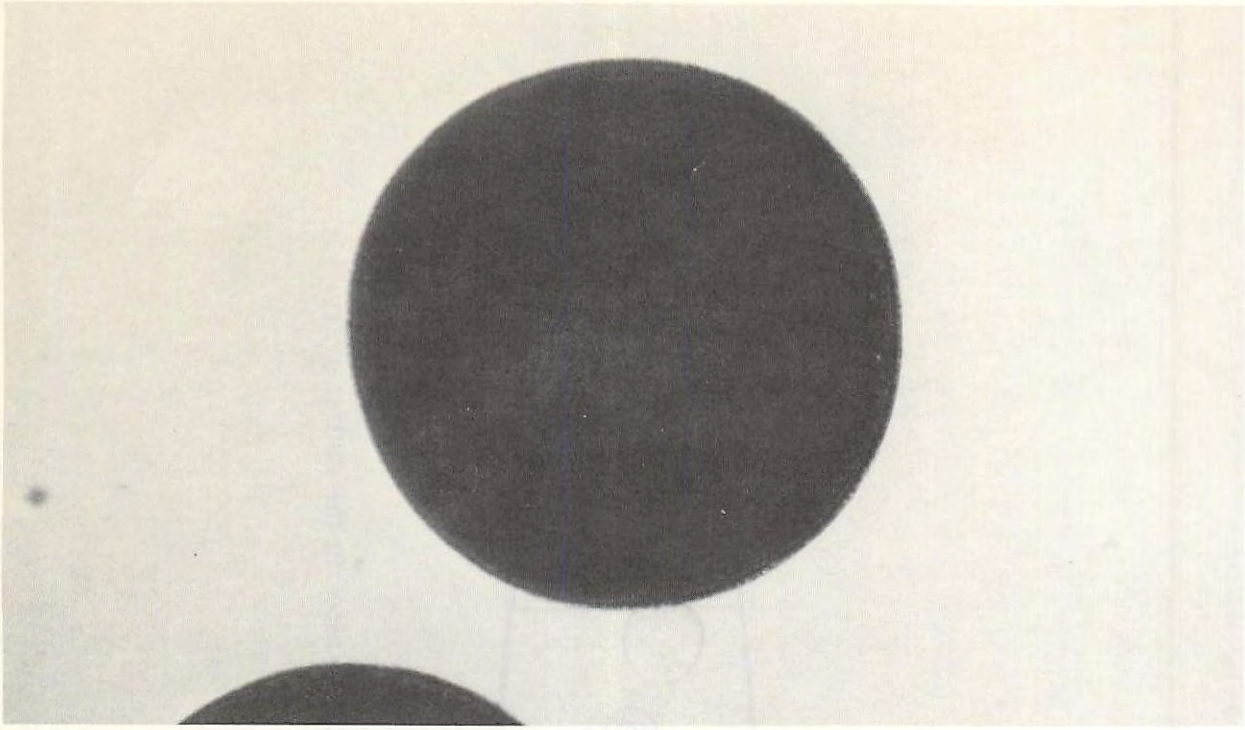

Fig. 2. Exteriorized (tertiary yolk stage) intra-ovarian oocyte under transmitted light. 155X magn.

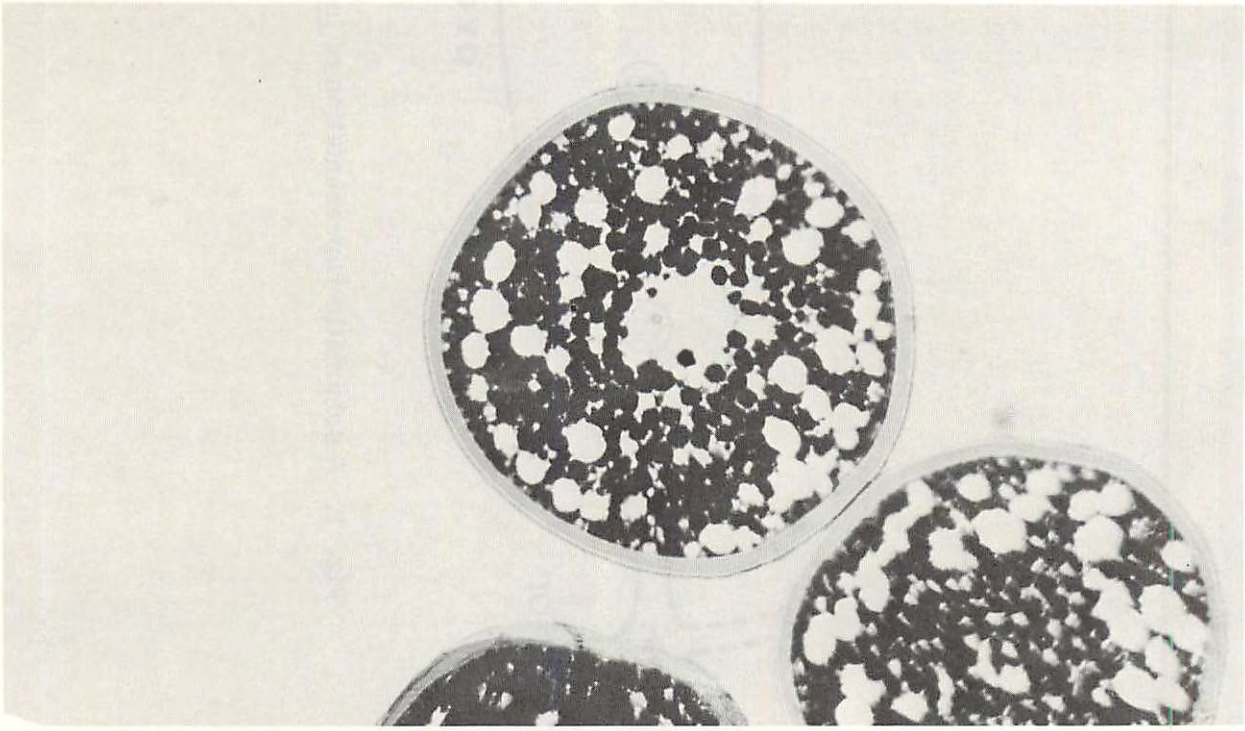

Fig. 3. Histological section of siphoned intra-ovarian oocyte at the tertiary yolk stage. Hematoxylin & Eosin, 135X magn.

of the whole oocyte is due to the packed yolk globules. The vacant spherical spaces apparent in the histological section represented oil droplets.

1. Multiple site analysis in single ovaries

Mean oocyte diameter values and diameter frequency distributions from seven ovarian sites (Fig. 1) in each of three females are shown in Table 1. Mean oocyte diameter values, 95% confidence limit of the mean, standard deviation and diameter value range are present in Fig. 4. Mean oocyte diameter values revealed a trend toward larger oocytes in the posterior-anterior direction. Statistical evaluation (Table 2) showed no significant differences in mean diameters of the samples except for sites 1 and 7, which differed from all other sites. Measured differences in mean oocyte diameters among sites 2 - 6 in three females ranged from 5.8 - 8.0 $\mu$ compared to 17 - 29 $\mu$ between sites 1 and 7. The oocytes of mullet appear to develop in synchrony and in vivo sampling error can be minimized by avoiding the extremities of the ovary.

2. Analysis of variation in duplicate in vivo samples

Statistical analyses of oocyte diameter data from duplicate samples taken from the same ovarian site in each of 17 females are shown in Table 3. No statistically significant differences were found in mean oocyte diameters or diameter frequency distributions between duplicate samples using t-test and chi-square analyses respectively.

3. Analysis of differences between in vivo and in vitro samples

Comparison of mean oocyte diameters and diameter frequency distributions between siphoned in vivo samples and in vitro samples removed from the central portion of each ovary in nine females is shown in Table 4. No statistically significant differences were detected in mean oocyte diameters or frequency distributions. Measured differences in diameter means ranged from 0.8 - 8.1 $\mu$ which is well within the range of variation noted among sites 2 - 6 (Fig. 1) of single ovaries in the multiple site analysis.

In vivo sampling is consequently an accurate alternative to current in vitro methods that involve sacrificing valuable brood stock.

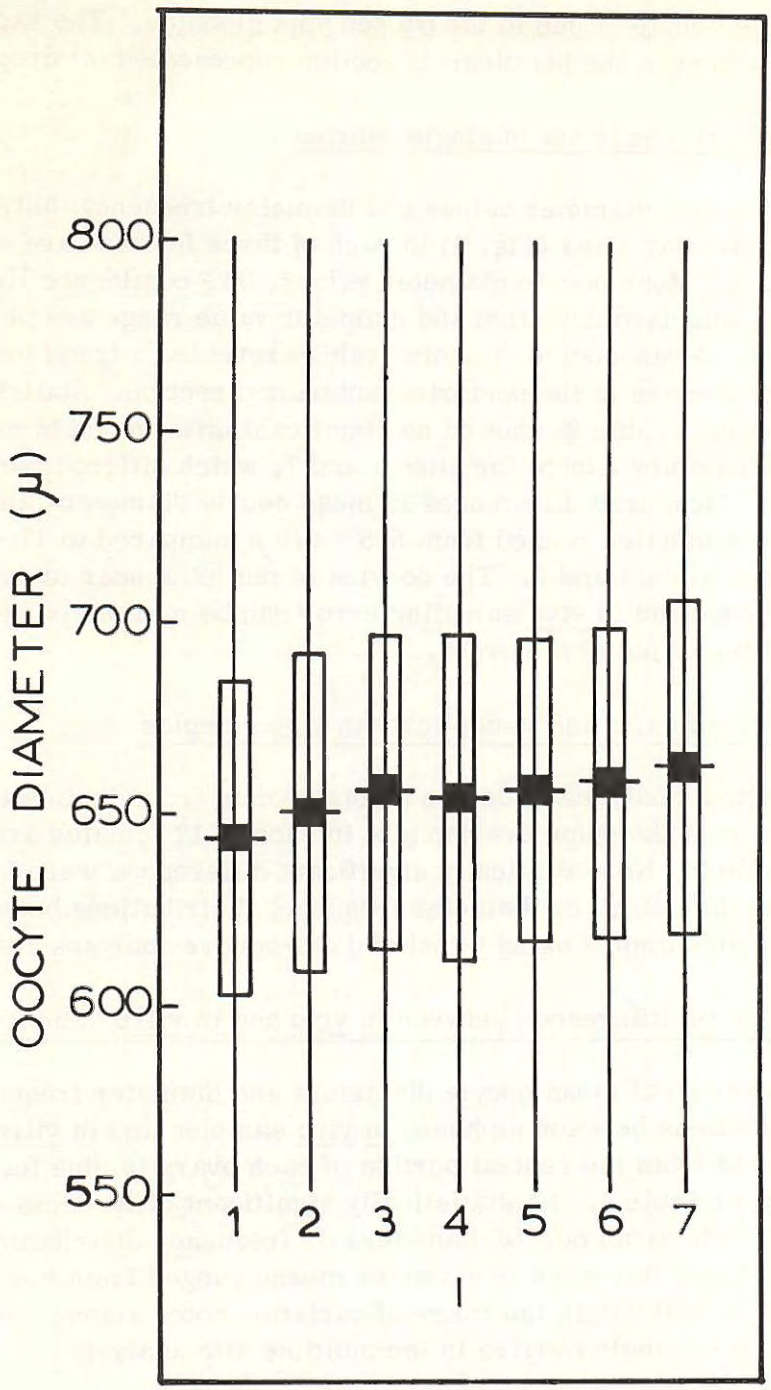


Fig. 4 (a, b, c). Oocyte measurements (mean oocyte diameter, 95% confidence limit of the mean, standard deviation and data range) from seven ovary sites in three female mullet (I, II, and III).

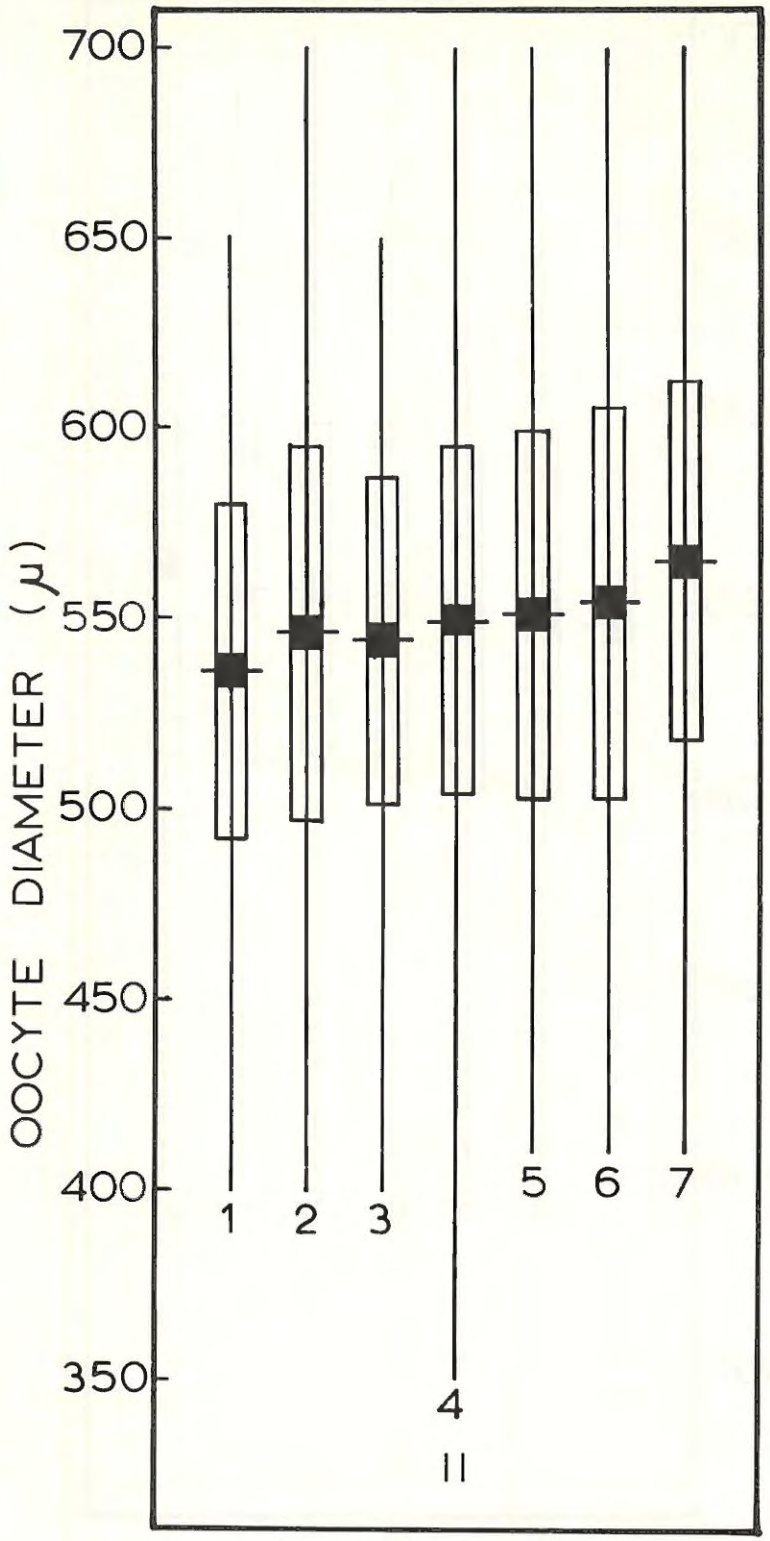


Fig. 4 (b)

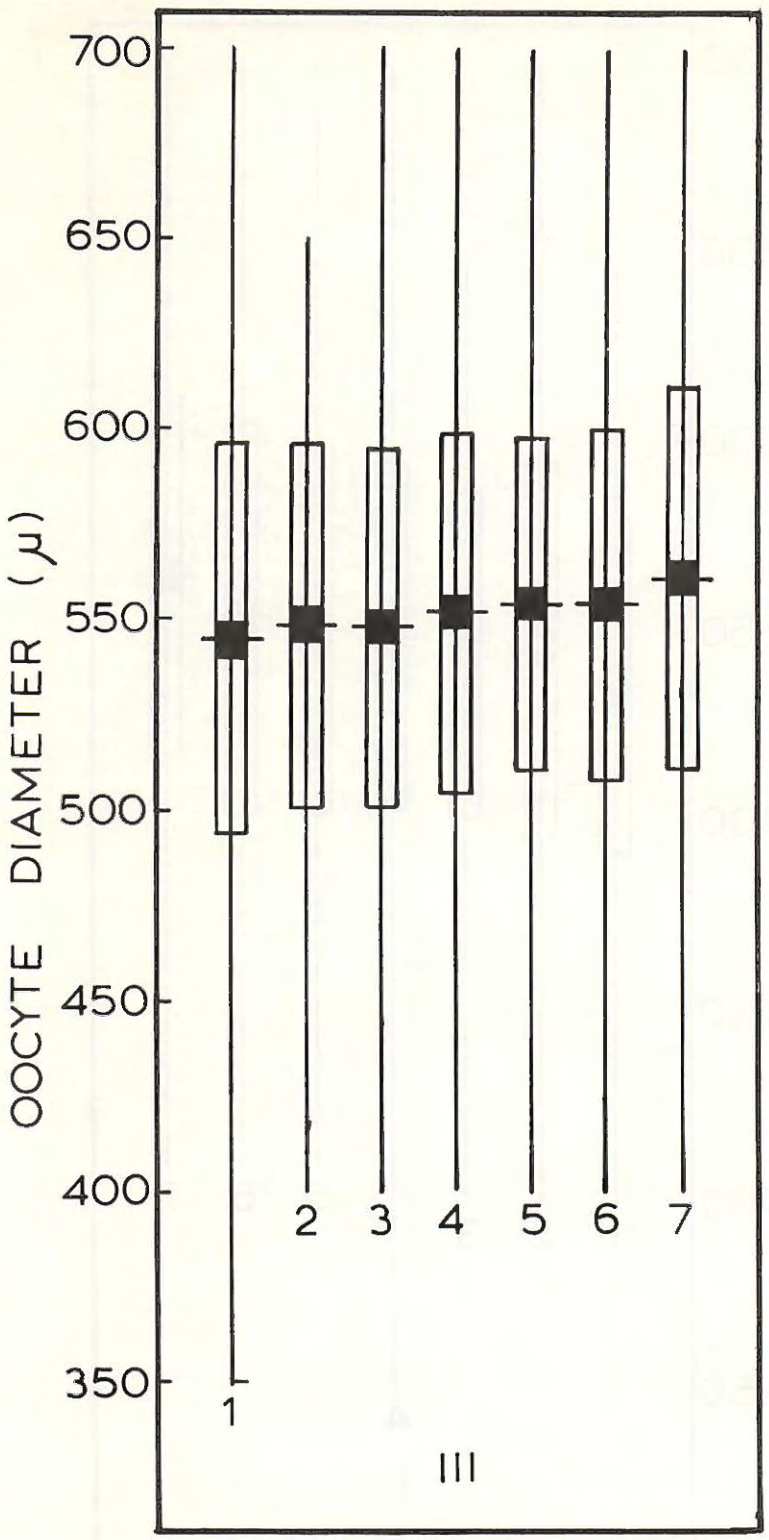


Fig. 4 (c)

Table 1. Oocyte diameter-frequency distribution and mean diameter values for seven ovary sites in three individual females.

| Fish No. | Site | oocyte diameter (μ) - frequency distribution 350\|400 | 400\|450 | 450\|500 | 500\|550 | 550\|600 | 600\|650 | 650\|700 | 700\|750 | 750\|800 | $\bar{x}$ (μ) | N | $S_{\bar{x}}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| I | 1 | | | | | 21 | 67 | 54 | 9 | 1 | 643 | 152 | 41 |
| | 2 | | | | | 15 | 65 | 58 | 15 | 1 | 650 | 154 | 41 |
| | 3 | | | | | 12 | 58 | 69 | 16 | 2 | 655 | 157 | 41 |
| | 4 | | | | | 14 | 60 | 62 | 19 | 1 | 654 | 156 | 42 |
| | 5 | | | | | 10 | 59 | 67 | 17 | 1 | 656 | 154 | 40 |
| | 6 | | | | | 11 | 55 | 70 | 20 | 1 | 658 | 157 | 41 |
| | 7 | | | | | 10 | 51 | 66 | 24 | 3 | 662 | 154 | 44 |
| II | 1 | | 3 | 25 | 53 | 47 | 7 | | | | 536 | 135 | 44 |
| | 2 | | 3 | 19 | 50 | 45 | 17 | 1 | | | 546 | 135 | 49 |
| | 3 | | 2 | 19 | 56 | 56 | 11 | | | | 544 | 144 | 43 |
| | 4 | 1 | 2 | 11 | 52 | 55 | 13 | 1 | | | 549 | 135 | 46 |
| | 5 | | 2 | 15 | 56 | 47 | 20 | 2 | | | 551 | 142 | 48 |
| | 6 | | 3 | 19 | 36 | 57 | 21 | 2 | | | 554 | 138 | 51 |
| | 7 | | 1 | 10 | 37 | 58 | 21 | 3 | | | 565 | 135 | 47 |
| III | 1 | 2 | 4 | 19 | 47 | 62 | 13 | 1 | | | 545 | 148 | 51 |
| | 2 | | 3 | 15 | 48 | 46 | 18 | | | | 548 | 130 | 48 |
| | 3 | | 2 | 17 | 51 | 48 | 16 | 1 | | | 548 | 135 | 47 |
| | 4 | | 1 | 17 | 51 | 49 | 21 | 1 | | | 552 | 140 | 47 |
| | 5 | | 1 | 11 | 60 | 54 | 21 | 1 | | | 554 | 148 | 44 |
| | 6 | | 1 | 15 | 48 | 55 | 18 | 2 | | | 554 | 139 | 46 |
| | 7 | | 5 | 8 | 37 | 66 | 21 | 4 | | | 561 | 141 | 50 |

Table 2. t-test evaluation of mean oocyte diameters from seven ovary sites in three mullet females. Numbers represent t-values.

| Fish No. | Site | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|
| I | 1 | 1.47 | 2.68 | 2.26 | 2.76 | 3.16 | 3.92 |
| | 2 | | 1.20 | 0.80 | 1.26 | 1.68 | 2.48 |
| | 3 | | | 0.38 | 0.04 | 0.48 | 1.33 |
| | 4 | | | | 0.43 | 0.85 | 1.68 |
| | 5 | | | | | 0.44 | 1.30 |
| | 6 | | | | | | 0.88 |
| II | 1 | 1.76 | 1.53 | 2.43 | 2.71 | 3.09 | 5.13 |
| | 2 | | 0.36 | 0.57 | 0.86 | 1.30 | 3.17 |
| | 3 | | | 1.00 | 1.30 | 1.75 | 3.79 |
| | 4 | | | | 0.30 | 0.78 | 2.70 |
| | 5 | | | | | 0.49 | 2.37 |
| | 6 | | | | | | 1.78 |
| III | 1 | 0.66 | 0.58 | 1.25 | 1.72 | 1.60 | 2.79 |
| | 2 | | 0.09 | 0.57 | 1.02 | 0.93 | 2.14 |
| | 3 | | | 0.09 | 1.13 | 1.03 | 2.26 |
| | 4 | | | | 0.43 | 0.36 | 1.62 |
| | 5 | | | | | 0.06 | 1.28 |
| | 6 | | | | | | 1.29 |

Table 3. Chi-square and t-test analyses of duplicate in vivo samples from 17 females.

| Sample No. | oocyte diameter (μ) - frequency distribution | | | | | | | | | | | $\bar{x}$ (μ) | N | t | $X^2$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 350 \| 400 | 400 \| 450 | 450 \| 500 | 500 \| 550 | 550 \| 600 | 600 \| 650 | 650 \| 700 | 700 \| 750 | 750 \| 800 | 800 \| 850 | 850 \| 900 | | | | |
| 1a | 2 | 5 | 17 | 62 | 48 | 15 | 1 | | | | | 541 | 150 | | 6.80 |
| 1b | 1 | 2 | 10 | 70 | 58 | 8 | 1 | | | | | 545 | 150 | 0.74 | |
| 2a | 2 | 9 | 42 | 52 | 18 | 1 | | | | | | 506 | 124 | | 4.66 |
| 2b | 1 | 3 | 40 | 64 | 17 | | | | | | | 512 | 125 | 1.10 | |
| 3a | | 4 | 13 | 63 | 70 | 16 | 1 | | | | | 546 | 167 | | 6.74 |
| 3b | 2 | 7 | 26 | 60 | 62 | 14 | 2 | | | | | 543 | 173 | 0.52 | |
| 4a | | 3 | 31 | 58 | 48 | 10 | | | | | | 535 | 150 | | 2.42 |
| 4b | | 4 | 22 | 61 | 49 | 13 | 1 | | | | | 541 | 150 | 1.06 | |
| 5a | | | 3 | 10 | 36 | 57 | 19 | 2 | | | | 607 | 127 | | 5.36 |
| 5b | | | 1 | 6 | 34 | 69 | 11 | 1 | | | | 610 | 122 | 0.66 | |
| 6a | | | | 4 | 21 | 63 | 29 | 3 | | | | 628 | 120 | | 2.07 |
| 6b | | | | 8 | 27 | 59 | 30 | 2 | | | | 621 | 126 | 1.14 | |
| 7a | | | | 2 | 20 | 63 | 37 | 4 | | | | 633 | 126 | | 0.10 |
| 7b | | | | | 25 | 72 | 41 | 2 | | | | 632 | 140 | 0.26 | |
| 8a | | | | | 5 | 92 | 82 | 3 | | | | 648 | 182 | | 1.55 |
| 8b | | | | | 4 | 63 | 57 | 5 | | | | 649 | 129 | 0.45 | |

(Table 3 continued next page)

Table 3. (continued)

| Sample No. | oocyte diameter (μ) - frequency distribution 350 \| 400 | 400 \| 450 | 450 \| 500 | 500 \| 550 | 550 \| 600 | 600 \| 650 | 650 \| 700 | 700 \| 750 | 750 \| 800 | 800 \| 850 | 850 \| 900 | $\bar{x}$ (μ) | N | t | $X^2$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 9a | | | | | 5 | 57 | 123 | 7 | | | | 659 | 192 | | 0.13 |
| 9b | | | | | 5 | 60 | 127 | 6 | | | | 659 | 198 | 0.20 | |
| 10a | | | | | 1 | 32 | 99 | 17 | 1 | | | 670 | 150 | | 1.25 |
| 10b | | | | | 2 | 22 | 104 | 16 | 1 | | | 672 | 145 | 0.63 | |
| 11a | | | | | | 1 | 48 | 112 | 5 | | | 711 | 166 | | 0.58 |
| 11b | | | | | | 3 | 43 | 87 | 4 | | | 709 | 137 | 0.88 | |
| 12a | | | | | | | 20 | 85 | 25 | 4 | | 730 | 134 | | 1.06 |
| 12b | | | | | | 2 | 25 | 112 | 24 | 5 | | 726 | 168 | 0.87 | |
| 13a | | | 2 | 13 | 55 | 108 | 4 | | | | | 602 | 182 | | 4.23 |
| 13b | | | 3 | 10 | 40 | 92 | 10 | | | | | 606 | 155 | 0.98 | |
| 14a | | | | 3 | 38 | 91 | 52 | 5 | | | | 630 | 189 | | 1.84 |
| 14b | | | | 1 | 31 | 88 | 41 | 2 | | | | 629 | 163 | 0.25 | |
| 15a | | | | 10 | 49 | 50 | 17 | | | | | 604 | 126 | | 4.19 |
| 15b | | | | 13 | 39 | 60 | 10 | | | | | 602 | 122 | 0.37 | |
| 16a | | | | | 9 | 57 | 78 | 13 | 1 | | | 656 | 158 | | 3.23 |
| 16b | | | | | 13 | 63 | 68 | 7 | 1 | | | 649 | 152 | 1.72 | |
| 17a | | | | | | 2 | 22 | 79 | 40 | 5 | | 733 | 148 | | 1.30 |
| 17b | | | | | | 2 | 23 | 87 | 42 | 9 | 1 | 736 | 164 | 0.67 | |

Table 4. Chi-square and t-test comparison of paired *in vivo* and *in vitro* samples from each of nine female mullet. The first sample in each pair represents the *in vitro* sample.

| Fish & Sample Number | oocyte diameter (μ) - frequency distribution | | | | | | | | $\bar{x}$ | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 400 \| 450 | 450 \| 500 | 500 \| 550 | 550 \| 600 | 600 \| 650 | 650 \| 700 | 700 \| 750 | 750 \| 800 | (μ) | N | t | $X^2$ |
| 1a | 2 | 9 | 55 | 65 | 19 | | | | 555 | 150 | 0.61 | 3.47 |
| 1b | 1 | 17 | 51 | 49 | 21 | 1 | | | 552 | 140 | | |
| 2a | 4 | 22 | 61 | 49 | 13 | 1 | | | 541 | 150 | 1.59 | 1.45 |
| 2b | 2 | 19 | 56 | 56 | 11 | | | | 544 | 144 | | |
| 3a | | | | 3 | 55 | 102 | 10 | | 660 | 170 | 1.83 | 4.21 |
| 3b | | | | 5 | 78 | 112 | 5 | | 654 | 200 | | |
| 4a | | | | 1 | 49 | 99 | 13 | | 663 | 162 | 1.73 | 2.98 |
| 4b | | | | 4 | 52 | 92 | 6 | | 658 | 154 | | |
| 5a | | | | 9 | 64 | 54 | 10 | 5 | 653 | 142 | 1.68 | 8.08 |
| 5b | | | | 9 | 51 | 78 | 19 | 3 | 661 | 160 | | |
| 6a | | | | 9 | 57 | 78 | 13 | 1 | 656 | 158 | 0.19 | 1.09 |
| 6b | | | | 8 | 59 | 69 | 17 | 1 | 657 | 154 | | |
| 7a | | | | 15 | 68 | 55 | 15 | 1 | 649 | 154 | 1.01 | 1.38 |
| 7b | | | | 14 | 60 | 62 | 19 | 1 | 654 | 156 | | |
| 8a | | | | 1 | 13 | 83 | 31 | 6 | 689 | 137 | 1.01 | 1.02 |
| 8b | | | | 3 | 15 | 89 | 28 | 4 | 684 | 142 | | |
| 9a | | | | | 9 | 59 | 35 | 8 | 694 | 111 | 0.57 | 1.24 |
| 9b | | | | | 12 | 81 | 35 | 11 | 691 | 139 | | |

## DISCUSSION

The results demonstrate that, for the grey mullet, in vivo sampling of intra-ovarian oocytes is an accurate and reliable method for monitoring ovarian development.

The method offers two advantages over in vitro techniques. Ovarian development can be determined without sacrificing female fish and the method provides a means to follow oocyte development in individual fish thus precluding variations between females in the broodstock.

Chen et al. (1969) cautioned against possible inducement of atresion in developing ovaries by frequent in vivo sampling. Since completing this study continual in vivo sampling of fish receiving exogenous hormone treatment has been practiced. Weekly sampling of ovaries in 23 female fish over a period of 60 days had no deleterious effect on oocyte development (Shehadeh et al., in preparation). However, atresion did occur if individuals were mishandled.

Properly used, in vivo sampling can replace the need for histological examination of oocytes to determine the state of development. Samples of oocytes can be assessed for development by diameter measurement, photographed and preserved for later sectioning and histological examination.

A comparative atlas of dimension, appearance and histological section of oocytes at various stages of development can be prepared. Certain biochemical data might also be included. Such an atlas would be invaluable as a guide to the laboratory investigator and field biologist. An atlas for the grey mullet, Mugil cephalus L., is presently being compiled.

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III.

# THE ESTABLISHMENT OF A CAPTIVE BROOD STOCK OF GREY MULLET, Mugil cephalus


## Abstract

A total of 40 females and 10 males were maintained in 10.2 $m^3$ rubber-lined ponds under running sea water (32 $^{o}/_{oo}$) conditions. A flow rate of 10 gpm and a turnover rate of 40.8 $m^3$/24 hours were maintained in all ponds throughout the year. A natural diet of benthic diatoms, blue-green algae and filamentous green algae was provided by the installation of vertical plastic sheets (6.5 $m^2$/kg fish) which accumulated the required mixed flora. Water chemistry and daily fluctuations in pH, dissolved oxygen and temperature were recorded and the results are discussed. Mortalities resulting from oxygen super-saturation (embolism) occurred during May - October and were subsequently precluded by doubling the flow rates during the dry season between the hours of 9 a.m. and 9 p.m. All males and females matured in February of 1970 and 1971 and were induced to spawn by hormone injection.

## INTRODUCTION

Induced breeding of pond-reared grey mullet has been reported in Taiwan (Liao et al., 1971) and Israel (Yashouv, 1969). Previous efforts in this field were mostly restricted to wild adults captured during the annual breeding season (Liao, 1969, Shehadeh and Ellis, 1970). The successful breeding of captive mullet consequently makes possible the establishment of captive adult stock for breeding purposes only.

Research on the induced breeding of grey mullet in this laboratory, during the past three years, has focused on three major objectives: the establishment of statistically reliable methods for the assessment of gonad maturation in individual hormone-recipient fish, the development of spawning methods other than by manual extrusion of eggs and sperm and the establishment of a captive brood stock to permit controlled selective breeding and eventually genetic selection. The development of spontaneous spawning and natural fertilization in laboratory tanks was a crucial consideration since very few adult males and females survive the manual breeding method and consequently preclude repeated spawning of the same marked individuals in successive years. The first two objectives of the program have been developed successfully (Shehadeh and Kuo, 1972a,b).

The present report documents the conditions under which 50 captive adult mullet were maintained, attained sexual maturity and were artificially spawned repeatedly during the period April 1969 - February 1972.

## MATERIALS AND METHODS

Six ponds, each measuring 9 m x 4 m x 1 m (length x width x depth) were excavated and their entire surface lined with butyl rubber. Each pond was provided with a sea water inlet and drain pipe of polyvinyl chloride construction. A total volume of 10.2 $m^3$ and a flow rate of 10 gpm were maintained in each pond. Water flow provided a pond turnover rate of 40.8 $m^3$/24 hours.

The vertical plastic surfaces were made from 3-mil translucent polyethylene sheets. Each length was prepared by heat-sealing the bottom of the sheet over a lead line, then subdividing the plastic along its width at 15-cm intervals. The net result was separate vertical surfaces measuring 15 cm (width) x 80 cm (vertical length) anchored to the same lead line. Several three meter lengths of this "plastic pasture" were prepared and

stretched across the width of each pond. The total surface area of the pasture in each pond was 163 $m^2$. The ponds were filled and the plastic pasture allowed to accumulate a growth of algae and diatoms, under static water conditions, over a period of two weeks after which water flow was initiated at 10 gpm.

A total of five males and 20 females were stocked in each of two ponds in April 1969. The ratio of plastic pasture surface area to fish mass was 6.5 $m^2$/kg body weight. All fish received a feeding supplement of Purina Trout Chow at 2% of the body weight per day.

Chemical analysis of pond water was carried out to determine levels of nitrate, phosphate, nitrite, ammonia and hydrogen sulfide. Dissolved oxygen levels, water pH and temperature fluctuations were monitored daily at four hour intervals during the wet (November - April) and dry (May - October) seasons. Feeding preference and gut contents were determined by examination of fish fecal pellets. Separation of gut contents was carried out according to Pillay (1953).

The state of gonad development was determined in February 1970 by *in vivo* sampling of intra-ovarian oocytes according to Shehadeh and Kuo (1972a). Gonadosomatic indices (GSI) were determined in 10 sacrificed fish.

## RESULTS AND DISCUSSION

### Pond chemistry

The rubber-lined ponds and plastic pasture are shown in Figures 1 and 2. Chemical analysis of pond water revealed stable low levels of ammonia, hydrogen sulfide and nitrite indicating an adequate pond flushing rate. Sea water pH was initially low at 7.65 but gradually increased and fluctuated between 8.00 and 9.00 as algae and diatoms became established. The high nitrate and phosphate levels, which contributed to the rapid establishment of algae and diatoms, were traced to organic contamination of the sea water well. The initial chemistry analysis is shown in Table 1.

Mean dissolved oxygen, pH and water temperature values during the dry (summer) and wet (winter) seasons are shown in Figures 3 and 4 respectively. All three parameters peaked at 3 p.m. and reached their lowest values at 4 a.m. in both seasons. Greater differences between day and night were reflected in the steeper slope of the daily cycle during the dry season (Fig. 3). Peak mean daily (3 p.m.) values for pH, temperature and dissolved oxygen during the dry season were 9.10, 28° and 16.0 respectively compared to 8.76, 25° and 14.0 in the wet season. Oxygen supersaturation conditions (<8 ppm) prevailed in the ponds during

Fig. 1. A view of the butyl rubber-lined, sea water brood stock ponds.

Fig. 2. Vertical plastic pasture strips in a brood stock pond.

Table 1. Pond water chemistry

| | | ppm |
|---|---|---|
| pH | 7.65 | |
| Salinity | 32 $^{o}/_{oo}$ | |
| Nitrate | | 1.4500 |
| Phosphate | | 0.1400 |
| Nitrite | | 0.0002 |
| Ammonia | | 0.0180 |
| Hydrogen sulfide | | 0.0060 |

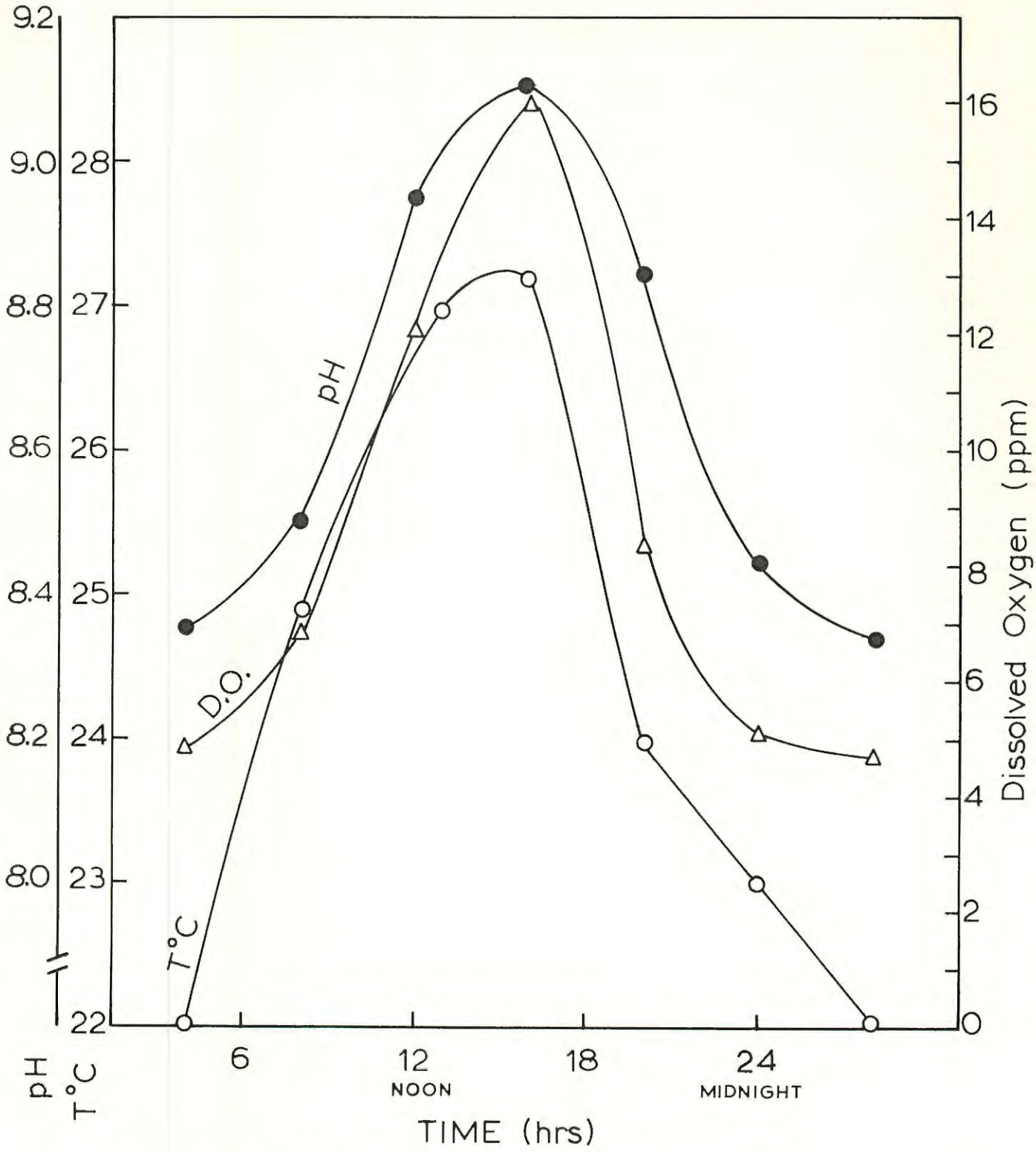


Fig. 3. Mean daily pond conditions during the dry season (May - October)

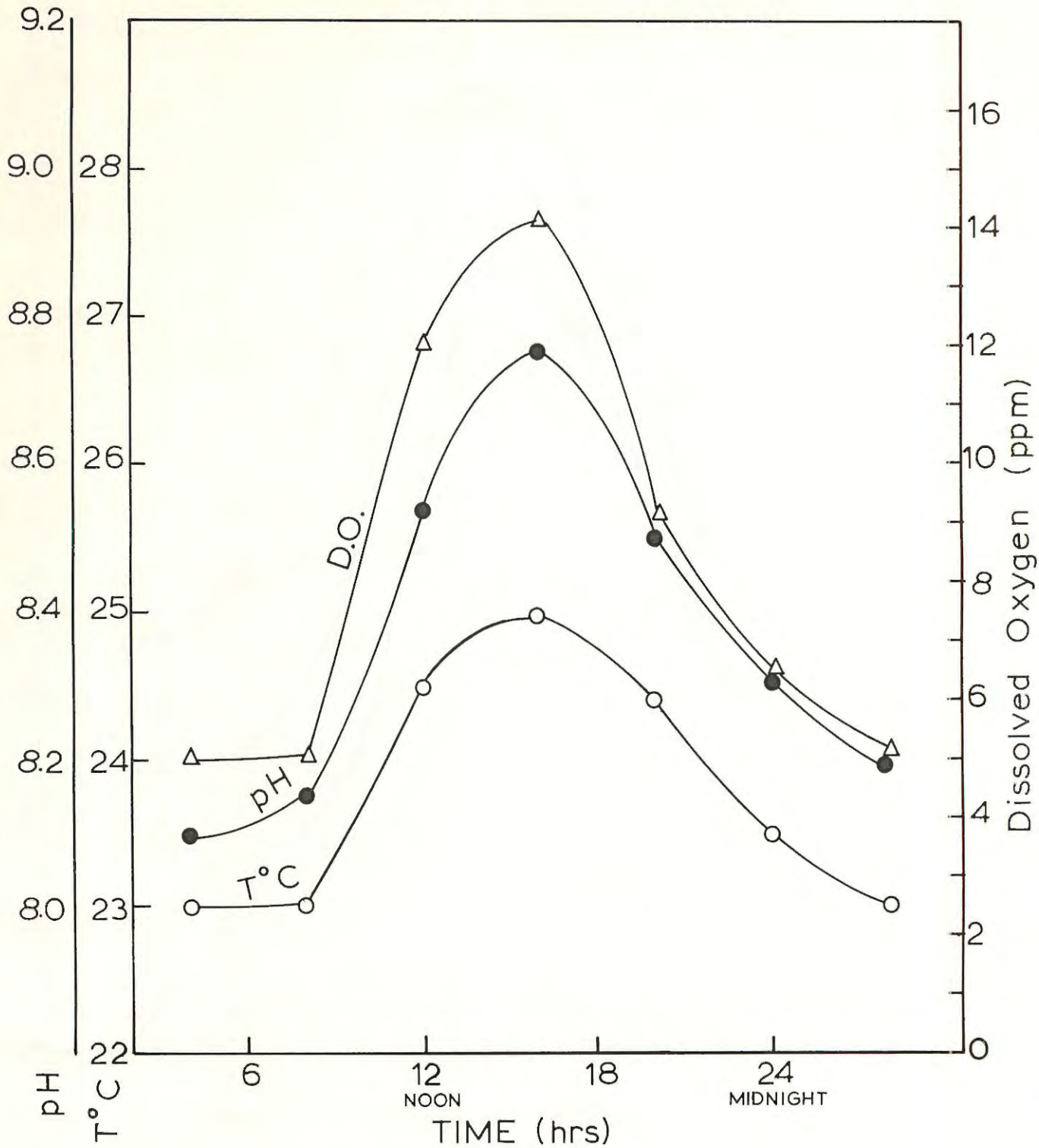


Fig. 4. Mean daily pond conditions during the wet season (November - April)

both seasons from 9 a.m. to 9 p.m. Mortalities due to air embolism occurred during the summer months only. This was probably due to the more pronounced fluctuations in dissolved oxygen content (minimum of 4.8 ppm from 4 a.m. to 9 a.m. to a maximum of 16.0 ppm at 4 p.m.) that were apparent during that time of the year. Corresponding minimum and maximum oxygen content during the wet season were 5.0 ppm and 14.0 ppm respectively. The embolism problem was subsequently avoided by doubling water flow through the ponds from 10 gpm to 20 gpm during the hours of 9 a.m. to 9 p.m.

Daily temperature and pH profiles closely resembled that of dissolved oxygen. The highest and lowest mean daily temperatures were 22-28° C and 22-25° C for the dry and wet seasons respectively. Water temperatures of 30-32° C occurred frequently, notably during the months of June - August. Water pH fluctuated between 8.30 and 9.10 in the dry season and between 8.13 and 8.76 in the wet season.

Feeding and sexual maturation

Mullet were observed to graze on the plastic pasture regularly (Fig. 5). Examination of fecal pellets revealed an average mass composition of 31% sand, 17% diatoms and 52% algae (Fig. 6). Corresponding volume composition was 23%, 33%, and 44% respectively. Diatoms consisted of Melosira, Achnanthes, Thalassiosira, Navicula, Thalassionema, Nitzchia and Amphiphora, with Navicula being the most dominant form. Algal forms consisted of Lyngbia, Enteromorpha and Cladophora, with Lyngbya predominating. These findings are in close agreement with gut content reports by Hiatt (1944) and Ghazzawi (1933) for the grey mullet.

Examination of gonads in February revealed GSI values of 13.27 - 15.28% in males and 7.6 - 16.53% in females (Table 2). The appearance of in vivo sampled eggs and egg diameter-frequency distributions in six sampled females are shown in Figures 7 and 8 respectively. Gonad condition in males and females are shown in Figure 9. Mean egg diameter values (605 - 702 $\mu$; Table 2) indicated that all sampled females were at the proper stage of development for spawning induction by means of hormone injection (see Shehadeh et al., 1972). All females stocked in the rubber-lined ponds were subsequently spawned in February 1970 and again in February and September 1971 according to procedures reported by Shehadeh and Kuo (1972b)

It is concluded that proper environmental conditions, such as the absence of metabolite accumulation, together with a proper diet, are adequate to ensure the seasonal maturation of captive grey mullet. This permits the establishment of a permanent brood stock and gives impetus to selective breeding and genetic selection efforts.

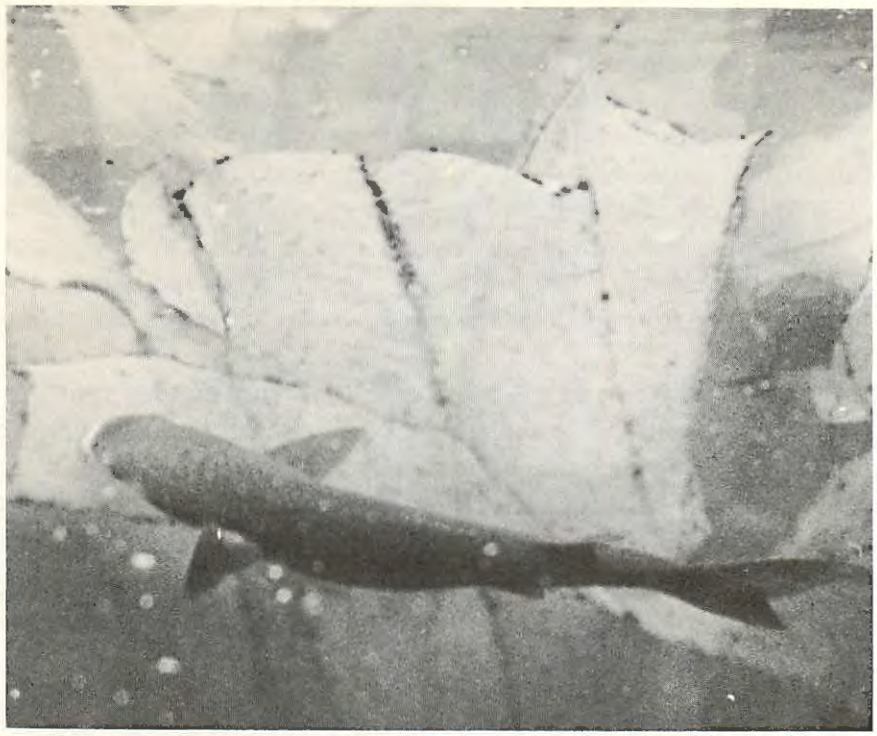

Fig. 5. Mullet grazing on plastic pasture.

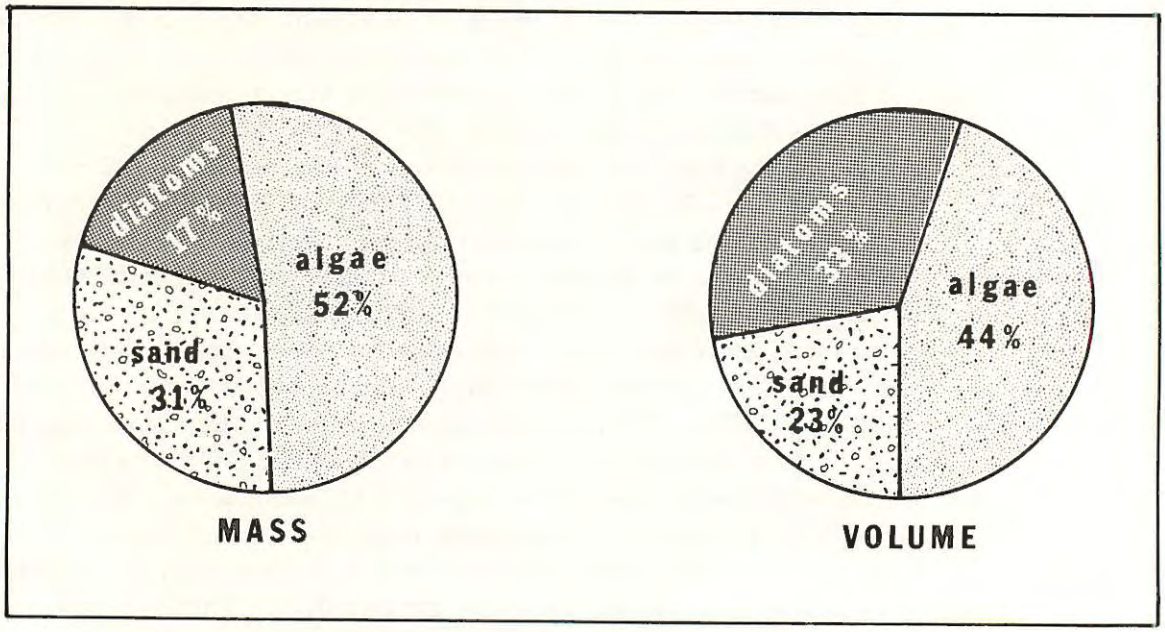


Fig. 6. A schematic representation of fecal pellet composition collected from pond mullet feeding on plastic pasture.

Table 2. Body measurements and gonad development data for sample pond fish sacrificed in February.

| Fish No. and sex | Body weight (g) | Fork length (cm) | Gonad weight (g) | Mean oocyte diameter ( $\mu$) | GSI (%) |
|---|---|---|---|---|---|
| 1 M* | 1025 | 390 | 136 | | 13.27 |
| 2 F** | 950 | 345 | 157 | 694 | 16.53 |
| 3 F | 923 | 355 | 110 | 675 | 11.92 |
| 4 F | 869 | 340 | 121 | 702 | 13.92 |
| 5 F | 1031 | 360 | 79 | 605 | 7.66 |
| 6 F | 1071 | 370 | 163 | | 15.22 |
| 7 F | 921 | 355 | 118 | 660 | 12.81 |
| 8 F | 859 | 335 | 122 | 654 | 14.20 |
| 9 M | 785 | 340 | 120 | | 15.28 |
| 10 M | 932 | 350 | 132 | | 14.16 |

* M - male ** F - female

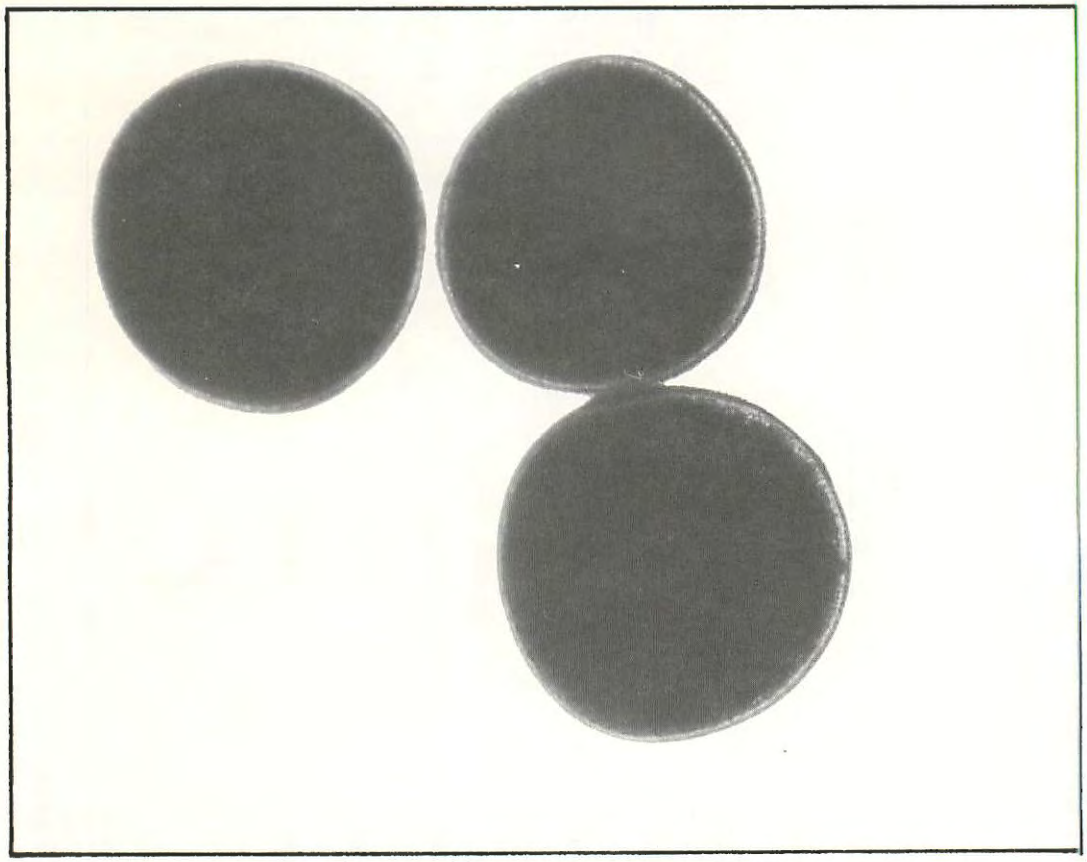

Fig. 7. Mullet eggs sampled in vivo in February showing densely packed yolk.

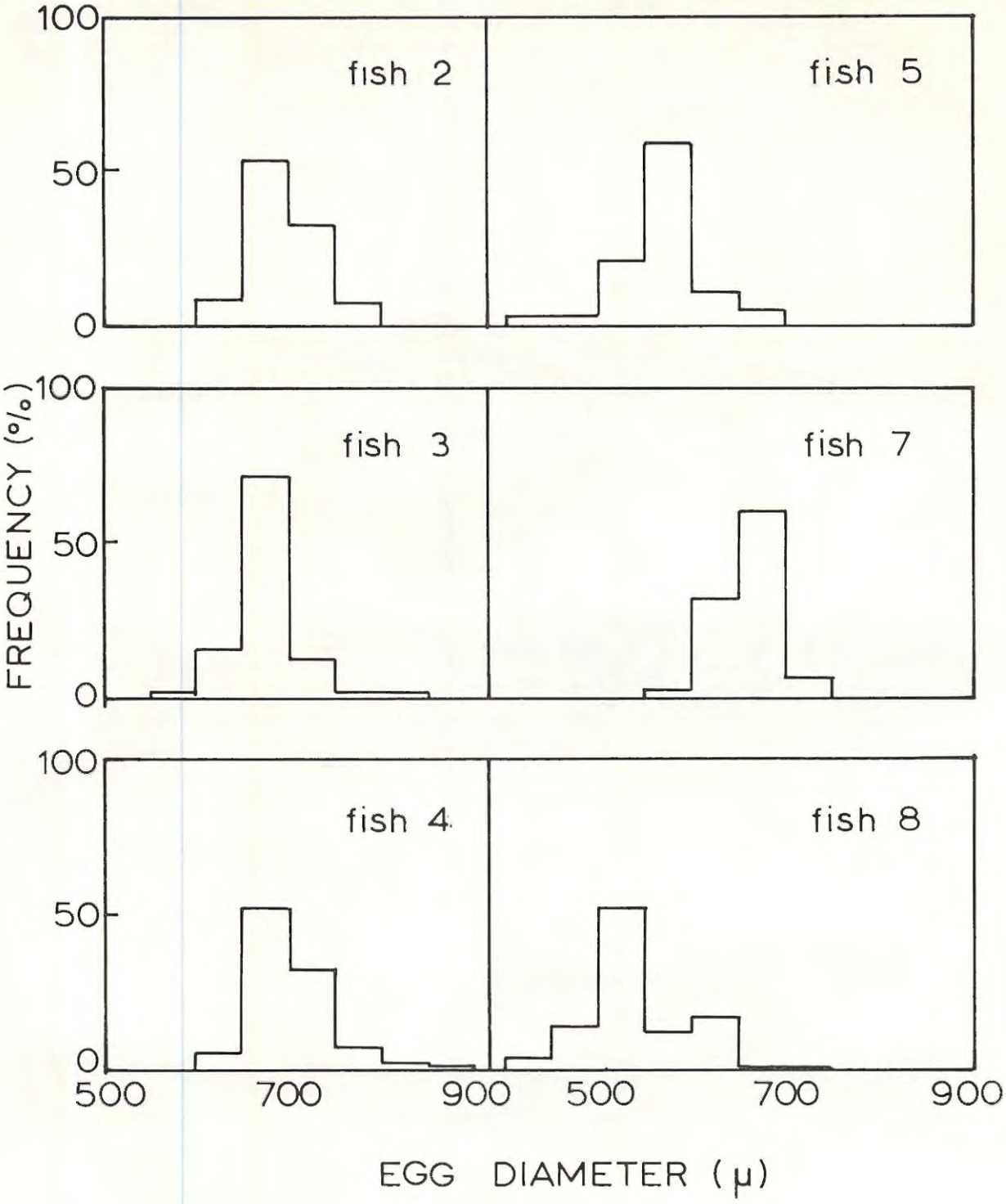


Fig. 8. Egg diameter frequency distributions in six sampled females.

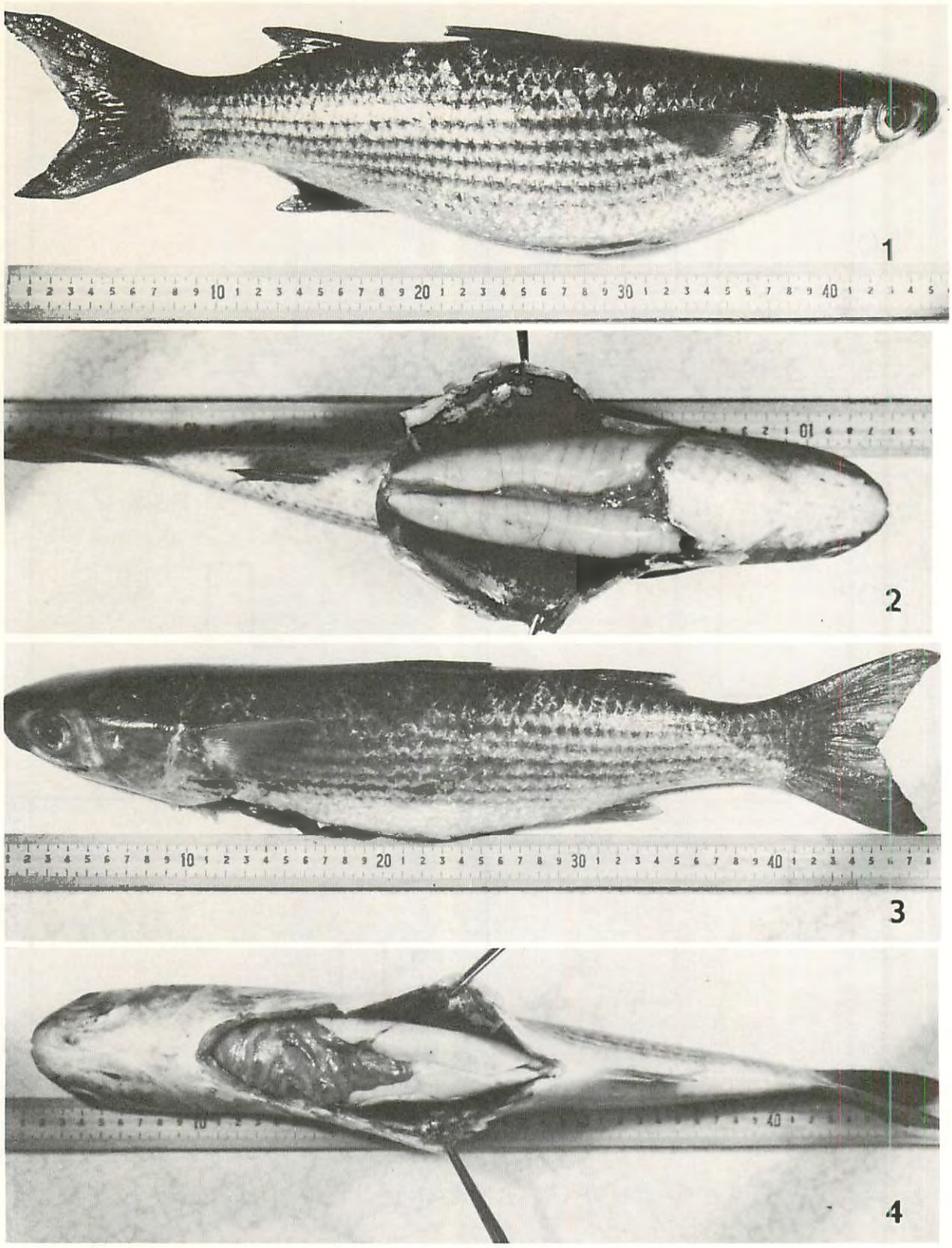


Fig. 9. State of gonad development in pond mullet during February: female (1, 2) and male (3, 4)

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IV.

# THE EFFECTS OF EXOGENOUS HORMONE TREATMENT ON SPERMIATION, VITELLOGENESIS AND OVULATION IN THE GREY MULLET, Mugil cephalus L.


## Abstract

The effects of 17 methyltestosterone and HCG on spermiation and prevention of milt resorption in grey mullet have been studied. Spermiation was achieved with 17 methyltestosterone in October - November (0.1 mg/100 g body wt) and in December - January (5 mg/100 g body wt). HCG was less effective at a dose of 20 IU/100 g body weight. Both hormones prevented milt resorption under laboratory conditions. HCG (50 IU/500 g body wt), FSH + LH (15 µg + 5 µg/500 g body wt), mullet pituitary extract + Synahorin (100 µg + 30 RU/500 g body wt) were used to induce oocyte maturation and prevent onset of atresion in the laboratory with equal success. Differences in oocyte development between controls and experimental fish are discussed, and a critical oocyte diameter beyond which spawning injection can be effective is suggested. Partially purified salmon pituitary gonadotropin applied at the rate of 100 µg/kg body wt was effective in inducing ovulation in fish with mean oocyte diameters of 750 µ. Eggs were fertile and gave viable larvae.



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## INTRODUCTION

Successful spawning of mullet, Mugil cephalus L., has been achieved in Taiwan (Tang, 1964; Liao, 1969, 1971), Israel (Abraham et al., 1967; Yashouv, 1969) and Hawaii (Shehadeh and Ellis, 1970). The breeders used in these tests were collected at sea with one exception where pond reared mullet were used (Yashouv, 1969).

Several problems have so far hampered the development of precise and repeatable breeding methods for this and other species. Of these, the accurate determination of ovarian development in individual recipient fish and the gonadotropic activity of the injected pituitary homogenates and/or extracts, are most prominent. An additional problem encountered in our laboratory has been the viscous, non-dispersing characteristics of sperm from both captive and wild adults even when collected during the natural breeding season. Yashouv (1966) experienced the same difficulty with Mugil cepito and suggested early and prolonged hypophysation of the males. Treatment with mullet pituitary homogenates did not alleviate this condition in Mugil cephalus L. (Shehadeh and Ellis, 1970). The possible use of mammalian steroids and gonadotropins as replacements for pituitary material would be most advantageous in coping with the lack of uniformity in gonadotropic potency of pituitary homogenates and extracts. Recent successes and problems in using mammalian hormones for this purpose have been reviewed by Shehadeh (1970).

The present work was undertaken to evaluate the efficacy of prepared mammalian hormones (FSH, LH, HCG, Synahorin) in (a) stimulating spermatogenesis and inducing hydration of milt in adult male mullet; (b) stimulating completion of vitellogenesis in female broodstock maintained in laboratory tanks which otherwise undergo atresion at early stages of oögenesis; and (c) to test the feasibility of using a partially purified salmon gonadotropin as an ovulating agent during the breeding season. The long range objective of these tests was the establishment of a captive mullet brood stock which would permit breeding for genetic selection.

## MATERIALS AND METHODS

Male and female mullet used in these tests were taken from a captive population maintained in outdoor, rubber-lined ponds (30' L x 15' W x 3' D) and circular fiberglass tanks under running sea water conditions. Fish were fed Purina Trout Chow at the rate of 4% body weight daily.

## Males

Three separate experiments were conducted on males. One group was treated with 17 methyltestosterone (Brown Pharmaceutical Co.) during October - November, immediately before the onset of the spawning season. The steroid was administered intraperitoneally at a dose of 0.1 mg/100 g body weight in 0.5 ml pure peanut oil on alternate days for a period of 30 days. Control males received intraperitoneal injections of 0.5 ml peanut oil.

A second group of males was treated with the same steroid during the spawning season (December - January). A higher dose of 5 mg/100 g body weight was injected intraperitoneally as a suspension in 0.5 ml of 0.6% NaCl. The change in dose and carrier was dictated by the results obtained from the first (October - November) experimental group. The hormone was administered on alternate days for a period of 42 days. Controls received 0.5 ml saline intraperitoneally.

The third group of males was treated with human chorionic gonadotropin (HCG, Sigma Chemical Co.) during the breeding season. Fish received intramuscular injections of 20 IU HCG/100 g body weight in 0.5 ml saline (0.6% NaCl) on alternate days for a period of 42 days. Controls received 0.5 ml saline.

Males in all three groups were checked for the presence of sperm initially and at weekly intervals during the test period. Terminal gonad weights were determined at the end of each test.

## Females

Females were divided into five sets: one control and four experimental groups. The hormones used, also the doses and frequency of injection, are listed in Table 4. All injections were intramuscular (IM) and applied daily for a period of 60 days (February - April). The initial state of ovarian development was ascertained by in situ aspiration of 100-200 oocytes with a sterile polyethylene cannula. Mean oocyte diameter was determined for each fish and the sampling repeated at weekly intervals during the test period. Details of this sampling method and its statistical validity will be published elsewhere (Shehadeh and Kuo, MS).

A purified salmon gonadotropin preparation was tested on three females during the breeding season. Initial mean oocyte diameter was determined

as described above. All three fish received 50 RU Synahorin (Teikoku Hormone Mfg. Co. Ltd.)/kg body weight/day for 40 days followed by 100 μg/kg/day salmon gonadotropin for four days. Synahorin was used for a period of six weeks in order to determine whether it would be effective in preventing the usual atresion of oocytes encountered in ripe females maintained in the laboratory.

All experimental fish were kept in fiberglass tanks with running sea water and fed on Purina Trout Chow at the rate of 4% body weight per day.

## RESULTS

### 17 Methyltestosterone

The response of immature male mullet to 17 methyltestosterone treatment in October - November is shown in Table 1. At the beginning of the test period, milt could not be expressed from any of the fish by gentle abdominal pressure or by aspirating with a polyethylene cannula. After 30 days of treatment all experimental animals developed milt while controls remained at their initial state. Two experimental males gave copious amounts of running milt; two others exhibited only small quantities. Terminal gonad weights were significantly higher ( $p<0.05$) in the experimental group. It was observed that both control and experimental groups developed peritoneal granuloma. Since control males did not receive the steroid, the peanut oil carrier was assumed to be responsible. Methyltestosterone was consequently administered as a suspension in 0.6% NaCl in subsequent tests.

The results of steroid injection in mature male mullet during the spawning season are expressed in Table 2. All experimental and control males exhibited at least traces of milt at the outset. Control males receiving saline injections began to deteriorate (dry) by the fourth week and no milt could be detected at the end of the sixth week. This agrees with our earlier observations on laboratory-held ripe males during the past four years. Such animals lost all milt during a two- to four-week sojourn in 50-gallon laboratory tanks in running sea water at 24-26° C. Steroid treated males began to show increased fluidity and volume of milt after four weeks of injection. Four males gave copious amounts of milt by the sixth week, one died from handling and another gave moderate amounts of milt. Examination of four steroid treated males did not reveal any peritoneal granuloma. Apparently the peanut oil was responsible for this symptom in the first experimental (October - November) group. With one exception, gonad weights were surprisingly low in the sacrificed experimental fish.

Table 1. Reaction of immature male mullet to 17 methyltestosterone treatment (0.1 mg/100 g body weight) during October - November

| Fish No. | Body Wt (g) | Initial State | Terminal State** | Terminal Gonad Wt (g) |
|---|---|---|---|---|
| 1 | 397 | dry | ++ | |
| 2 | 316 | dry | ++ | 2.22 |
| 3 | 349 | dry | +++ | 0.82 |
| 4 | 325 | dry | +++ | 2.13 |
| 5C | 308 | dry | dry | 0.64 |
| 6C | 382 | dry | dry | 0.58 |
| 7C | 400 | dry | dry | 0.61 |

C: Control fish injected with 0.5 ml peanut oil; ** +: traces of viscous milt, ++: small amounts of running (hydrated) milt, +++: copious running milt

Table 2. Reaction of mullet males to 17 methyltestosterone treatment (5 mg/100 g body weight) during December - January

| Fish No. | Body Wt (g) | Initial State | Results ** I* | II | III | IV | V | VI | Terminal Gonad Wt (g) |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 391 | + | + | + | + | ++ | +++ | +++ | 3.12 |
| 2 | 324 | + | + | + | + | ++ | +++ | +++ | 2.20 |
| 3 | 346 | + | + | +++ | +++ | +++ | +++ | +++ | 1.80 |
| 4 | 364 | + | + | + | + | ++ | ++ | ++ | 13.11 |
| 5 | 773 | + | + | + | + | + | ++ | died | -- |
| 6 | 423 | ++ | ++ | + | + | ++ | ++ | +++ | -- |
| 7C | 356 | ++ | ++ | + | + | + | dry | dry | -- |
| 8C | 410 | ++ | ++ | ++ | ++ | + | + | dry | -- |
| 9C | 425 | ++ | ++ | + | + | dry | dry | dry | -- |

* Roman numerals denote weeks; ** legend as in Table 1; C: denotes control fish sham injected with 0.5 cc saline

Table 3. Reaction of immature male mullet to human chorionic gonadotropin (HCG) treatment (20 IU/100 g body weight) during December - January

| Fish No. | Body Wt (g) | Initial State | Results** I* | II | III | IV | V | VI | Terminal Gonad Wt (g) |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 349 | dry | dry | dry | dry | + | ++ | ++ | < 1 |
| 2 | 311 | dry | dry | dry | + | + | + | ++ | < 1 |
| 3 | 312 | dry | dry | dry | dry | + | ++ | ++ | 1 |
| 4 | 327 | dry | dry | dry | + | + | ++ | ++ | 1.2 |
| 5 | 308 | dry | dry | dry | dry | + | ++ | ++ | < 1 |
| 6C | 318 | dry | dry | dry | dry | dry | dry | dry | 1 |
| 7C | 370 | dry | dry | dry | dry | dry | dry | dry | < 1 |
| 8C | 356 | dry | dry | dry | dry | dry | dry | dry | < 1 |

* Legend as in Table 1; ** legend as in Table 1; C: denotes control fish sham injected with 0.5 cc saline

HCG

Males injected with HCG were initially dry but all began to show traces of milt after four weeks of treatment and gave small amounts of hydrated (fluid) milt after six weeks (Table 3). Control males remained at their initial condition. There was no significant difference in terminal gonad weights between experimental and control fish and all gonad weights of experimental males were, again, surprisingly low.

Effects of exogenous hormone treatment on oocyte growth

These tests were conducted in February - April since previous observations showed that captive female mullet attained peak ovarian development 30 to 45 days later than wild populations. Initial examination of the 23 females used in this test revealed mean oocyte diameters ranging from 0.44 mm to 0.70 mm (Table 4). The normal mean diameter of mature ovulated oocytes is 0.93 mm. Body weights varied between 800-1200 kg.

Females survived the prolonged treatment with little or no physical damage; only two of 23 fish died from handling within the 60 day period. Oocyte growth in individual fish, determined by weekly sampling is shown in Fig. 1. Mean oocyte diameters and standard deviation values for each of the five groups is plotted in Fig. 2. With the exception of controls, all groups exhibited gradual and significant growth (increase in oocyte diameter). This was true when fish were considered singly or in groups (Figs. 1, 2). Differences in initial mean egg diameters among all five groups were not statistically significant ($p \geqslant 0.075$). An interesting phenomenon observed in all experimental groups is the decrease in the standard deviations around the means that accompanied oocyte growth. This was not evident in control fish.

Atresion developed first in groups II and V, FSH/LH-treated fish and controls respectively (Figs. 1,2). Maximum mean oocyte diameter attained was $0.72 \pm 0.02$ mm (mean $\pm$ standard deviation) in group II and $0.62 \pm 0.07$ mm (mean $\pm$ standard deviation) in group V. This difference in oocyte diameter was statistically significant ($p \leqslant 0.05$) indicating a positive effect from FSH/LH treatment over saline injected controls. In the experimental groups, I and IV showed the best results in so far as four of five females in the former (terminal mean oocyte diameters of 0.75, 0.74, 0.79, and 0.74 mm) and two of five females in the latter (terminal mean oocyte diameters of 0.75 and 0.74 mm) did not undergo atresion during the 60-day test. On closer examination, however, terminal results were found to be misleading as an indication of hormone effectiveness. Comparison of oocyte diameters

Table 4. Effects of exogenous hormone treatment on oocyte growth in female mullet during February - April

| Group No. | Fish No. | $D^{o}$* (mm) | Treatment (60 days) | Cumulative Treatment | | | | | $D^{t}$** (mm) (60 days) |
|---|---|---|---|---|---|---|---|---|---|
| | | | | HCG (IU) | FSH/LH (IU) | Mullet pituitaries | Salmon Gonadotropin (mg) | Synahorin (RU) | |
| I | 1 | 0.52 | HCG @ 50 IU/500 g daily | 6900 | | | | | 0.75 |
| | 2 | 0.44 | (IM)/500 g body wt | 6900 | | | | | 0.74 |
| | 3 | 0.62 | | 8000 | | | | | 0.79 |
| | 4 | 0.62 | | 6000 | | | | | atresion |
| | 5 | 0.70 | | 6900 | | | | | 0.74 |
| II | 6 | 0.61 | FSH/LH (15 µg/5 µg) | | 650/216 | | | | atresion |
| | 7 | 0.58 | daily (IM)/500 g body wt | | 650/216 | | | | atresion |
| | 8 | 0.69 | | | 775/216 | | | | atresion |
| | 9 | 0.62 | | | 650/216 | | | | atresion |
| | 10 | 0.60 | | | 80/36 | | | | died |
| III | 11 | 0.62 | 1/2 mullet pituitary + | | | 19.5 | | 1660 | atresion |
| | 12 | 0.56 | 35 RU Synahorin (IM)/ | | | 16.0 | | 1410 | atresion |
| | 13 | 0.65 | 500 g body wt | | | 16.0 | | 1410 | atresion |
| | 14 | 0.64 | | | | 19.5 | | 1410 | atresion |
| | 15 | 0.57 | | | | 16.0 | | 1410 | atresion |
| IV | 16 | 0.62 | 100 µg purified salmon | | | | 4.5 | 1350 | 0.73 |
| | 17 | 0.56 | gonadotropin + 35 RU | | | | 4.1 | 1230 | atresion |
| | 18 | 0.65 | Synahorin daily (IM)/ | | | | 4.5 | 1350 | 0.74 |
| | 19 | 0.64 | 500 g body wt | | | | 3.6 | 1080 | atresion |
| | 20 | 0.57 | | | | | 6.5 | 850 | died |
| V | 21 | 0.49 | 1/2 cc saline daily (IM)/ | | | | | | atresion |
| control | 22 | 0.63 | 500 g body wt | | | | | | atresion |
| | 23 | 0.58 | | | | | | | atresion |

* $D^{o}$ = initial mean oocyte diameter; ** $D^{t}$ = terminal mean oocyte diameter

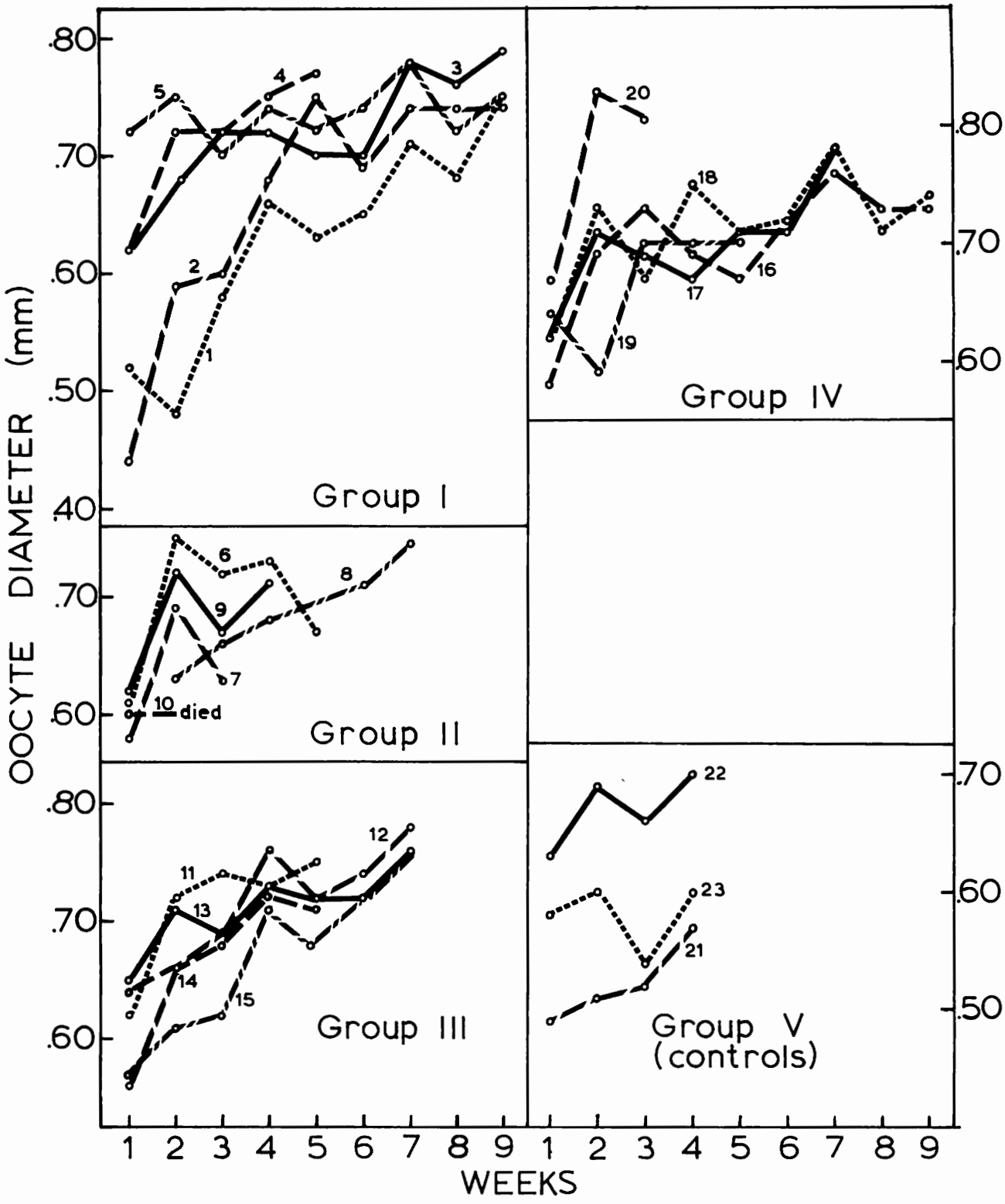



Fig. 1. Weekly oocyte growth record for individual females by group. Group legends as in Table 4.

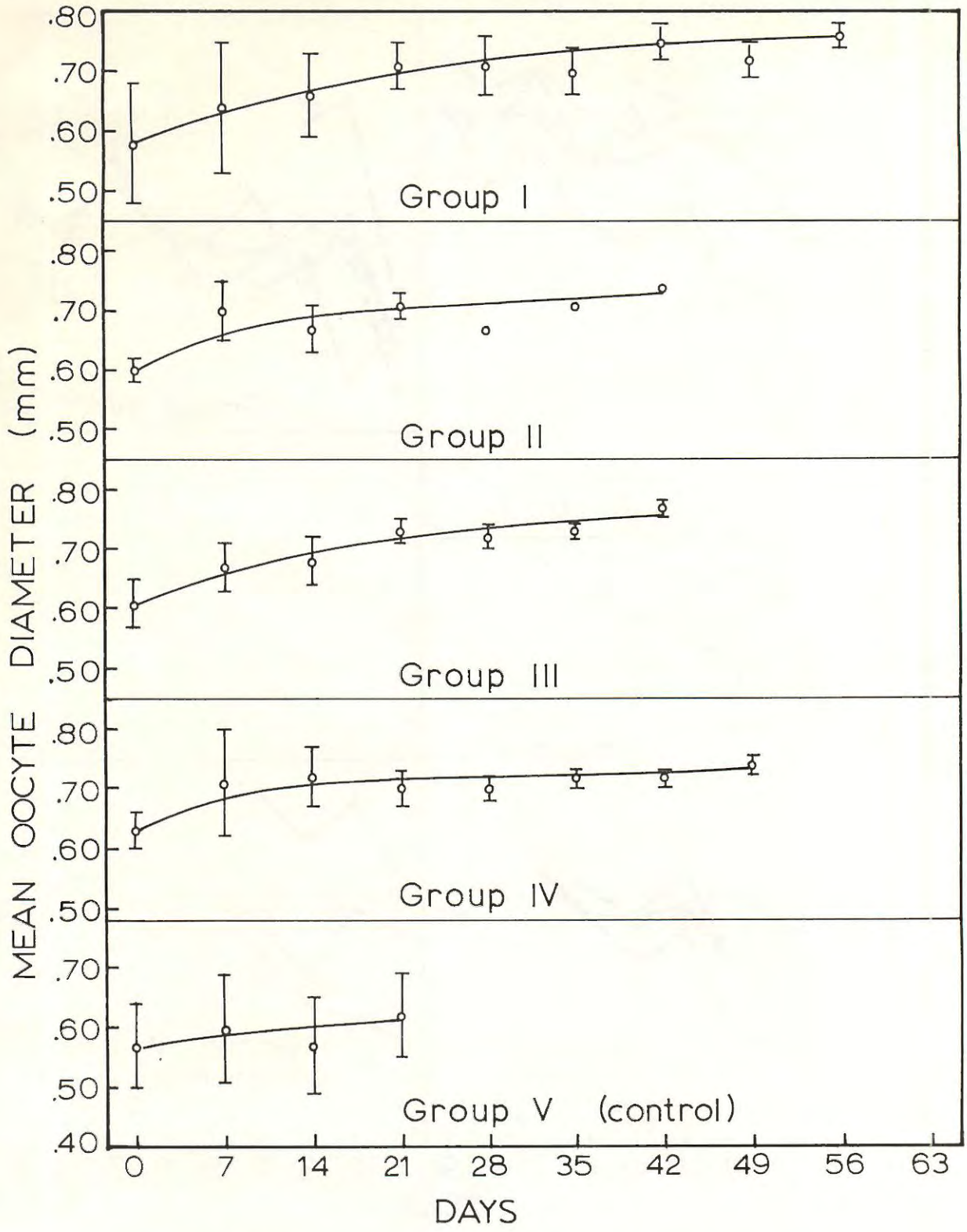



Fig. 2. Weekly changes in mean (± standard deviation) oocyte diameters. Group legends as in Table 4.

before onset of atresion in groups I - IV showed no statistically significant differences ($p \geqslant 0.075$). In all four groups, atresion set in when oocyte diameters reached values of $0.75 \pm 0.03$ mm (mean $\pm$ standard deviation), as shown in Fig. 3. This indicates that HCG, FSH/LH, Synahorin in conjunction with mullet pituitary homogenates, and partially purified salmon gonadotropin in combination with synahorin were equally effective, at the doses used, in promoting oocyte growth. The data further suggests that at mean oocyte diameters of $0.75 \pm 0.03$ mm (mean $\pm$ standard deviation) vitellogenesis is probably completed and females are ready for spawning. Hormone injection beyond this developmental stage precipitates atresion, at least at the doses used. This conclusion was further supported by subsequent spawning trials (see Results, next section). Data from the control group show that during the breeding season and in the absence of exogenous hormone treatment, atresion begins, in laboratory held animals, within a period of four weeks. This conforms to data from ripe males, under similar conditions during the breeding season (Table 2) and is in agreement with previous observations in this laboratory.

Spawning trials with partially purified salmon gonadotropin

Initial examination of ovarian development in three female mullet revealed mean oocyte diameters of 0.68, 0.70 and 0.67 mm respectively. After six weeks of treatment with Synahorin all oocytes were normal but mean diameters did not exceed 0.75 mm. One female died from handling damage. These results are in agreement with the critical egg diameter beyond which atresion was found to begin, and indicate that Synahorin alone is effective in inducing slow oocyte growth and preventing the onset of early atresion experienced with non-treated fish (see Results above).

Ovulation, as evidenced by abdominal distension, occurred in both females after the administration of 400 $\mu$g of partially purified salmon gonadotropin per fish over a period of four days. Gradual abdominal distension took place during a 17 hour period. Following ovulation, females were stripped manually and fertilized with milt from methyltestosterone treated males (Fig. 4). Fertilized eggs were then placed on a 35 $\mu$ mesh nitex screen and rinsed free of sperm and mucus with clean sea water. Eggs were incubated in a 50-gallon fiberglass tank equipped with an air supply. Fertilization and hatch-out rates were 68% and 60% respectively. Larvae hatched out 36-40 hours after fertilization at 24° C in static sea water (32 $^{o}/_{oo}$). Hatched larvae (Fig. 5) were transferred to outdoor fiberglass tanks (10' diameter x 4' deep) but were not offered any food. It should be stated here that the purpose of this study was to test the effectiveness of the salmon gonadotropin as an ovulating agent and to ascertain the fertility of

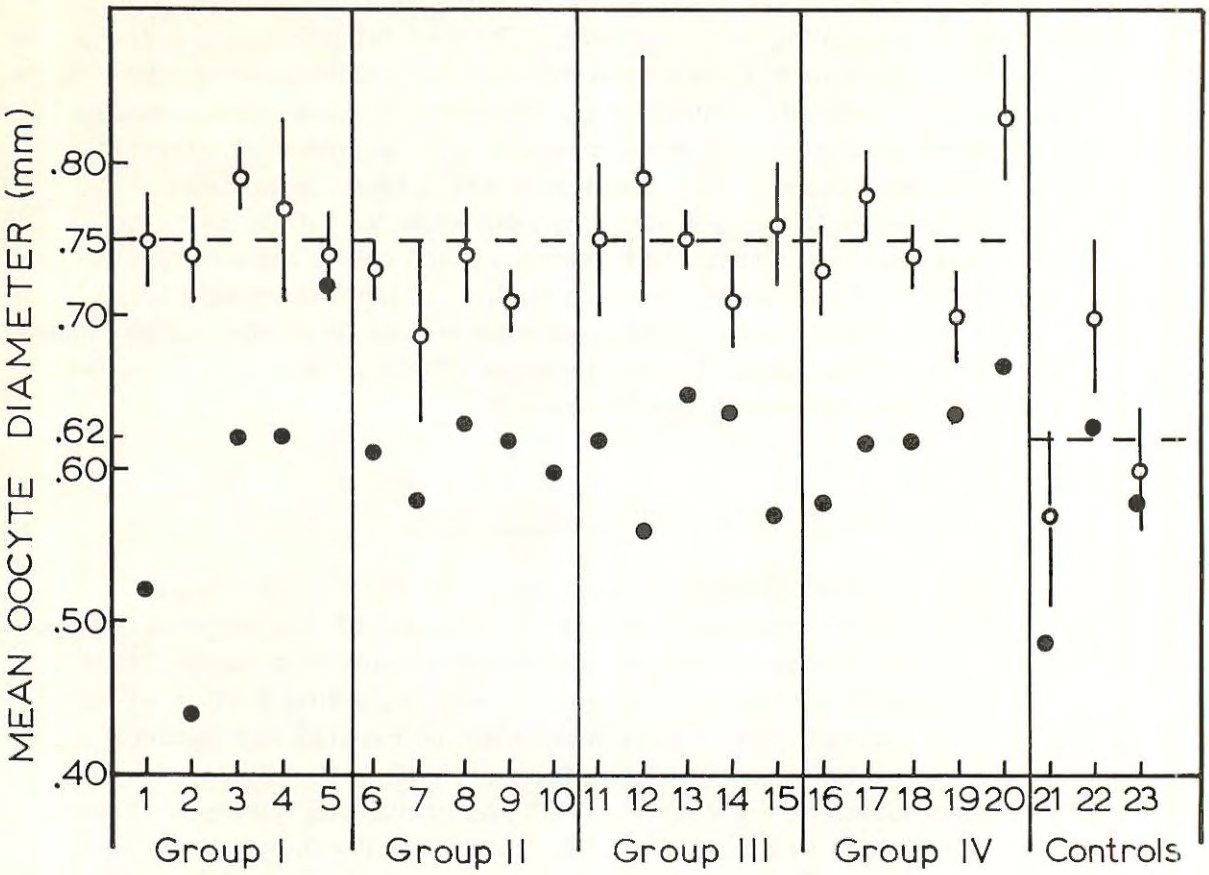



Fig. 3. Mean initial oocyte diameter (•) and mean oocyte diameter attained before onset of atresion (o) in individual fish from all experimental groups. Group legends as in Table 4.

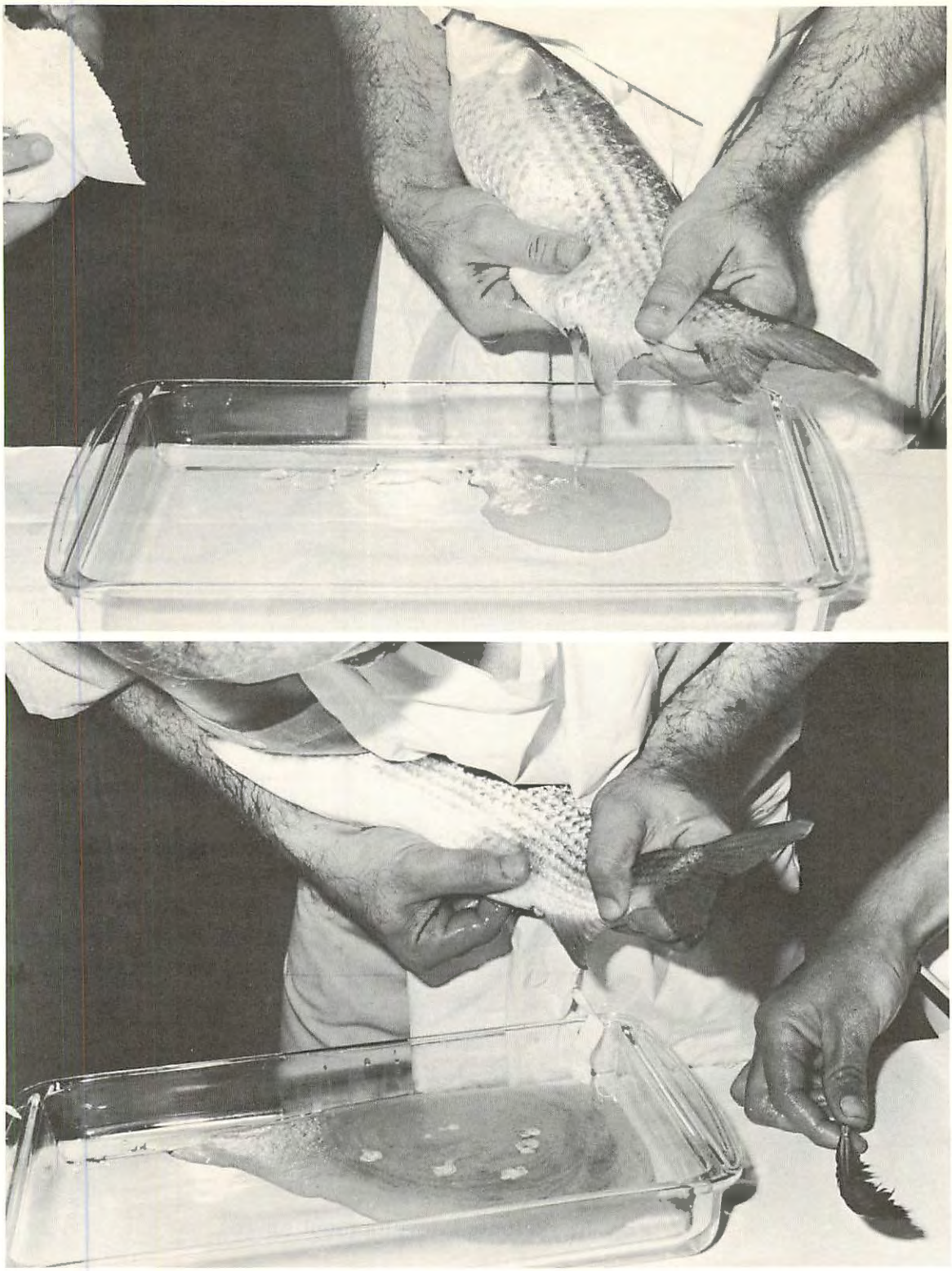
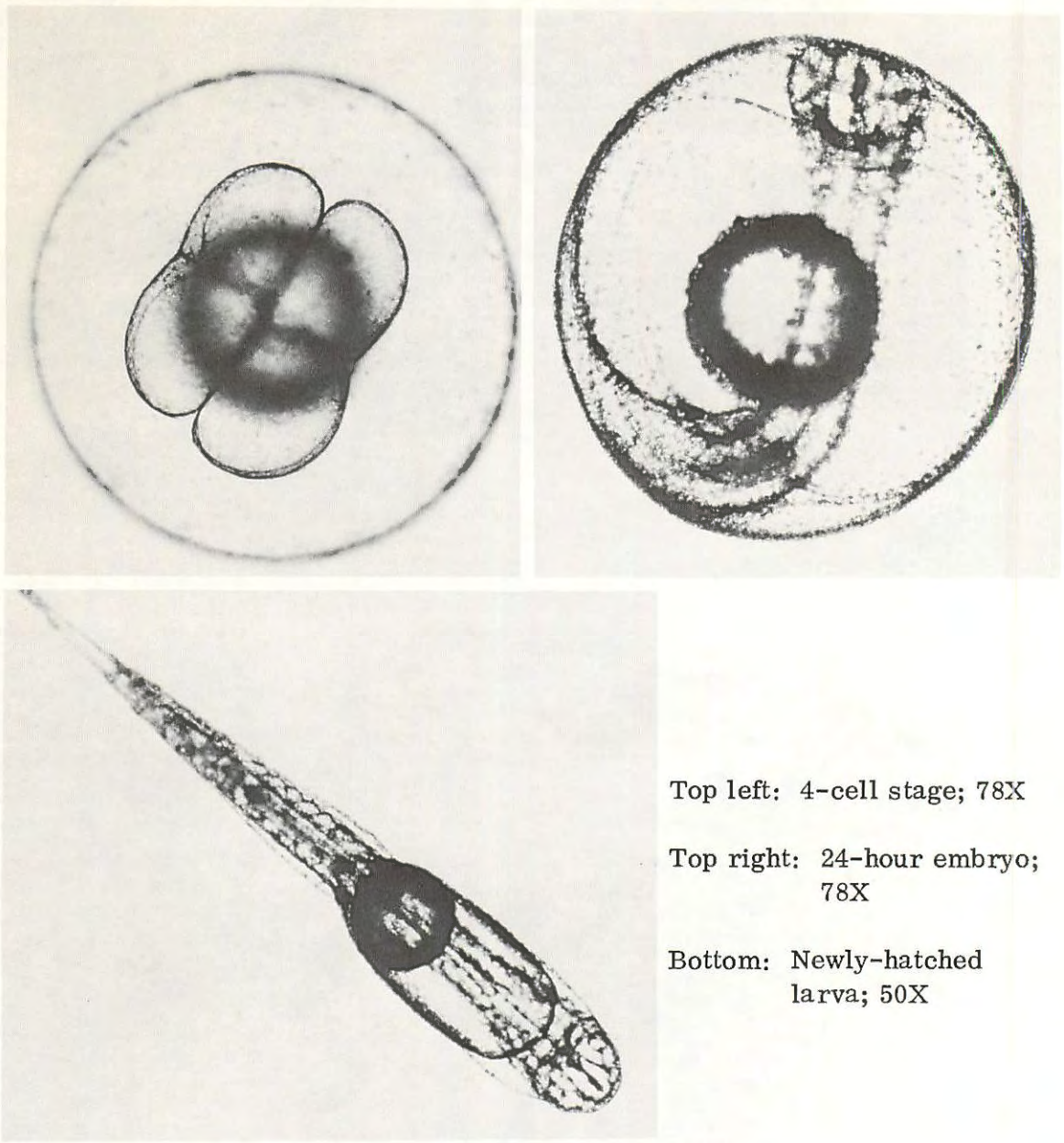


Fig. 4. The manual stripping of (top) ovulated female, and (bottom) a mullet male. Feather was used to mix sperm and eggs.



Top left: 4-cell stage; 78X

Top right: 24-hour embryo; 78X

Bottom: Newly-hatched larva; 50X

Fig. 5. Selected development stages.

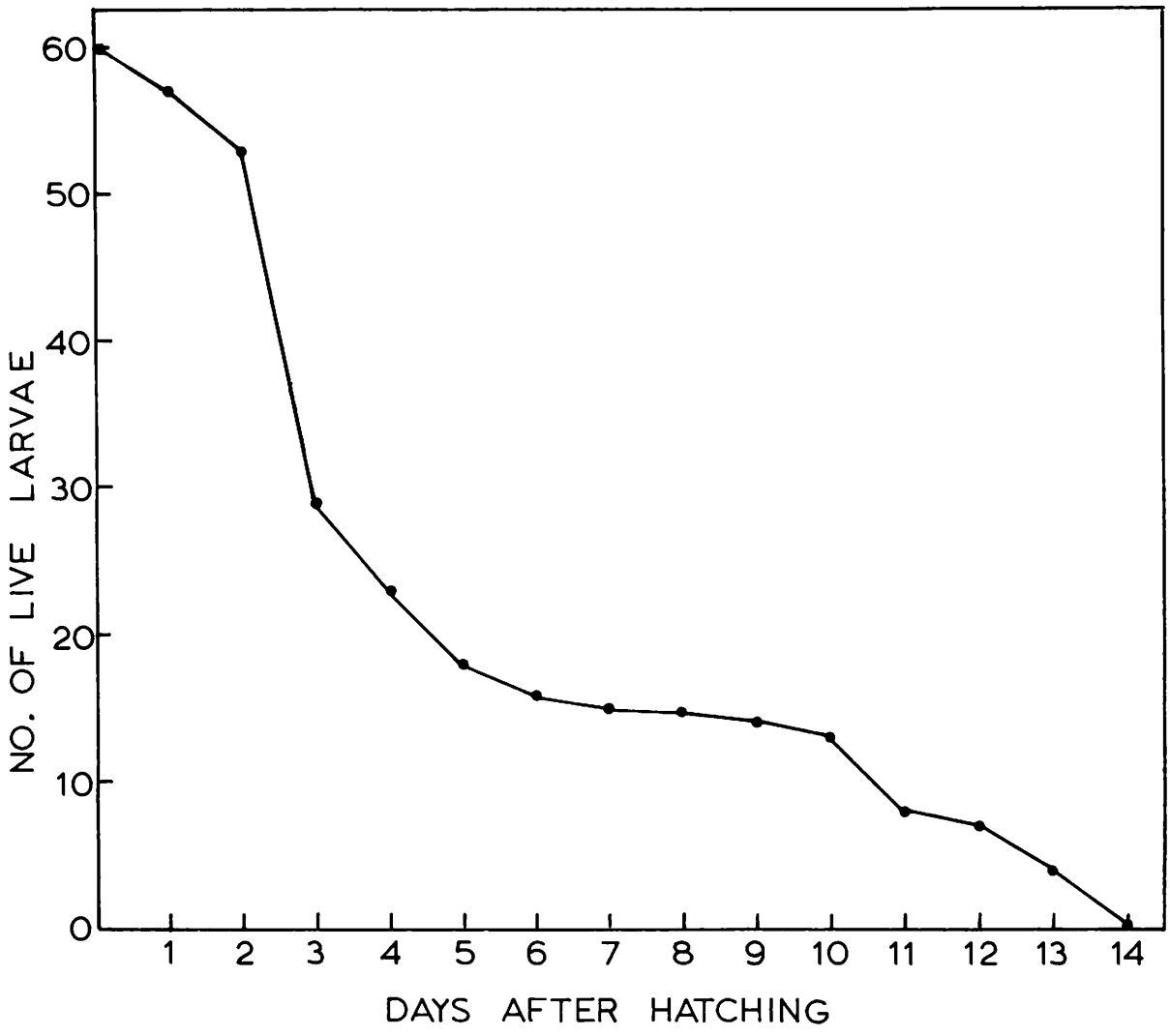



Fig. 6. Survival of newly hatched larvae maintained without food.

spawned eggs. Adequate measures were not taken to cope with the larvae. Observations on larvae were consequently restricted to examination of gut contents in outdoor tanks and survival rates in the absence of food.

Sixty larvae were placed in a 10-gallon tank filled with filtered sea water (32 $^{o}/_{oo}$) and daily mortality counts kept. No food was offered. Maximum larval survival was 13 days with the highest mortality (40%) occurring between the second and third days from hatching (Fig. 6). The high mortality was preceded by the larvae sinking to the bottom of the tank. This occurred towards the end of the second day at which time the yolk sac was completely absorbed. Up to this time (second day) the larvae were inactive and suspended in the water column in a heads-down position. The mouth opened on the third day, the larvae became active swimmers and aggregated near the surface. A second major mortality occurred on the tenth day. This was presumably due to one week of starvation.

Examination of gut contents of larvae maintained in outdoor tanks revealed that, when present, it consisted chiefly of undigested diatoms (Nitzchia and Amphiphora sp.), some unidentified flagellated algae, and digested veliger larvae of a gastropod (Aplysia sp.) which was abundant in the water (Fig. 7).

## DISCUSSION

The use of mammalian steroids and gonadotropins in the induced breeding of fish offers a number of advantages over fish pituitary homogenates or extracts. Mammalian hormones are (a) readily available, (b) are uniform and consequently amenable to exact quantitation of potency and dosage, and (c) could do away with the need to sacrifice sexually mature fish for pituitary material.

The experiments conducted in this study demonstrate that the androgen, 17 methyltestosterone, and the placental gonadotropin, HCG, are effective in inducing spermatogenesis in male mullet during and outside the breeding season. Both were effective in preventing milt resorption in laboratory held males. These findings are in agreement with other reports in the literature. Testosterone propionate, 17 methyltestostereone and HCG were successfully used to induce spermiation in Heteropneustes, Carassius, Fundulus and Anguilla (Etienne, 1959; Sundararaj and Goswami, 1965; Lofts et al., 1966; Sundararaj and Nayyar, 1967a, 1967b; Yamazaki and Donaldson, 1969). In this study the most effective androgen dose was 5 mg/100 g body weight compared to 10 mg/10 g body weight in the goldfish (Yamazaki and Donaldson, 1969). These authors stated that the high dose, far beyond

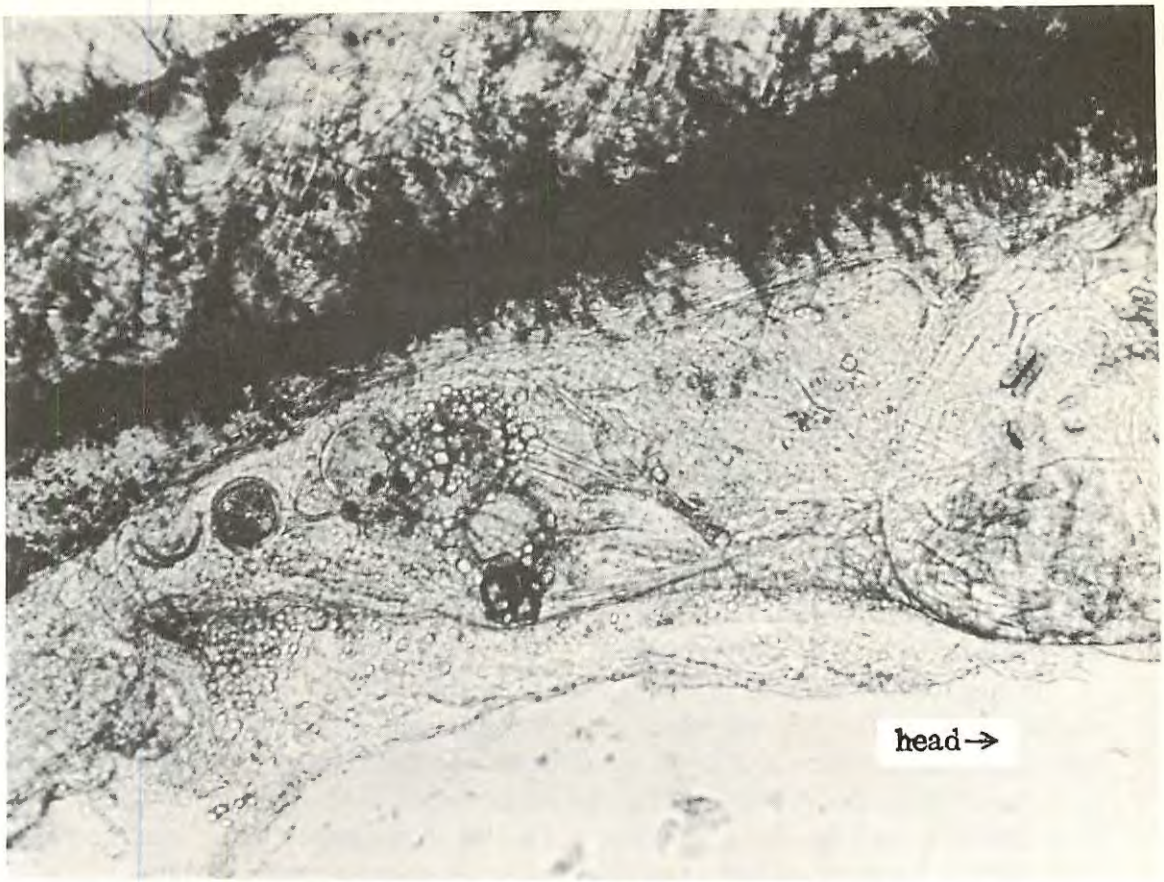



Fig. 7. Magnified section of terminal section of larval digestive tract showing ingested diatoms, veligers and algae. (224X)

physiological plasma levels, was required probably because the applied androgen was only slowly absorbed and/or, due to the close proximity of the Leydig cells and the androgen target cells, local androgen levels in this location might normally be much higher than in plasma.

The effectiveness of HCG in inducing spermiation is related to its LH-like properties. It is generally accepted that, of the two mammalian gonadotropins, only LH has a stimulating effect on the teleost testis (Hoar, 1965). The slower and less pronounced response of male mullet to HCG during the breeding season is probably due to the fact that HCG acts on interstitial cells to induce androgen secretion whereas methyltestosterone would act directly on the androgen target cells in the seminiferous lobules to stimulate spermatogenesis. A dose dependent response might also be involved. Sundararaj and Nayyar (1967a) reported such an effect in hypophysectomized Heteropneustes when doses of 1, 2 and 5 IU/day were administered. That higher levels of HCG might be more effective is

supported by findings that 60 IU/10 g body weight induced rapid spermiation in hypophysectomized goldfish (Yamamoto and Yamazaki, 1967), while a total of 500 mg was required to induce spermiation in Anguilla (Etienne, 1959).

The results of tests with mammalian gonadotropins in this study demonstrate the effectiveness of these hormones in inducing oocyte maturation in captive Mugil cephalus females. The eventual occurrence of atresion does not necessarily preclude the usefulness of these hormones in the induction of ovulation and spawning. A dose effect is probably involved. In the case of Heteropneustes fossilis, injection of 10-25 IU HCG failed to induce oocyte maturation, 50 IU yielded ripe eggs and 100 IU precipitated profuse spawning (Sundararaj and Goswami, 1966). A similar response was reported with LH and serum gonadotropin Equinex (Sundararaj and Goswami, 1966). Yamazaki (1965) induced spawning in goldfish with injections of HCG at a dose of 20 IU/10 g body weight while Sneed and Clemens (1956) spawned gravid Ictalurus punctatus with 700 IU/450 g body weight. These doses represent seven and ten times the HCG dose used in this study. It appears likely therefore that a tenfold increase in dose could have precipitated spawning in gravid female mullet with oocyte $< 0.75$ mm in diameter.

A significant finding in these tests was the critical oocyte diameter ($0.75 \pm 0.03$ S.D.) beyond which atresion took place in treated females. That this phenomenon signifies completion of vitellogenesis (or maturation) is supported by the results of the spawning trials (see Results). Three females with oocytes not exceeding 0.75 mm in diameter were spawned and gave fertile eggs following injection of 400 µg salmon gonadotropin. Furthermore, previous work in this laboratory demonstrated that females with mean oocyte diameters $< 0.60$ mm will not spawn even when very high hormone doses are administered (Shehadeh and Kuo, unpublished data). Abraham (1963) reported that the largest oocytes observed in Mugil cephalus females, collected from sea water close to the spawning season, measured 523 µ. If allowance is made for shrinkage during preservation and sectioning, oöcyte diameters would agree with those of control females (Fig. 3) before the onset of atresion (mean of $620 \pm 70$ µ standard deviation). Abraham (1963) concluded that ovaries of sampled females had not received a needed stimulus to complete the maturation process. Presumably, the protracted absence of this "stimulus" would precipitate early atresion as in the case of saline injected controls in this study. Exogenous hormone treatment apparently mimics the requisite "stimulus" and induces completion of maturation. Ovulation and spawning require higher levels of hormones.

Oocyte maturation and spawning results obtained with very low doses of a partially purified salmon gonadotropin (Donaldson and Yamazaki, 1968) are very encouraging. The same preparation was also successfully used to spawn Heteropneustes (B.I. Sundararaj, private communication) and to induce

vitellogenesis and ovulation in the goldfish (Yamazaki and Donaldson, 1968). However, the practicality of purification procedures for use in applied fish breeding is yet to be demonstrated.

It is very difficult to make any statements about larval survival due to absence of feeding. However, the high mortality associated with vertical movement of the larvae before the mouth opens is worthy of special attention.

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V.

# EFFECT OF SALMON GONADOTROPIN ON OVARIAN AND TESTICULAR DEVELOPMENT IN IMMATURE GREY MULLET Mugil cephalus


## Abstract

The purpose of the present experiment has been to determine the feasibility of inducing sexual development in immature mullet using a partially purified preparation of salmon gonadotropin. Immature mullet were captured and held in ponds for one year preceding the experiment. The animals were divided into three groups, each having equal numbers of males and females. Three times per week for four weeks the three groups were injected with 0.5 mg partially purified salmon gonadotropin in 0.5 ml 0.9% NaCl, 5.0 mg partially purified salmon gonadotropin, and 0.5 ml 0.9% NaCl respectively. Measurements of body weight, gonad weight, GSI, and PI, together with observations of the animals, established that the gonadotropin induced both spermatogenesis and spermiation in males. Response of females varied from neglible to pronounced, possibly due to individual fish's maturity at the beginning of the experiment. Alternatively, some mullet may develop antigen in response to the exogenous gonadotropin or to another protein in the preparation. Daily injections or lower water temperature may improve the response of females to the gonadotropin preparation.

## INTRODUCTION

While the grey mullet has been cultured for many years in artificial impoundments, they have invariably failed to reproduce, thus necessitating the replacement of harvested stocks with captured wild fry. The supply of wild fry is unreliable and dependence upon it has prevented establishment of the selective breeding programs which are characteristic of all agricultural operations.

To date, investigators have concentrated on the induction of spawning in mature mullet (Tang, 1964; Abraham et al., 1967; Liao, 1969; Yaskouv, 1969; Shehadeh and Ellis, 1970; and Ling, 1970). The purpose of the present experiment has been to determine the feasibility of inducing sexual development in immature mullet using a partially purified preparation of salmon (Oncorhynchus tshawytscha) gonadotropin (SG-G100) which has previously been shown to be effective in inducing gonad development in male and female goldfish (Yamazaki and Donaldson, 1968a,b, 1969), the female guppy (Liley and Donaldson, 1969), the male catfish (Sundararaj et al., 1971a), and the female catfish (Sundararaj et al., 1971b).

## MATERIALS AND METHODS

Grey mullet (Mugil cephalus) were captured when immature and held for one year in an outdoor, 4500-gallon aquarium, supplied with running seawater. The fish were fed Purina trout chow. This diet was supplemented by algal growth on vertical strips of polyethylene placed in the aquarium. Experimental fish and terminal controls were transferred to 189-l laboratory aquaria at a density of not more than five fish per aquarium. The running seawater (32 $^{o}/_{oo}$) was at a temperature of 26° C. The fish were maintained on a natural photoperiod and fed a diet of Purina trout chow 5% body weight per day for the duration of the experiment.

The first injections were given on October 10. The fish were not anaesthetized but were restrained using close-fitting black polyethylene bags which were slipped over the head. The hypodermic needle was inserted in the scale-free region at the base of the pelvic fins. Eight fish were injected interperitoneally with 0.5 mg partially purified salmon (Oncorhynchus tshawytscha) gonadotropin, SG-G100, (Donaldson and Yamazaki, 1968; Donaldson et al., 1972) in 0.5 ml 0.9% NaCl. Ten fish were injected with 5.0 mg SG-G100 and five fish received 0.5 ml of 0.9% NaCl. Owing to difficulty in sexing the fish, it was not possible to have equal numbers of

males and females in each group. Injections were repeated three times per week for four weeks, the last injection being given on November 5. Seven zero control fish were sampled on October 13, gonadotropin-injected fish and saline-injected terminal-control fish were killed 48 hours after the last injection. Body weight, fork length, and gonad weight were measured. Gonadosomatic index, GSI [(gonad weight/body weight) x 100] and ponderal index, PI [(body weight/body length$^3$) x 100], were calculated. Gonads were fixed in Bouins solution, paraffin embedded, sectioned at 7 $\mu$ and stained with hematoxylin and eosin.

## RESULTS

### Males

Mean values for body weight, gonad weight, GSI, and PI are presented in Table 1. In the zero control and terminal control males, no testes contained visible milt. There was however, considerable testicular growth in the control fish during the period of the experiment, GSI $P<0.01$. The GSI in the 0.5 mg gonadotropin group was no greater than that in the terminal controls but four of the six had white testes indicating presence of spermatozoa and two released milt when cut. In two of the three males in the 5.0 mg gonadotropin group, milt flowed from the cloaca when they were removed from the aquarium. Thus the GSI determined in these functionally mature fish, while higher than that in the terminal controls, is actually an underestimate. Microscopic examination of spermatozoa from one male in this group revealed that more than 90% were motile.

Histological examination of the zero control testes showed only minimal spermatogenesis with a small quantity of spermatozoa in the sperm duct (Fig. 1). Spermatogenesis in the terminal control testes was quite marked and was accompanied by a greater accumulation in the sperm duct. The males injected with 0.5 mg salmon gonadotropin showed the most active spermatogenesis and this was accompanied by a considerable accumulation of spermatozoa in the duct (Fig. 2). In the 5.0 mg dose group, the testes of fish 4, which had the highest GSI (Fig. 3), showed very active spermatogenesis and a large accumulation of spermatozoa. The testes of fish 8, which had the lowest GSI in the group (Fig. 3), appeared to have passed the point of maximum spermatogenesis and had entered the regressed phase.

Table 1. Body weight, gonad weight, gonadosomatic index (GSI) and ponderal index (PI) of male Mugil cephalus.

| | No. | Body Weight | Gonad Weight | GSI | PI |
|---|---|---|---|---|---|
| Zero controls | 4 | 381.3 ± 35.4 | 0.271 ± 0.132 | 0.0692 ± 0.02774 | 1.148 ± 0.17418 |
| Comparison with terminal controls | | | .10 > P > .05 | .01 > P > .005 | N.S. |
| 0.5 mg SG-G100 | 6 | 401.8 ± 73.8 | 1.11525 ± 0.32778 | 0.2785 ± 0.06268 | 1.3154 ± 0.04690 |
| Comparison with zero controls | | | .005 > P > .001 | P < .001 | .10 > P > .05 |
| Comparison with terminal controls | | | N.S. | N.S. | .025 > P > .02 |
| 5.0 mg SG-G100 | 3 | 391.0 ± 109.219 | 1.60560 ± 0.94489 | 0.4224 ± 0.24706 | 1.1737 ± 0.14642 |
| Comparison with zero controls | | | 0.5 > P > .025 | .05 > P > .025 | N.S. |
| Comparison with terminal controls | | | N.S. | N.S. | N.S. |
| Terminal controls | 3 | 422.3 ± 156.6 | 1.2392 ± 0.8765 | 0.2720 ± 0.09813 | 1.1737 ± 0.10540 |

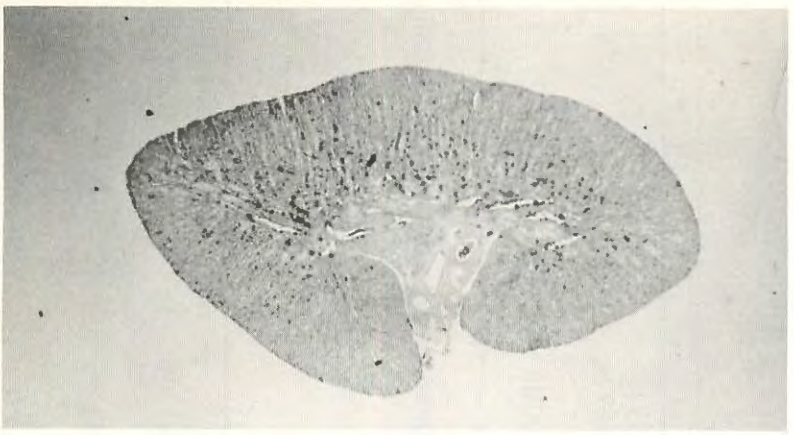

Fig. 1 Zero control testis. Transverse section through testis of fish 6 in zero control group showing minimal spermatogenesis with only a small amount of spermatozoa in the sperm duct. X30

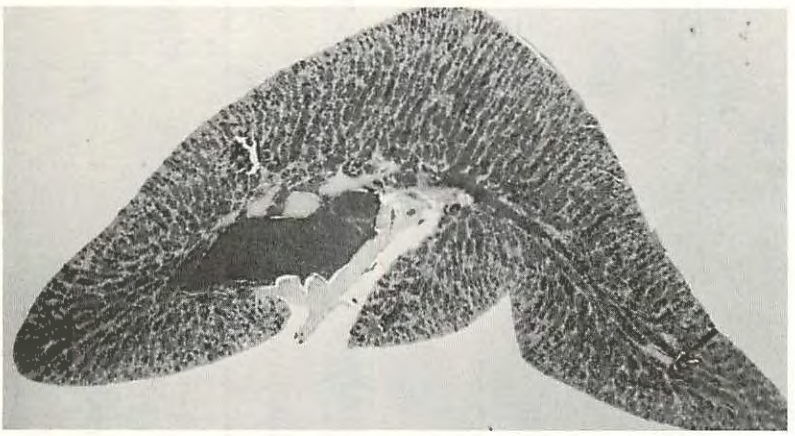

Fig. 2 0.5 mg gonadotropin treated testis. Transverse section through testis of fish 1 in 0.5 mg salmon gonadotropin dosage group showing active spermatogenesis and a considerable accumulation of spermatozoa in the sperm duct. X30

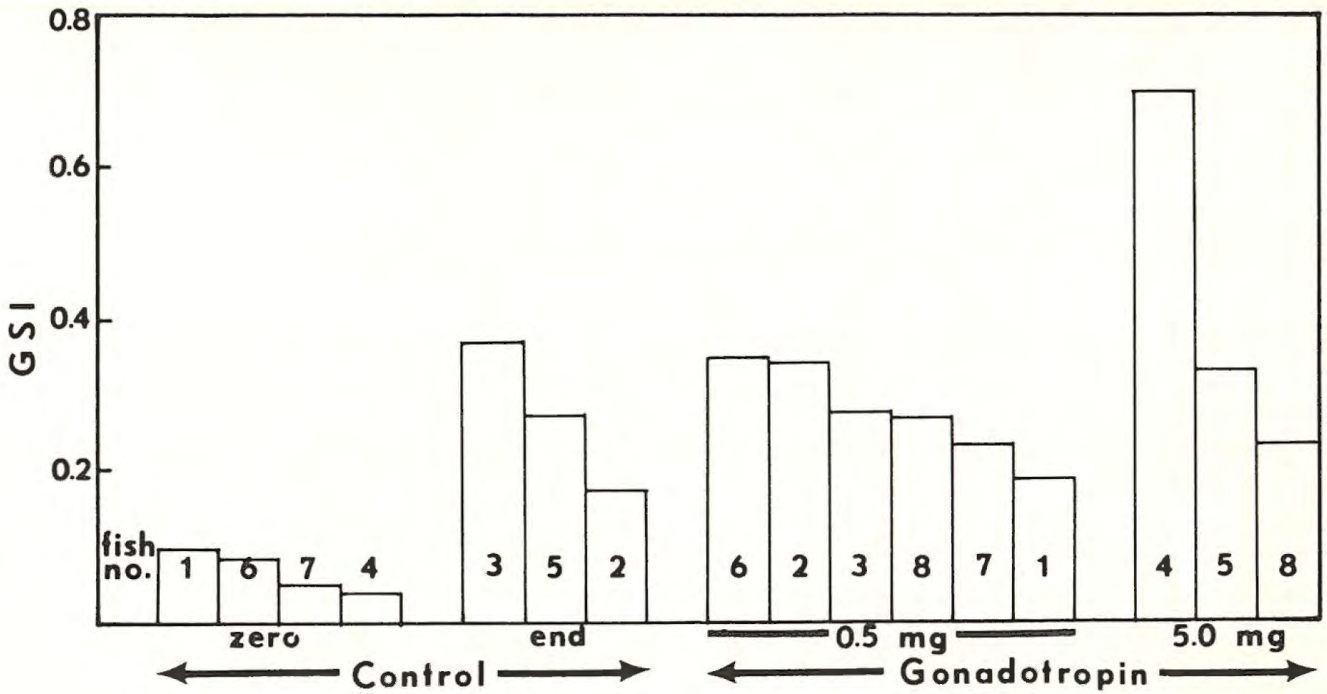


Fig. 3 Gonadosomatic indices (GSI) of individual male mullet treated with salmon gonadotropin.

Females

Mean values for body weight, gonad weight, GSI, and PI are presented in Table 2. The GSI was slightly but not significantly higher in the terminal controls than in the zero control females. The GSI of the 0.5 mg dose females was significantly higher than the GSI of the zero control females; $P > 0.02$ and approached the point of being significantly higher than the GSI of the end control females $P > 0.10$. The 0.5 mg dose females showed considerable variation within the group (Fig. 4); thus, while the mean GSI was twice that in the zero control females, the difference was hardly significant, $P > 0.10$. The changes in GSI were not sufficient to result in significant changes in the ponderal index. The mean diameter of the developing oocytes, i.e. late perinucleolar stage and beyond, was determined by measuring the largest and shortest diameter of twenty oocytes in each ovary. The diameter of each oocyte was obtained by taking the square root of the product of the two diameters (Braekevelt and McMillan, 1967). Mean oocyte diameters are presented in Fig. 5. The mean diameters of the developing oocytes in the two 0.5 mg dose group females are no different from those in the two control groups. On the other hand, most fish in the 0.5 mg group have higher mean oocyte diameters indicating that ovarian development is more advanced in this group. In the ovary of the zero control fish (Fig. 6) the oocytes ranged from early perinucleolar stage to early yolk vesicle stage. In the two terminal control fish, there were early and late perinucleolar stages but no oocytes in the early yolk vesicle stage. The ovaries of the females which received 0.5 mg salmon gonadotropin per injection contained oocytes up to the late perinucleolar stage. As was indicated above there was considerable variation in the 5.0 mg dose group. The oocytes of the least developed ovaries were between the early and late yolk vesicle stages while many oocytes in the most developed ovary were in the secondary yolk stage (Fig. 7).

Table 2. Body weight, gonad weight, gonadosomatic index (GSI) and ponderal index (PI) of female Mugil cephalus.

| | No. | Body Weight | Gonad Weight | GSI | PI |
|---|---|---|---|---|---|
| Zero controls | 3 | 425.0 ± 70.9 | 1.528 ± 0.351 | 0.3574 ± 0.02387 | 1.231 ± 0.02387 |
| Comparison with terminal controls | | | N.S. | N.S. | N.S. |
| 0.5 mg SG-G100 | 2 | 460.0 ± 42.4 | 2.04540 ± 0.12980 | 0.4453 ± 0.01264 | 1.3740 ± 0.17166 |
| Comparison with terminal controls | | | N.S. | .02>P>.01 | N.S. |
| Comparison with zero controls | | | N.S. | .10>P>.05 | N.S. |
| 5.0 mg SG-G100 | 7 | 454.9 ± 67.06 | 3.04561 ± 1.06534 | 0.67394 ± 0.23803 | 1.2505 ± 0.06610 |
| Comparison with zero controls | | | .05>P>.025 | .10>P>.05 | N.S. |
| Comparison with terminal controls | | | N.S. | N.S. | N.S. |
| Terminal controls | 2 | 420.0 ± 91.92 | 1.80575 ± 0.52601 | 0.3881 ± 0.02213 | 1.1687 ± 0.25575 |

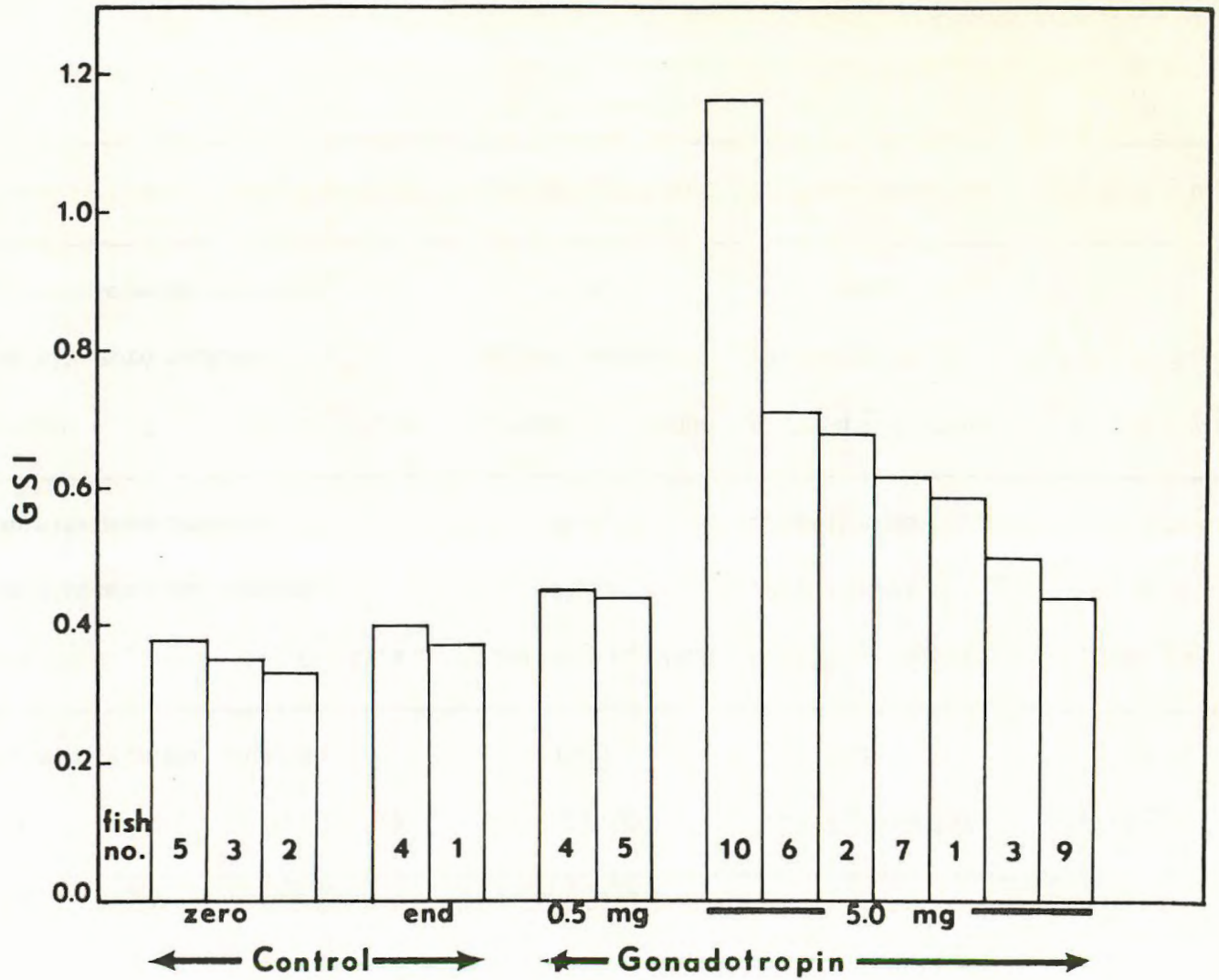


Fig. 4 Gonadosomatic indices (GSI) of individual female mullet treated with salmon gonadotropin.

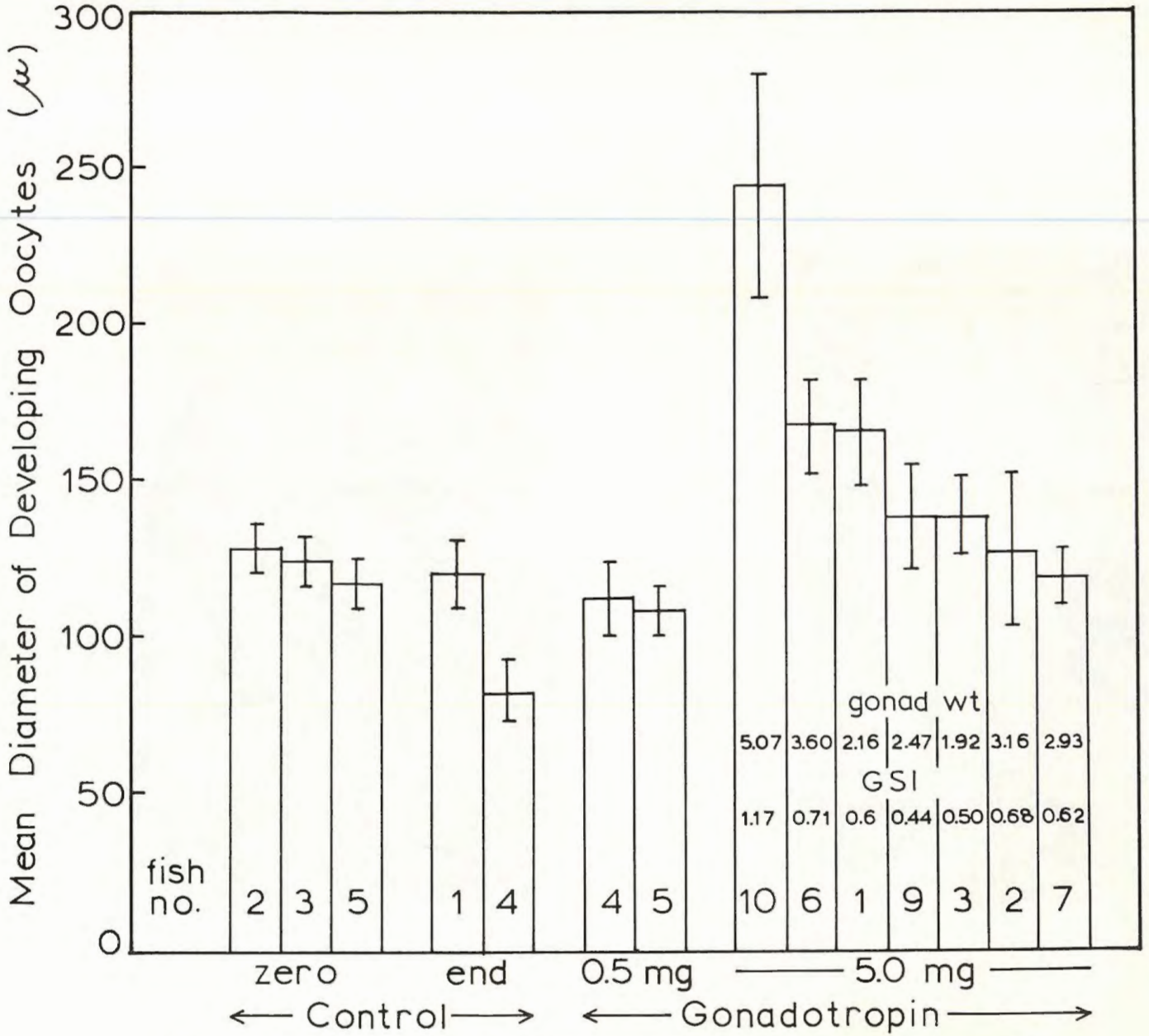


Fig. 5 Mean diameter of developing oocytes in individual female mullet treated with salmon gonadotropin.

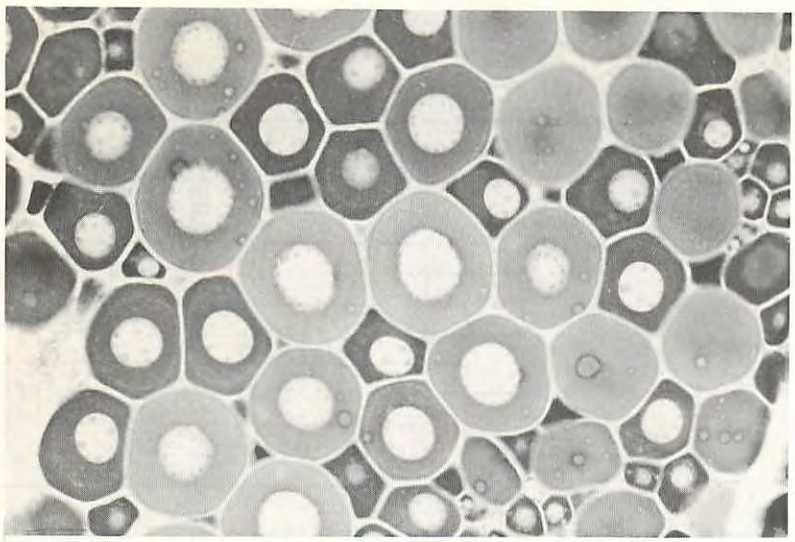

Fig. 6 Zero control ovary. Portion of ovary from zero control fish 5 showing oocytes ranging from early perinucleolar stage to early yolk vesicle stage. X300

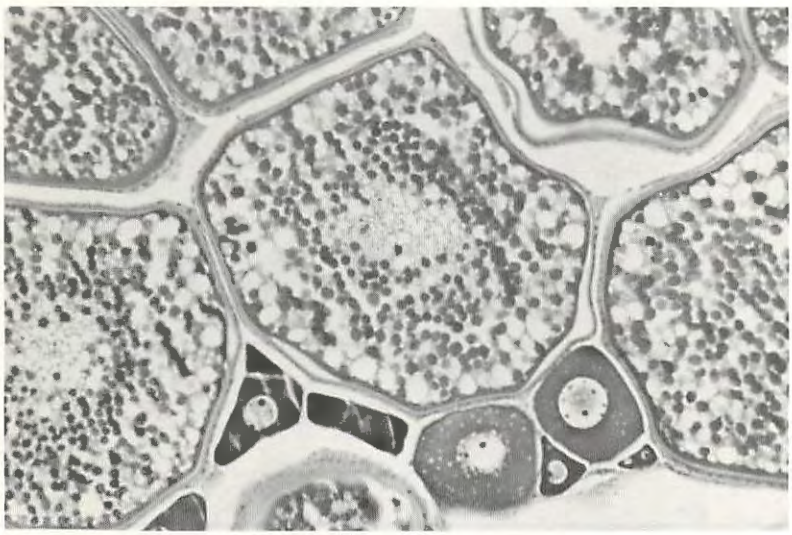

Fig. 7 5.0 mg gonadotropin treated ovary. Portion of ovary from fish 10 in 5.0 mg salmon gonadotropin dosage group showing oocytes in secondary yolk stage. X300

## DISCUSSION

The observation of free flowing milt in the testes of both groups of salmon gonadotropin injected male mullet combined with the observation of high sperm motility indicates that salmon gonadotropin is very effective for the induction of sexual development in male Mugil cephalus. The free flowing milt seen in these fish contrasts markedly with the thick non-flowing consistency of milt extruded from untreated mullet at the time of normal sexual maturity (Shehadeh and Ellis, 1970). In effect, the gonadotropin preparation has induced both spermatogenesis and spermiation as observed in Carassius auratus (Yamazaki and Donaldson, 1968a, b, 1969).

In the female mullet, the low dose of 0.5 mg salmon gonadotropin clearly had little effect, while the response to the 5.0 mg dose varied from a negligible response in some fish to a very pronounced effect in other fish. The oocytes in fish 10, in the 5.0 mg group, were in the secondary yolk stage and quite close to those in the tertiary yolk stage in the ovaries of mullet captured in the spawning season in waters off Oahu Island (Shehadeh and Ellis, 1970). The variation in response may have been a result of the individual fish being in different states of maturity at the beginning of the experiment. Alternatively, some mullet may develop antigen in response to the exogenous gonadotropin or to another protein in the partially purified preparation. Antibodies to heterologous gonadotropin have been shown to effectively block reproductive development in mammalian species (Pineda et al., 1968; Loewit et al., 1969). Another reason for the relatively low response may have been the interval between injections. The biological half-life of the exogenous gonadotropin after injection may be related to temperature, i.e. a shorter half-life may be expected in fish living in warm water. A dose regimen of three times per week has been shown to be suitable for pink salmon (Oncorhynchus gorbuscha) (Donaldson et al., 1972), at a temperature of 4-12° C and also in goldfish at 20° C (Yamazaki and Donaldson, 1968a, b). On the other hand, daily injections of salmon gonadotropin were found to be exceptionally effective in inducing sexual development in hypophysectomized male and female catfish (Heteropneustes fossilis) at 25° C (Sundararaj et al., 1971a, b). Thus a daily injection regime may have been much more effective in the present experiment which was performed at 26° C.

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VI.

# CHANGES IN BLOOD COMPONENTS OF THE MULLET, Mugil cephalus L., FOLLOWING TREATMENT WITH SALMON GONADOTROPIN AND METHYLTESTOSTERONE


## Abstract

1. Blood from twenty-six striped mullet (Mugil cephalus) injected with salmon gonadotropin and methyltestosterone was compared to blood from saline injected controls for changes in hematocrit, total dissolved plasma solids, plasma osmolarity, total plasma calcium and copper, and electrophoretic patterns of plasma proteins.
2. In all hormone treated fish, hematocrits increased slightly, plasma solids increased significantly ( $P \leq 0.05$) and osmolarity was not affected.
3. Total plasma calcium increased with the higher (5.0 mg) gonadotropin dose, and increased markedly in methyltestosterone treated fish. Plasma copper changes followed those of calcium but were not pronounced.
4. Methyltestosterone, in contrast to salmon gonadotropin, induced distinct qualitative and quantitative changes in plasma protein electropherograms.



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## INTRODUCTION

The spawning cycle in teleosts has been demonstrated to elicit alterations in a number of blood components. Serum calcium increases in females of a number of species during the natural spawning season (Garrod and Newall, 1958; Booke, 1964b; Flemming et al., 1964; Woodhead, 1968). Similar changes have been reported for total serum protein (Saito, 1957; Booke, 1964b), plasma lipids and vitamin A aldehydes (Plack and Woodhead, 1966) and mean erythrocyte counts (Slicker, 1958). Variations in the electrophoretic patterns of serum proteins have also been reported in a number of fish (Booke, 1964a; Thurston, 1967).

These changes are apparently related to sex hormone titers during sexual maturation (Hoar, 1965, 1969). Experimental injection of estrogens into non-spawning adult fish induced similar increases in serum calcium, phosphorus, total protein, lipids, vitamin A aldehydes and other parameters (Bailey, 1957; Ho and Vanstone, 1961; Urist and Schjeide, 1961; Plack and Woodhead, 1966; Plack and Pritchard, 1968; Woodhead, 1969). Slicker (1958) found that mean erythrocyte counts dropped below non-breeding levels in hypophysectomized Fundulus males, but could be increased to breeding levels after one month's treatment with methyltestosterone. Testosterone propionate, however, had no effect on serum calcium, phosphorus, proteins or lipids in mature Carassius (Bailey, 1957).

This study was undertaken to investigate the effects of partially purified salmon gonadotropin (Donaldson and Yamazaki, 1968) and methyltestosterone on hematocrit, plasma total dissolved solids, osmolarity, total plasma calcium and copper, and electrophoretic patterns of plasma proteins in the striped mullet, Mugil cephalus.

## MATERIALS AND METHODS

The blood samples examined in this study were obtained from twenty-six adult mullet, Mugil cephalus L., (424 g mean body wt.) at the termination of an experiment on hormone-induced gonad maturation (Donaldson and Shehadeh; data to be published elsewhere). The experimental regime involved intraperitoneal injection of 0.5 mg and 5.0 mg of partially purified salmon gonadotropin (in 0.5 ml saline; 0.995 g % NaCl) and 25 mg crystalline methyltestosterone (in 0.5 ml pure peanut oil) into three separate fish groups respectively. Controls received 0.5 ml saline. Injection was carried out on alternate days for a period of one month.

Blood samples were taken by cardiac puncture from anesthetized fish (MS 222; Sandoz Pharmaceuticals, N.J.). A sample of whole blood was removed for hematocrit determination and the remainder centrifuged to separate the plasma. Total dissolved solids were measured with a Goldberg refractometer and plasma proteins examined by cellulose acetate electrophoresis at pH 8.6 (sodium barbital buffer, ionic strength 0.05). Electrophoresis was carried out on 1 x 6 Gelman Separaphore III polyacetate strips in a Colab Universal Electrophoresis Chamber at 300 V for 20 min. Electropherograms were stained and prepared according to Smith & Goldstein (1967). In one control fish, two 0.1-ml plasma samples were incubated under refrigeration (12° C) for 2 hr with 0.01 ml of the methyltestosterone injection material (one sample) and with 0.01 ml of peanut oil (second sample) before electrophoresis.

Plasma calcium and copper were measured with an atomic absorption spectrophotometer (Perkin-Elmer, Model 303). Samples were diluted 250 times with 0.05 g % $La_2O_3$ in 0.25 % HCl and read at 4227 Å (calcium) and 2407 Å (copper) using hollow cathode source lamps and oxy-acetylene flame. Plasma osmolarity was read on a Fish Osmometer with a small sample operating head.

## RESULTS

Mean and standard deviations for hematocrit, total dissolved solids, osmolarity, calcium and copper concentrations for each experimental group are reported in Table 1. Hematocrit values showed an increase over controls but the difference was not statistically significant. Total dissolved solids, on the other hand, demonstrated a significant ( $P \leq 0.05$) increase over controls in all experimental groups. Plasma osmolarity remained constant within statistical limits. Total plasma calcium increased significantly ($P < 0.05$) in the 5.0 mg gonadotropin group but no significant changes were detected in the 0.5 mg gonadotropin group. The rise in plasma calcium was restricted to females in the former group. A three to fourfold increase in total plasma calcium occurred in two of three (two females and one male) fish in the methyltestosterone group. Values of 17.25, 48.38 and 41.38 mg % were obtained for two females and one male respectively. Copper concentrations showed a slight but non-significant increase in the 5.0 mg gonadotropin group and a near twofold increase in the methyltestosterone group.

Table 1. Changes in blood parameters in the various experimental groups (mean ± standard deviation)

| Injection | Sex | No. of fish | Hematocrit (% cell vol.) | Refractometry (% total solid) | Osmolarity (m-osmols) | Calcium (mg %) | Copper (mg %) |
|---|---|---|---|---|---|---|---|
| Saline Control | F | 2 | 36.5 ± 2.1 | 5.3 ± 0.4 | 366 ± 9 | 12.50 ± 0.88 | 0.25 ± 0.00 |
| | M | 3 | 45.0 ± 6.2 | 6.2 ± 1.4 | 370 ± 16 | 14.12 ± 2.07 | 0.28 ± 0.02 |
| | Total | 5 | 42.0 ± 6.5 | 5.8 ± 1.1 | 368 ± 10 | 13.47 ± 1.77 | 0.27 ± 0.02 |
| Gonadotropin (0.5 mg) | F | 2 | 47.5 ± 3.8 | 6.5 ± 0.4 | 356 ± 3 | 12.50 ± 0.88 | 0.29 ± 0.02 |
| | M | 6 | 48.3 ± 6.2 | 7.9 ± 0.8 | 353 ± 24 | 14.58 ± 1.87 | 0.28 ± 0.02 |
| | Total | 8 | 48.1 ± 5.6 | 7.6 ± 0.9* | 354 ± 19 | 14.06 ±1.88 | 0.28 ± 0.02 |
| Gonadotropin (5.0 mg) | F | 7 | 47.3 ± 3.5 | 6.7 ± 1.1 | 357 ± 27 | 16.59 ± 1.77 | 0.30 ± 0.03 |
| | M | 3 | 47.7 ± 6.0 | 6.5 ± 0.3 | 370 ± 31 | 14.00 ± 1.44 | 0.30 ± 0.02 |
| | Total | 10 | 47.4 ± 4.0 | 6.6 ± 0.9* | 361 ± 28 | 16.11 ± 1.74* | 0.30 ± 0.03 |
| Methyltestosterone (25.0 mg) | F | 2 | 45.5 ± 6.4 | 8.4 ± 0.6 | 384 ± 40 | 32.82 ± 22.00** | 0.50 ± 0.00 |
| | M | 1 | 54.0 | 11.7 | 359 | 41.38 | 0.37 |
| | Total | 3 | 48.3 ± 6.7 | 9.5 ± 2.0* | 376 ± 10 | 35.67 ± 16.30** | 0.44 ± 0.07 |

* Significant ( $P \leq 0.05$) increase above controls ( t test)

** The two females injected with methyltestosterone gave calcium values of 48.38 and 17.25 mg %.

The discrepancy in the numbers of males and females in each experimental group precluded a statistical evaluation of the differences in values of various parameters between the sexes. The problem stems from the fact that mullet do not exhibit sexual dimorphism and are difficult to sex. Nevertheless, the data indicate a slight tendency for males to have generally higher hematocrit and total dissolved plasma solids than females. Males also appeared to have higher total plasma calcium in both control and 0.5 mg gonadotropin groups. Where hypercalcemia was evident (5.0 mg gonadotropin and methyltestosterone groups), females showed higher total plasma calcium than males. Plasma copper changes were similar to those of calcium.

Considerable variation was evident in the plasma electropherograms obtained from the experimental fish. Differences in the mobility and staining intensity of fractions 3-6 (Fig. 1) could not be clearly correlated, for the most part, with sex or the type of administered hormone. As shown in Fig. 1, however, an important exception occurred in fish injected with methyltestosterone. In two of the three fish, there was a relatively high concentration of fraction 8 which was not observed in any other fish. Further differences were evident in all three fish, with fraction 3 being strongly stained, and fractions 4 and 6 absent. Control plasma incubated with methyltestosterone in peanut oil showed increased staining of fraction 3 (Fig. 1). This did not occur with peanut oil incubation.

## DISCUSSION

As indicated by results summarized in Table 1, hematocrit values were slightly elevated by injection of either salmon gonadotropin or methyltestosterone. Sano (1960) reported little seasonal variation in hematocrit values for the rainbow trout, Salmo gairdnerii, whereas mean erythrocyte counts were observed to increase during spawning and following injection of methyltestosterone in Fundulus heteroclitus (Slicker, 1958). It would be interesting to examine hematocrit changes during the normal spawning cycle in Hawaiian mullet since the winter decrease in sea surface temperature, postulated to be the cause for elevated hematocrit and blood cell count in Texas mullet (peak temperature drop of nearly 19° C; Cameron, 1970), is less than 3° C for most Hawaiian waters (Seckel, 1962)

The hormone induced increase in total dissolved plasma solids is probably related to an increase in macromolecules (proteins, lipids, etc.) since no significant change in plasma osmolarity was observed. This is further supported by the increase in certain plasma protein fractions (Fig. 1) in fish treated with methyltestosterone. This increase coincided with the highest total dissolved solids values (Table 1).

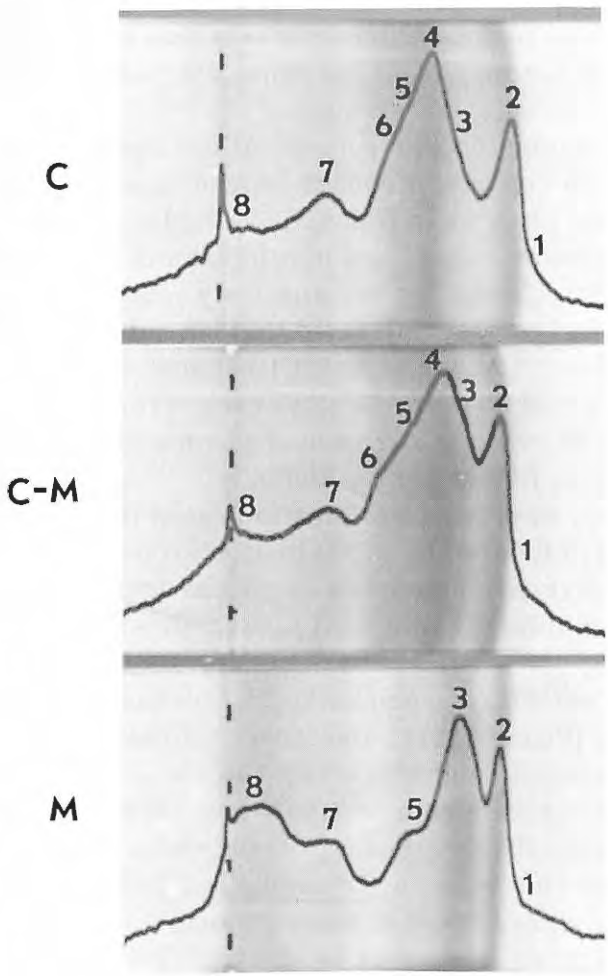


Fig. 1. Electrophoretic patterns of plasma from control fish (C), control fish after plasma incubation with methyltestosterone injection material (C-M) and methyltestosterone-injected fish (M).

Calcium variations, in general, were similar to those reported for estrogen injected fish (Bailey, 1957; Ho and Vanstone, 1961; Urist and Schjeide, 1961; Fleming et al., 1964). The hypercalcemia noted in females (5.0 mg gonadotropin and methyltestosterone groups) conforms with plasma changes reported in other fish in the course of the normal spawning cycle (Garrod and Newall, 1958; Booke, 1964b; Woodhead, 1968). In contrast to other fish (Fontaine, 1956; Garrod and Newall, 1958) non-spawning female mullet show lower total plasma calcium levels than non-spawning males, although such a condition may exist, at times, in yearling brook trout (Booke, 1964b).

Variations in plasma copper concentrations appear to simulate calcium changes. No previous reports of copper concentrations in relation to spawning or hormonal changes in fish are available. Evans and Wiederanders (1968) showed that plasma copper and ceruloplasmin (copper containing globulin) concentrations in the rat are similarly effected by other hormones. Evans et al. (1970) further demonstrated that estradiol induced increased de novo synthesis of ceruloplasmin in rat liver that subsequently caused an increase in serum ceruloplasmin. Methyltestosterone probably has a similar effect since 90 per cent or more of plasma copper appears to be bound to ceruloplasmin (White et al., 1964).

Electrophoresis of plasma proteins indicated that changes in the nature of these proteins were induced by methyltestosterone but not salmon gonadotropin. Similarly, injections of estrogens (estradiol-17$\beta$ 3-benzoate or estrone) were found to increase total plasma proteins as well as induce the appearance of new proteins (Urist and Schjeide, 1961; Bailey, 1957; Ho and Vanstone, 1961). Testosterone and estrogen increase in the plasma during the spawning season (Hoar, 1969), and induce increased protein synthesis (Davidson, 1965; Rosenfeld and O'Malley, 1970).

The work of Urist and Schjeide (1961) and Liao et al. (1965) support Bailey's (1957) findings that estrone increases serum levels of calcium, phosphorus, proteins and lipids in Carassius but testosterone propionate does not. The discrepancy between these reports and our results [and possibly those of Slicker (1958)] may be due to differences in methyl-testosterone and testosterone propionate with respect to their target tissues. As reviewed by Hoar (1969), changes in the qualitative nature of testosterones are known to occur during the spawning cycle in fishes, particularly in the ratio of testosterone to testosterone derivates or other androgens.

The mode of action of gonadotropins is not known, but they apparently function in the control of gonadal steroidogenesis (Hoar, 1969). Thus, plasma hyperproteinemia, hypercalcemia, hyperlipemia, etc., are not expected unless sufficient gonadal steroid synthesis and release has occurred to effect

these changes. The relatively smaller changes in total dissolved solids and total plasma calcium and copper in the gonadotropin groups, compared to the methyltestosterone group, and the lack of hypercalcemia in the 0.5 mg gonadotropin group can be explained on this basis.

The appearance, or increase, of fraction 3 in methyltestosterone-treated fish is suggested to be due, at least in part, to a conjugation of plasma proteins and methyltestosterone. This is evidenced by the appearance of this fraction following in vitro incubation of the hormone with control plasma (Fig. 1) and further supported by similar reports in the literature (Grajcer and Idler, 1961, 1963). The large increase in fraction 8 (Fig. 1) in these fish is considered to be related to a protein(s) containing bound calcium, as demonstrated by the clear correlation with the pronounced hypercalcemia. In this last regard, estrogen injection is known to increase non-ultrafiltrable calcium but not "free" calcium due to increases in colloidal $Ca_3(PO_4)_2$ and protein bound calcium (Bailey, 1957; Urist and Schjeide, 1961). Similarly the copper increase noted with methyltestosterone, presumably reflecting an increase in plasma ceruloplasmin, may also be instrumental in causing alterations in these electrophoretic patterns.

## SUMMARY


1. Changes in hematocrit, total dissolved plasma solids, plasma osmolarity, total plasma calcium and copper and electrophoretic patterns of plasma proteins were examined in the striped mullet, M. cephalus, following injections of 0.5 mg and 5.0 mg dosages of partially purified gonadotropin and 25 mg methyltestosterone.

2. Increased hematocrit following hormone injection was apparent, but not statistically significant.

3. Plasma solids increased significantly ( $P<0.05$) with hormone treatment. This increase is suggested to reflect an increase in macromolecules such as proteins, lipids and vitamin A aldehydes.

4. Total plasma calcium increased three to fourfold in the methyltestosterone group and slightly in the females of the 5.0 mg gonadotropin group. Plasma copper variations mimicked calcium changes, but were less pronounced.

5. Qualitative and quantitative changes were observed in plasma protein electropherograms of the methyltestosterone group only.

6. These changes are discussed in relation to similar findings in other species during the natural spawning cycle in fish and following injection of sex hormones.

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VII.

# INDUCED SPAWNING OF GREY MULLET (Mugil cephalus L.) WITH FRACTIONATED SALMON PITUITARY EXTRACT


## Abstract

The efficacy of fractionated salmon pituitary gonadotropin as a spawning agent in Mugil cephalus was tested. Natural spawning was induced in all females with a total dose of 11.9 - 20.9 µg/g body wt. Spawning dose varied inversely with initial mean egg diameters of recipient females. A "critical" mean egg diameter of 650-700 µ was observed to precede the hormone dose that induced spawning. A "priming" effect was observed following the initial injection and is discussed. The "latency period" was determined to be 10 - 15 hours; fecundity was estimated at 648 eggs/g body wt. Courtship, spawning and fertilization occurred naturally with uninjected males.



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# INTRODUCTION

The artificial induction of spawning in the grey mullet has been the subject of study in Taiwan, Korea, Israel and the United States (Hawaii) for the past decade. Sanzo (1936) and Anderson (1957) reported the first successful artificial fertilization of mullet eggs at sea. Yang and Kim (1962) described similar success with ripe fish collected in the Yungsan estuary, Korea. In all three cases, eggs and sperm were removed from ripe wild fish without hormone injection.

The first hypophysation-induced spawning was reported by Tang (1964) in Taiwan. Homogenates of mullet pituitary glands collected during the breeding season from migrating spawners were used successfully. Since then artificial spawning has been repeated with carp pituitary homogenates in Israel (Yashouv, 1969), mullet pituitary homogenates (Liao et al., 1970), and with Pacific salmon pituitary homogenates in Hawaii (Shehadeh and Ellis, 1970). However, a standard spawning procedure delineating dose rate of applied hormone(s) and/or pituitary extracts required to induce spawning of grey mullet at clearly defined stages of sexual maturity is yet to be described. The problem arises from the difficulty in determining the gonadotropic potency of crude pituitary homogenates and the absence of a reliable method with which ovarian development can be determined in individual, hormone-recipient females.

More recently, partially purified teleost pituitary gonadotropins have been used successfully in the induction of spawning in Puntius gonionotus, Hypophthalamichthys molitrix, Aristichthys nobilis, Mugil cephalus, Cyprinus carpio, and Heteropneustes fossilis (Sinha, 1969, 1971; Sundararaj et al., 1972; Shehadeh et al., 1972). The advantage of these fractions over crude pituitary homogenates is that standard and precise methods are used in their preparation and the purified fractions can be quantitated in dose rate descriptions in induced spawning work.

The present study attempts to:

(1) Test a partially purified salmon pituitary gonadotropin fraction as a spawning agent in the grey mullet, and

(2) Determine the dose rates of gonadotropin fraction required to induce spawning in female mullet at given stages of egg development.

## MATERIALS AND METHODS

### Female Treatment

Eight females were chosen for this study from a captive population maintained in running sea water (32 $^{o}/_{oo}$) in outdoor, rubber-lined ponds. Each female was weighed and placed in a separate 50-gallon fiberglass tank in running sea water (32 $^{o}/_{oo}$) at 24 $^{o}$C. Ovarian development was determined by *in vivo* siphoning of intra-ovarian eggs according to Shehadeh and Kuo (1972). Fish weights and initial mean egg diameters are shown in Table 1. The fish were allowed to acclimatize to the new environment for 24 hours before hormone injection was initiated.

Purified salmon gonadotropin (courtesy of Fisheries Research Board of Canada, Vancouver Laboratory) was injected in the dorsal musculature near the base of the dorsal fin in a constant volume of 0.50 cc of 0.60% NaCl. The fractionation and purification of the salmon (*Oncorhynchus tshawytscha*) pituitary gonadotropin utilized in this study have been described by Donaldson and Yamazaki (1968a) and Donaldson *et al.* (1972).

In the absence of established guidelines, dose rates were chosen arbitrarily at the beginning of the study and subsequently modified according to initial results. Injections were arbitrarily spaced 24 hours apart. In order to determine the effect of single gonadotropin injections in each female, *in vivo* egg samples were taken before and 24 hours after each injection. Egg diameters were measured and diameter-frequency distributions plotted. Specific dose rates ($\mu$g/g body wt) and injection schedules are listed in Table 2 (see Results section).

### Male Treatment

The males used in this study were taken from the same captive population as the females. Eight milting males were chosen on the basis of the presence of small amounts of viscous milt. None were injected during the study.

One or two males were placed with each injected female when the latter began to exhibit abdominal distention (ovulation). At this time water flow was stopped to guard against loss of eggs following spawning, and air was introduced in the tank to help in mixing of eggs and sperm. Spawning and fertilization of released eggs were permitted to proceed naturally in the tanks.

The female and two males were removed from the spawning tank after spawning and natural fertilization was completed. Fecundity was estimated by counting the number of eggs in a measured volume of water removed from the spawning tanks. Thorough aeration helped minimize error resulting from unequal distribution of eggs in the water. Fertilization rates were determined by counting the number of cleaving eggs under the microscope in 10 individual samples (total of 100 eggs).

Table 1. Body weights and initial mean egg diameters of experimental female mullet.

| Fish No. | Body wt (g) | Initial mean egg diameter (μ) |
|---|---|---|
| 1 | 596 | 630 |
| 2 | 968 | 702 |
| 3 | 1371 | 606 |
| 4 | 1093 | 670 |
| 5 | 1010 | 665 |
| 6 | 980 | 711 |
| 7 | 838 | 723 |
| 8 | 758 | 659 |

Table 2. Spawning by injections of purified salmon pituitary gonadotropin

| Fish No. | Dose injected (mg) | Date | Cumulative specific dose (μg/g body wt) | Mean egg diameter (μ) | Time to spawning from last injection | Spawning | Fecundity (thousand) | Fertilization rate(%) | Hatching |
|---|---|---|---|---|---|---|---|---|---|
| 1 | - | 2/3/71 | - | 630 | | | | | |
| | 0.5 | 2/4/71 | 0.839 | 629 | | | | | |
| | 0.5 | 2/5/71 | 1.677 | 637 | | | | | |
| | 0.5 | 2/6/71 | 2.517 | 648 | | | | | |
| | 1.0 | 2/7/71 | 4.195 | 649 | | | | | |
| | 5.0 | 2/8/71 | 12.584 | 649 | | | | | |
| | 5.0 | 2/9/71 | 20.973 | 940 | 14 hrs. 15 min. | natural | 340 | 60 | + |
| 2 | - | 2/5/71 | - | 702 | | | | | |
| | 3.0 | 2/6/71 | 3.099 | 713 | | | | | |
| | 10.0 | 2/7/71 | 13.429 | 925 | 13 hrs. 50 min. | natural | ? | ? | + |
| 3 | - | 2/8/71 | - | 606 | | | | | |
| | 3.0 | 2/9/71 | 2.188 | 602 | | | | | |
| | 10.0 | 2/11/71 | 9.482 | 609 | | | | | |
| | 10.0 | 2/13/71 | 16.776 | 666 | | | | | |
| | 5.0 | 2/13/71 | 20.423 | 900 | 14 hrs. 20 min. | natural | 759 | 96 | + |
| 4 | - | 2/8/71 | - | 670 | | | | | |
| | 10.0 | 2/9/71 | 9.147 | 672 | | | | | |
| | 5.0 | 2/10/71 | 13.723 | 934 | 12 hrs. 30 min. | natural | 795 | 53 | + |
| 5 | - | 2/20/71 | - | 665 | | | | | |
| | 5.0 | 2/21/71 | 4.950 | 676 | | | | | |
| | 10.0 | 2/22/71 | 14.851 | 921 | 10 hrs. | natural | 434 | 93 | + |

(Table 2. continued)

| Fish No. | Dose injected (mg) | Date | Cumulative specific dose (μg/g body wt) | Mean egg diameter (μ) | Time to spawning from last injection | Spawning | Fecundity (thousand) | Fertilization rate(%) | Hatching |
|---|---|---|---|---|---|---|---|---|---|
| 6 | - | 2/21/71 | - | 711 | | | | | |
| | 5.0 | 2/22/71 | 5.102 | 714 | | | | | |
| | 10.0 | 2/23/71 | 15.306 | 940 | 11 hrs. 45 min. | natural | 659 | 83 | + |
| 7 | - | 2/21/71 | - | 723 | | | | | |
| | 5.0 | 2/22/71 | 5.966 | 723 | | | | | |
| | 5.0 | 2/23/71 | 11.933 | 915 | 14 hrs. 40 min. | natural, partial spawning | 309 | 93 | + |
| 8 | - | 2/28/71 | - | 659 | | | | | |
| | 5.0 | 3/1/71 | 6.596 | 687 | | | | | |
| | 10.0 | 3/2/71 | 19.788 | 933 | 10 hrs. 30 min. | natural | 594 | 81 | + |

## RESULTS

The effects of various gonadotropin dose rates on mean egg diameters and spawning are shown in Table 2. Partially purified salmon pituitary gonadotropin proved to be a potent spawning agent. All injected females spawned naturally in the tanks and released a great number of eggs. Fecundity was determined to be 648 $\pm$ 62 eggs/g body wt (mean $\pm$ standard deviation). Attempts to siphon eggs from spawned-out females failed. Females apparently dropped all their eggs. Spawned fertile eggs measured 926 $\pm$ 5 $\mu$ (mean $\pm$ standard deviation).

The total quantity of gonadotropin required to induce spawning was inversely proportional to the initial mean egg diameter of recipient females (Fig. 1) and varied between 11.9 - 20.9 $\mu$g/g body wt.

Close examination of the relationship between dose rates and changes in egg diameter (and spawning) revealed a number of interesting facts. The "effective" gonadotropin dose, which precipitated natural spawning 10 - 15 hours after injection, was always preceded by mean egg diameters of 650 - 700 $\mu$ (690 $\pm$ 9 $\mu$, mean $\pm$ standard deviation) in all recipient females. In females with initial mean egg diameters $<$650 $\mu$ (females no. 1, 3, and 8) gonadotropin injection first induced a shift in egg diameter-frequency distributions (Fig. 2) and an increase in mean egg diameters (Table 2) towards the "critical" range of 650 - 700 $\mu$. In these females, the "effective" gonadotropin dose was found to range between 5 and 13 $\mu$g/g body wt. In the case of females with initial mean egg diameters equal to or greater than the "critical" value, the first gonadotropin dose, which ranged between 3 and 10 $\mu$g/g body wt, failed to induce any change in mean egg diameters (Table 2) or diameter-frequency distributions (Fig. 2). The "effective" gonadotropin dose under these circumstances was a total of 11.9 - 15.3 $\mu$g/g body wt applied in two doses 24 hours apart; one third of the dose first, followed by the remaining two thirds 24 hours later, or vice versa. This sequence appears to be critical since injection of half the dose at a time resulted in partial spawning (female no. 7). Administration of two-thirds the "effective" dose initially did not alter mean egg diameter or diameter-frequency distribution (female no. 4, Table 2, Fig. 2). The reason(s) for this "priming" effect are not clearly understood.

The "latency period" (i.e., the time to spawning after injection of the "effective" dose) varied within a relatively narrow time range of 10 hrs, 0 min. and 14 hrs. 40 min. During this time all females exhibited gradual abdominal distention and protrusion of the cloacal region. This distention was apparently due to egg hydration since egg water content increased from 55% at egg diameters of 690 $\pm$ 9 $\mu$, to 85% at egg diameters of 926 $\pm$ 5 $\mu$ (spawned eggs).

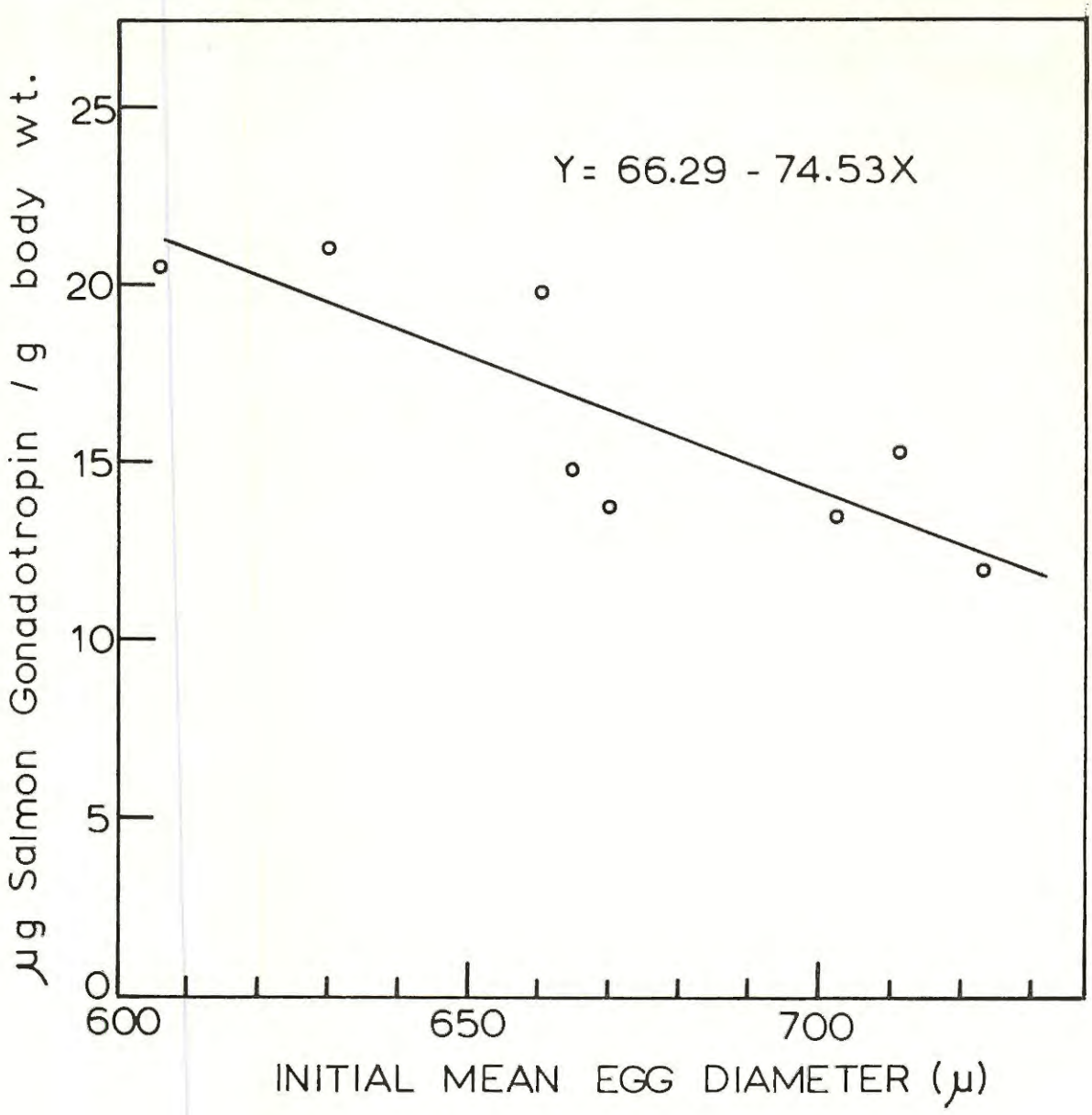


Fig. 1. The relationship between initial mean egg diameter of recipient females and the amount of gonadotropin required to induce spawning. For further details see Table 2.

Fig. 2. Effect of gonadotropin injection on egg diameter-frequency distributions in eight individual females. Injection protocol for each female is listed in Table 2.
$\overline{X}$: mean egg diameter.
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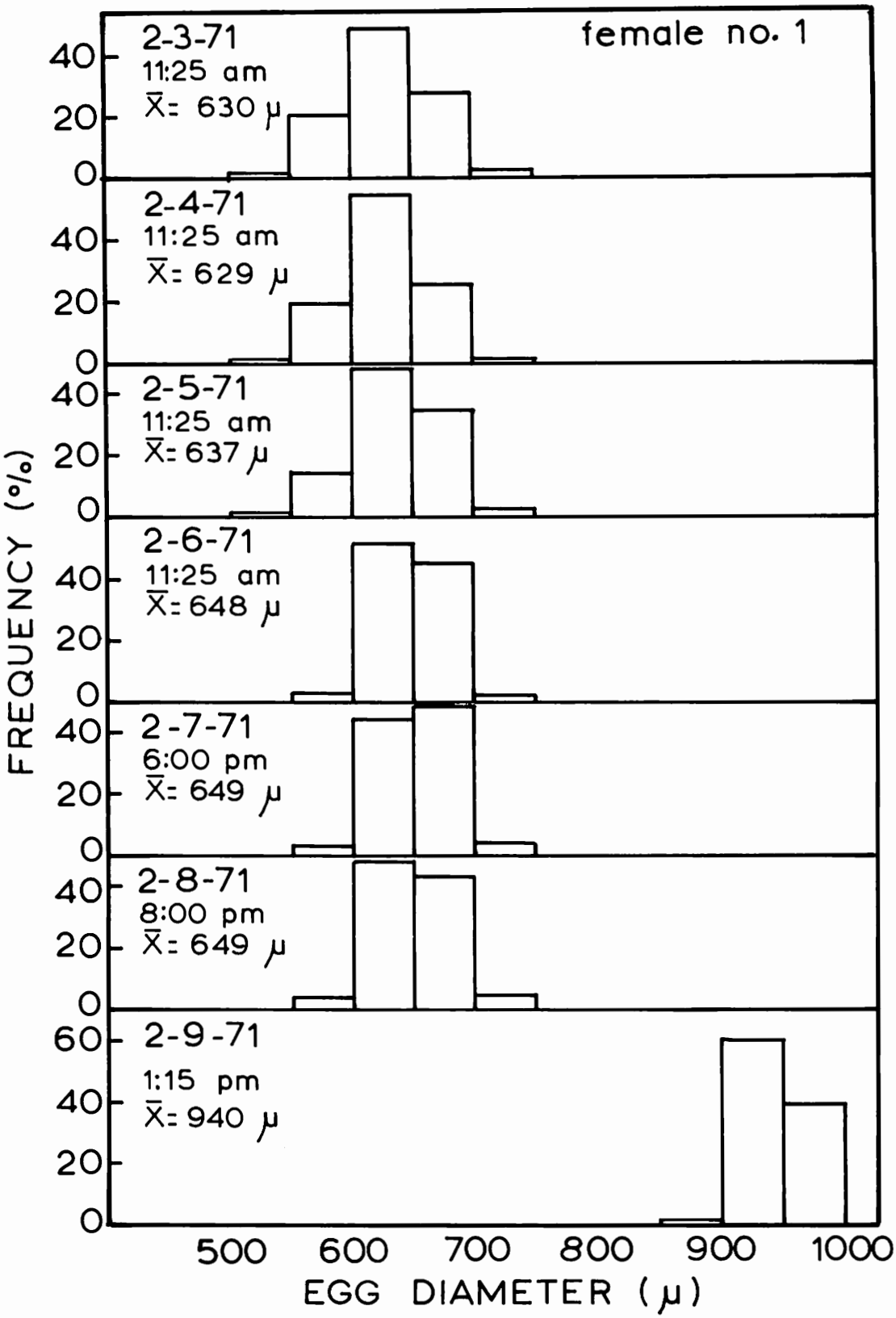
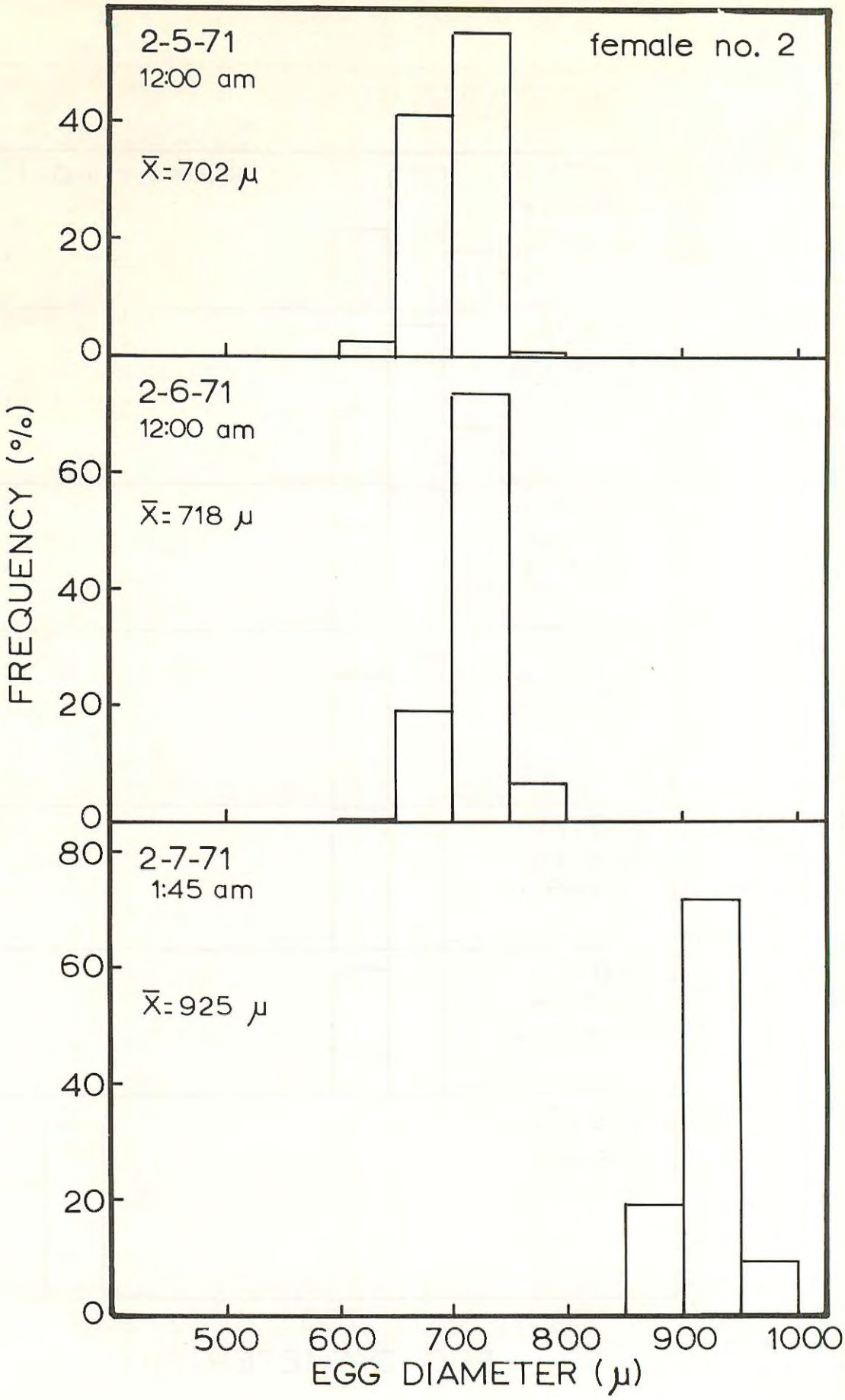
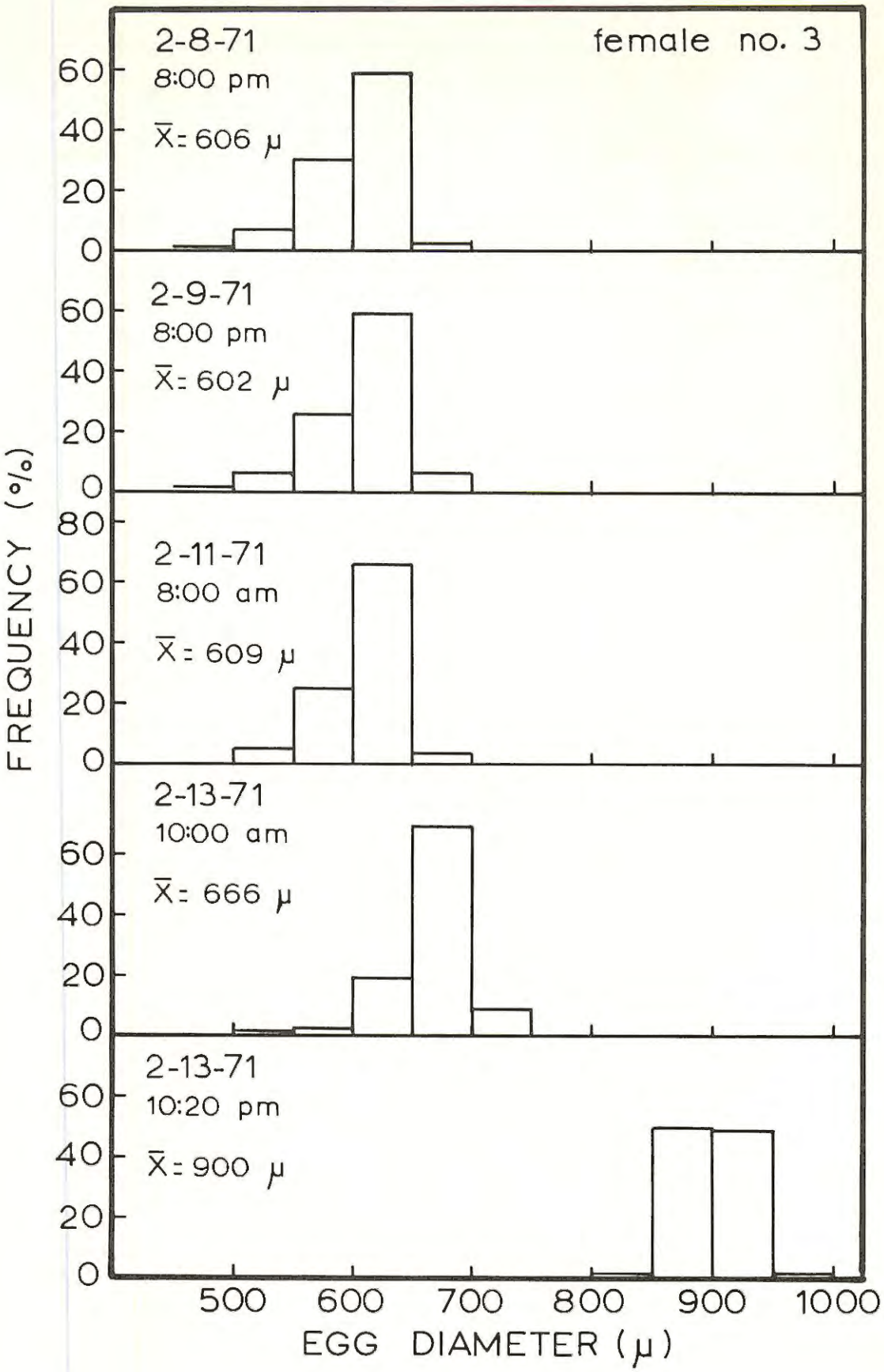
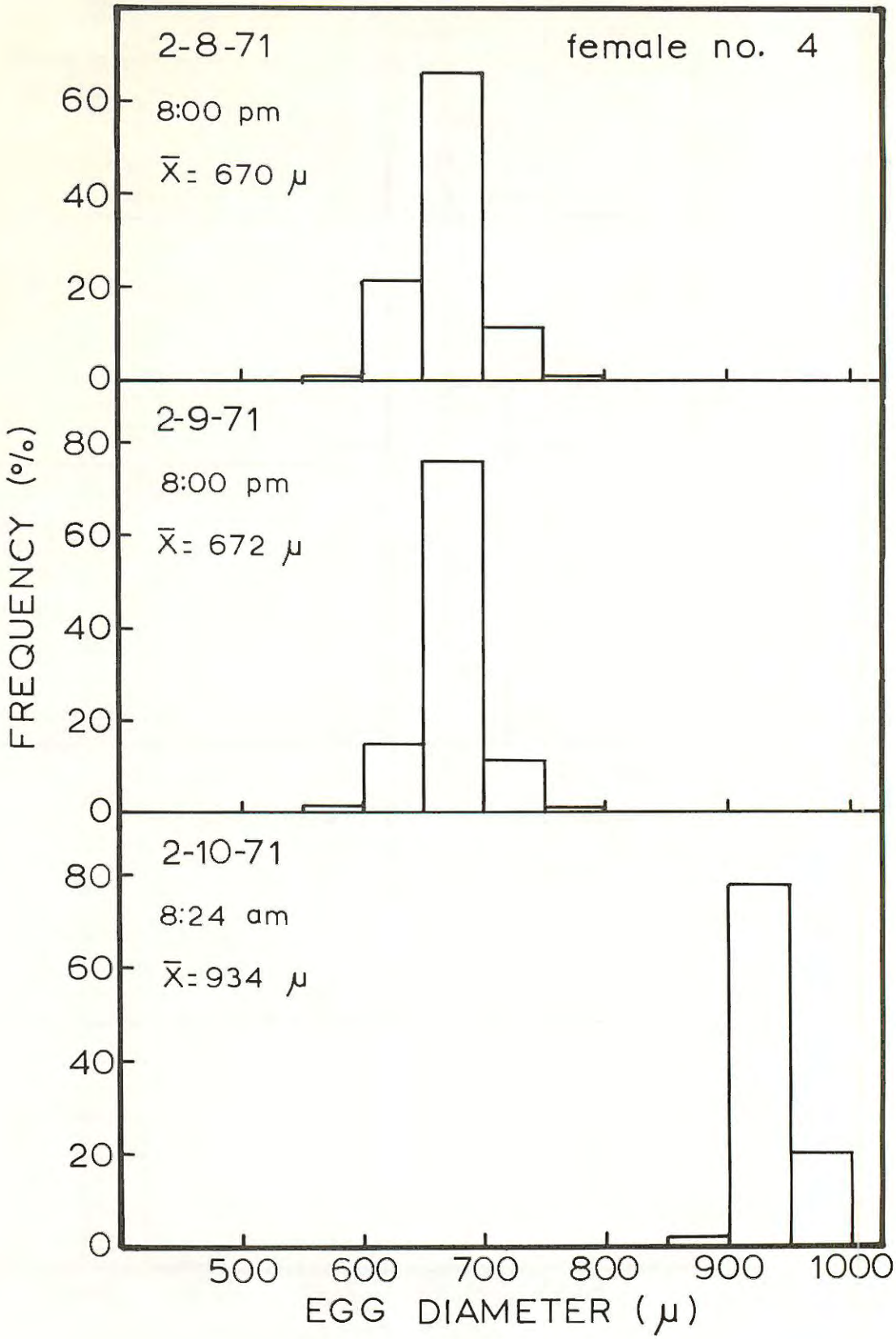
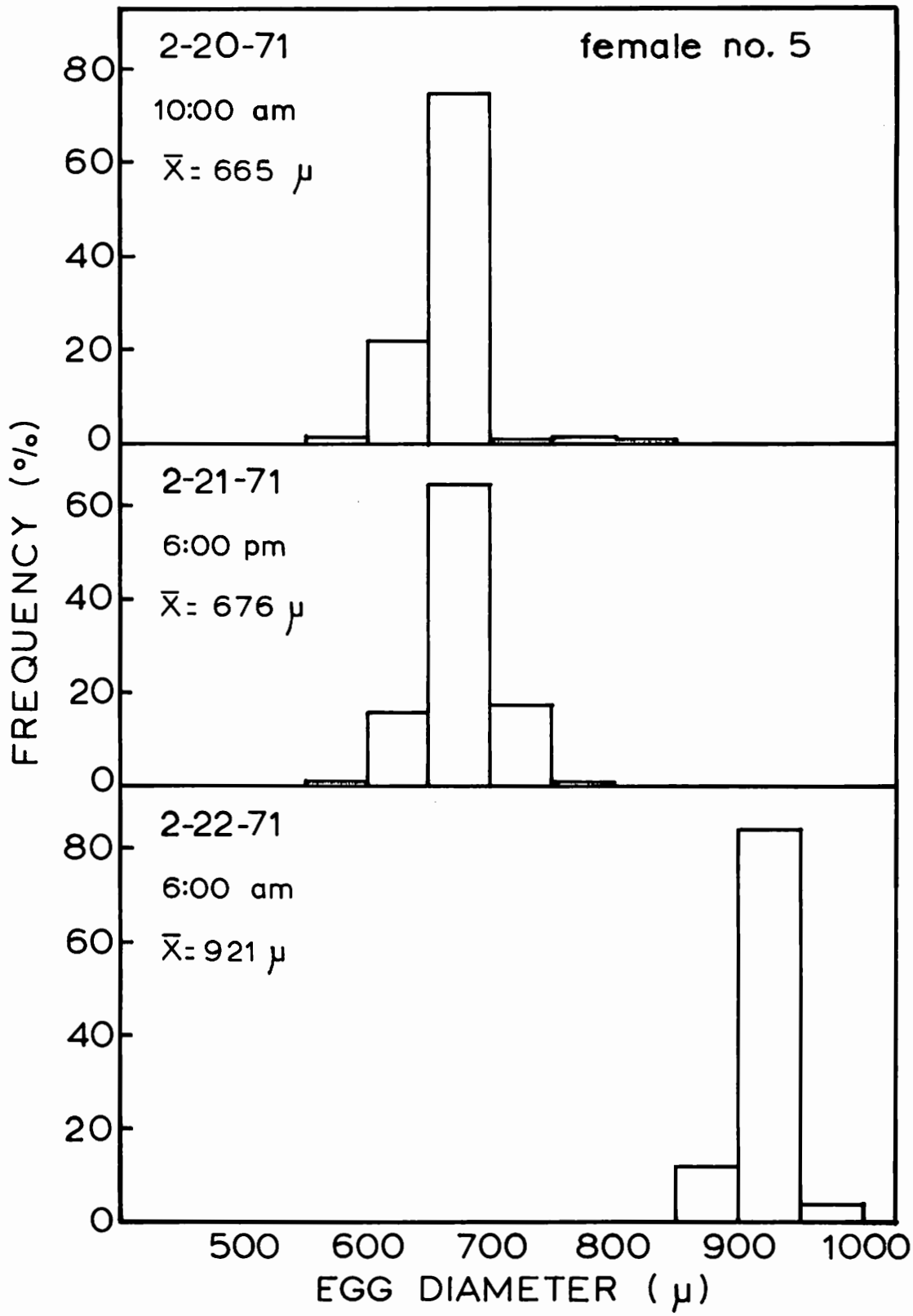
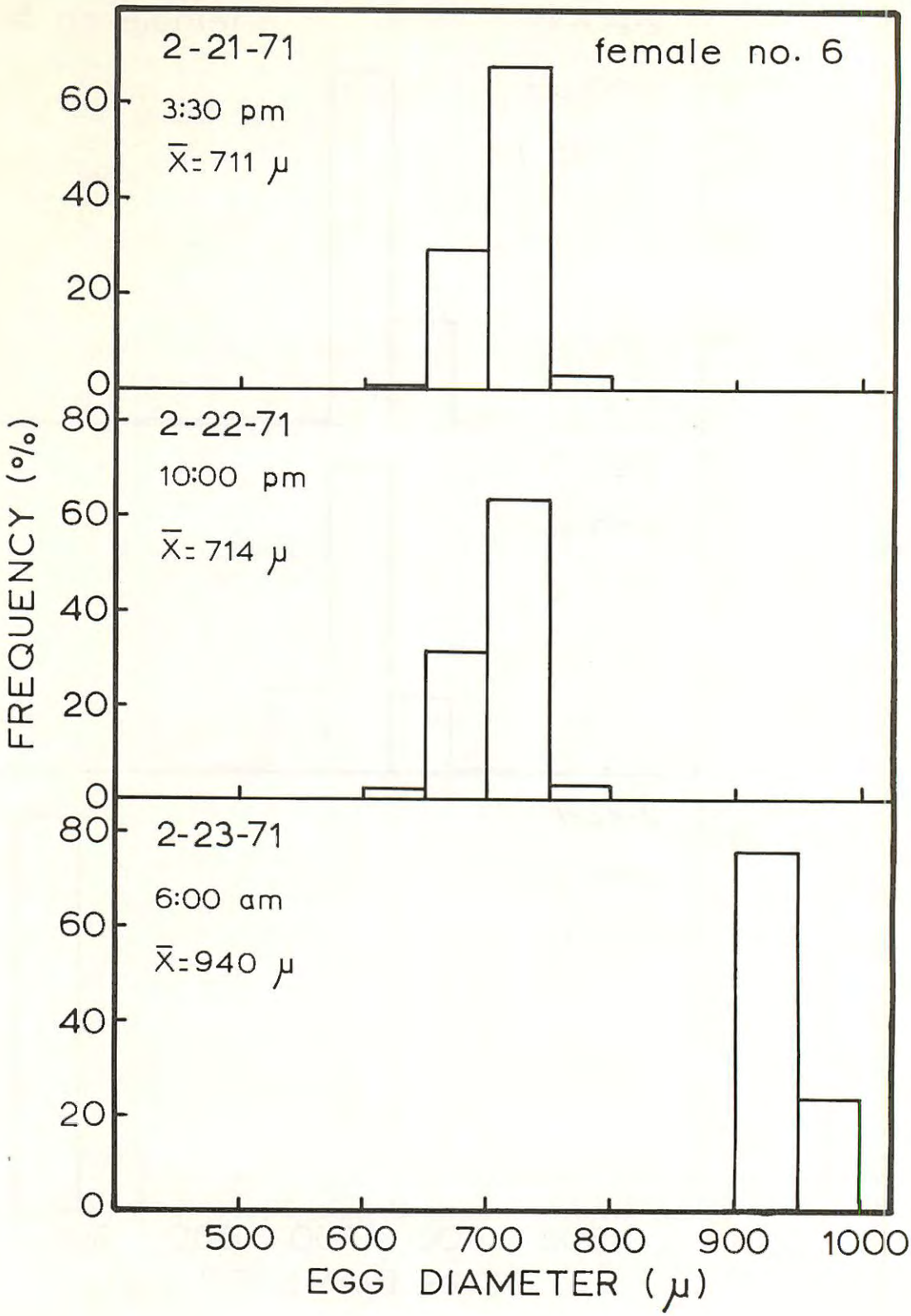

female no. 1
2-3-71
11:25 am
X̄= 630 μ
2-4-71
11:25 am
X̄= 629 μ
2-5-71
11:25 am
X̄= 637 μ
2-6-71
11:25 am
X̄= 648 μ
2-7-71
6:00 pm
X̄= 649 μ
2-8-71
8:00 pm
X̄= 649 μ
2-9-71
1:15 pm
X̄= 940 μ
FREQUENCY (%)
EGG DIAMETER (μ)
500
600
700
800
900
1000

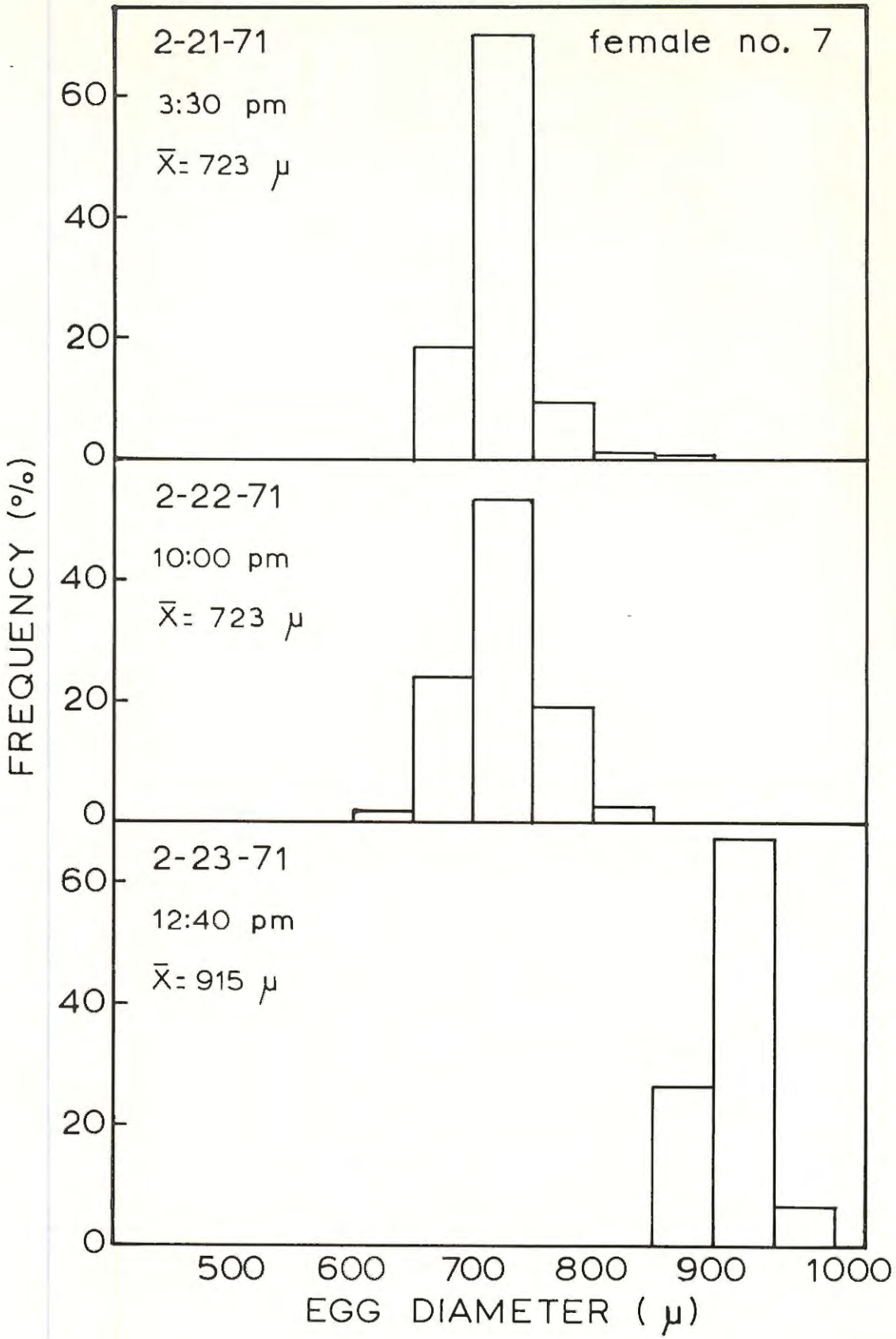

2-5-71
12:00 am
X̄= 702 μ
female no. 2
2-6-71
12:00 am
X̄= 718 μ
2-7-71
1:45 am
X̄= 925 μ
FREQUENCY (%)
EGG DIAMETER (μ)
0
20
40
60
80
500
600
700
800
900
1000

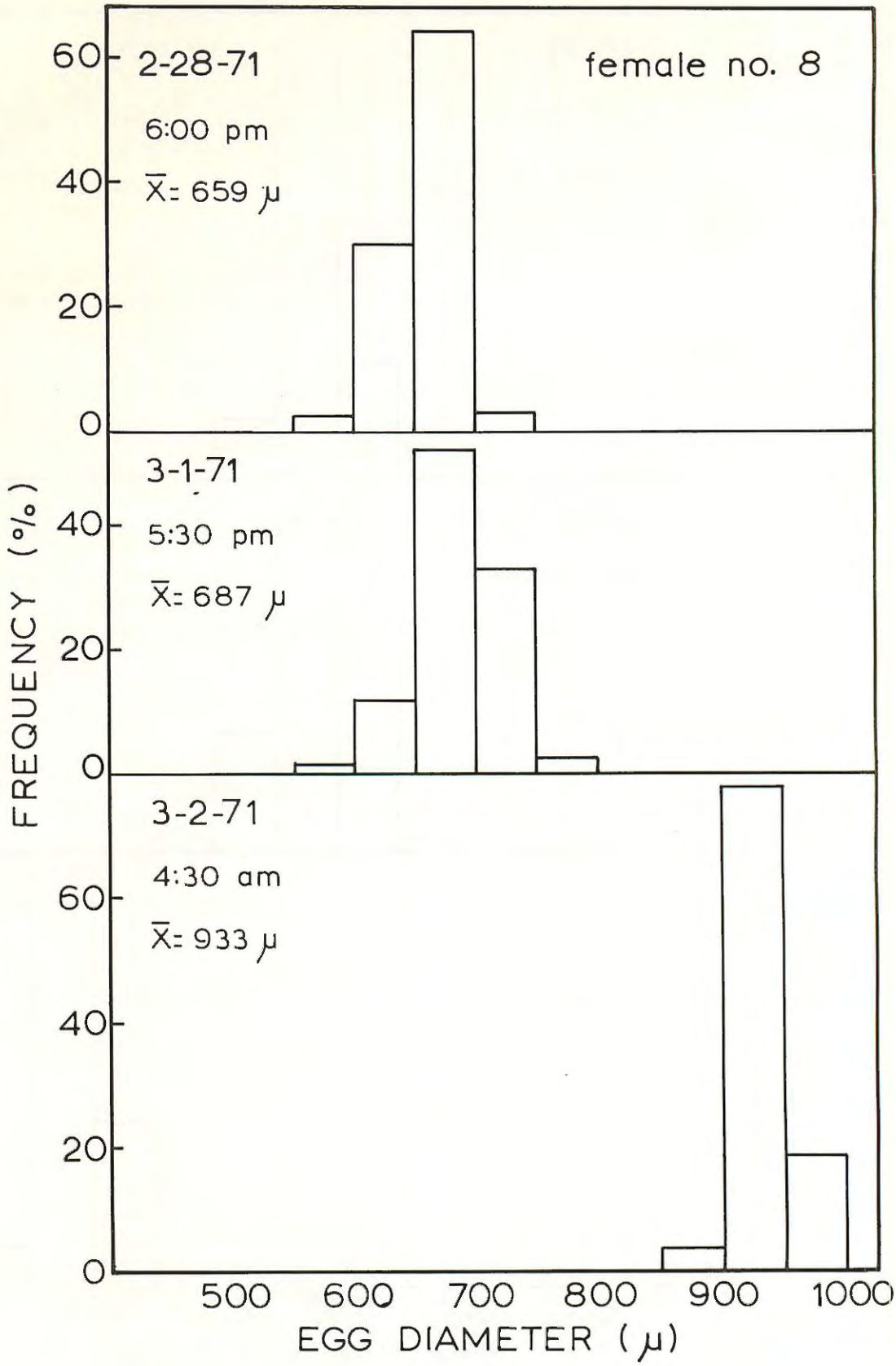

2-8-71
8:00 pm
X̄= 606 μ
female no. 3
2-9-71
8:00 pm
X̄= 602 μ
2-11-71
8:00 am
X̄= 609 μ
2-13-71
10:00 am
X̄= 666 μ
2-13-71
10:20 pm
X̄= 900 μ
FREQUENCY (%)
EGG DIAMETER (μ)
500
600
700
800
900
1000


2-8-71
female no. 4
8:00 pm
X̄= 670 μ
2-9-71
8:00 pm
X̄= 672 μ
2-10-71
8:24 am
X̄=934 μ
FREQUENCY (%)
0
20
40
60
80
500
600
700
800
900
1000
EGG DIAMETER (μ)


2-20-71
female no. 5
10:00 am
X̄= 665 μ
2-21-71
6:00 pm
X̄= 676 μ
2-22-71
6:00 am
X̄= 921 μ
FREQUENCY (%)
0
20
40
60
80
500
600
700
800
900
1000
EGG DIAMETER (μ)


2-21-71
female no. 6
3:30 pm
X̄= 711 μ
2-22-71
10:00 pm
X̄= 714 μ
2-23-71
6:00 am
X̄=940 μ
FREQUENCY (%)
0
20
40
60
80
500
600
700
800
900
1000
EGG DIAMETER (μ)


2-21-71
female no. 7
3:30 pm
X̄= 723 μ
2-22-71
10:00 pm
X̄= 723 μ
2-23-71
12:40 pm
X̄= 915 μ
FREQUENCY (%)
0
20
40
60
500
600
700
800
900
1000
EGG DIAMETER (μ)


female no. 8
2-28-71
6:00 pm
X̄= 659 μ
3-1-71
5:30 pm
X̄= 687 μ
3-2-71
4:30 am
X̄= 933 μ
FREQUENCY (%)
0
20
40
60
500
600
700
800
900
1000
EGG DIAMETER (μ)

During the "latency period", and as each female began to exhibit abdominal distention, the males became more active and tended to remain in close contact with the female (Fig. 3). Occasionally the males would spin around the female or nudge her cloacal region but no specific courtship pattern was noticeable in any of the eight one female-two male sets. When the female eventually dropped her eggs the males were always closely applied to her sides with the caudal fins near the female's cloaca. As egg fertilization commenced the bodies of the males vibrated rapidly and the caudal fins tended to mix the eggs and disperse them in the tank. Sperm was not visible during natural spawning. The complete spawning sequence is shown in Fig. 3. Fertilization rates ranged between 53 and 93% with a mean of 77% (Table 2). Hatching success was estimated to be 75 %.

## DISCUSSION

The efficacy of purified salmon pituitary gonadotropin in the induction of egg maturation, courtship behavior and natural spawning in the grey mullet, Mugil cephalus, in this study confirms similar findings in the goldfish, Carassius auratus (Yamazaki and Donaldson, 1968), the female guppy, Poecilia reticulata (Liley and Donaldson, 1969), and the Indian catfish, Heteropneustes fossilis (Sundararaj et al., 1972).

In the present study comparatively high doses were required to induce spawning even in females with oocyte diameters of 690 $\pm$ 9 $\mu$ . A spawning dose of 11.9 - 15.3 $\mu$g/g body wt was required compared to 0.67 - 1.34 $\mu$g/g body wt in the case of intact Heteropneustes (calculated from data presented by Sundararaj et al., 1972), a 10-15 fold difference. It is unlikely that this difference can be attributed to salmon gonadotropin specificity since Mugil and Heteropneustes are both far removed phylogenetically from Oncorhynchus. It is possible, however, that the higher dose is related to the failure of mullet to spawn in captivity without hormone treatment and the absence of this "block" in the case of Heteropneustes and Carassius. It is not known whether lower gonadotropin dose rates applied at shorter time intervals in mullet would reduce the spawning dose.

The critical egg diameter of 690 $\pm$ 9 $\mu$, which was observed to precede the effective spawning dose in this study, is in close agreement with an earlier report of a critical egg diameter of 750 $\pm$ 3 $\mu$ in the grey mullet, beyond which atresion occurred in the absence of a spawning trigger (Shehadeh et al., 1972). The "priming" effect noted in this study is not clearly understood. This phenomenon was absent in Carassius, Heteropneustes, Aristichthys and Hypophthalmichthys (Yamazaki and Donaldson, 1968; Sundararaj et al., 1972; Sinha, 1971). Doubling of the initial gonadotropin dose did not cancel the "priming" effect and the phenomenon cannot be explained by time-dependent hydration of eggs since no change in egg water content was detected during the 24 hours following the initial injection (see Results section).

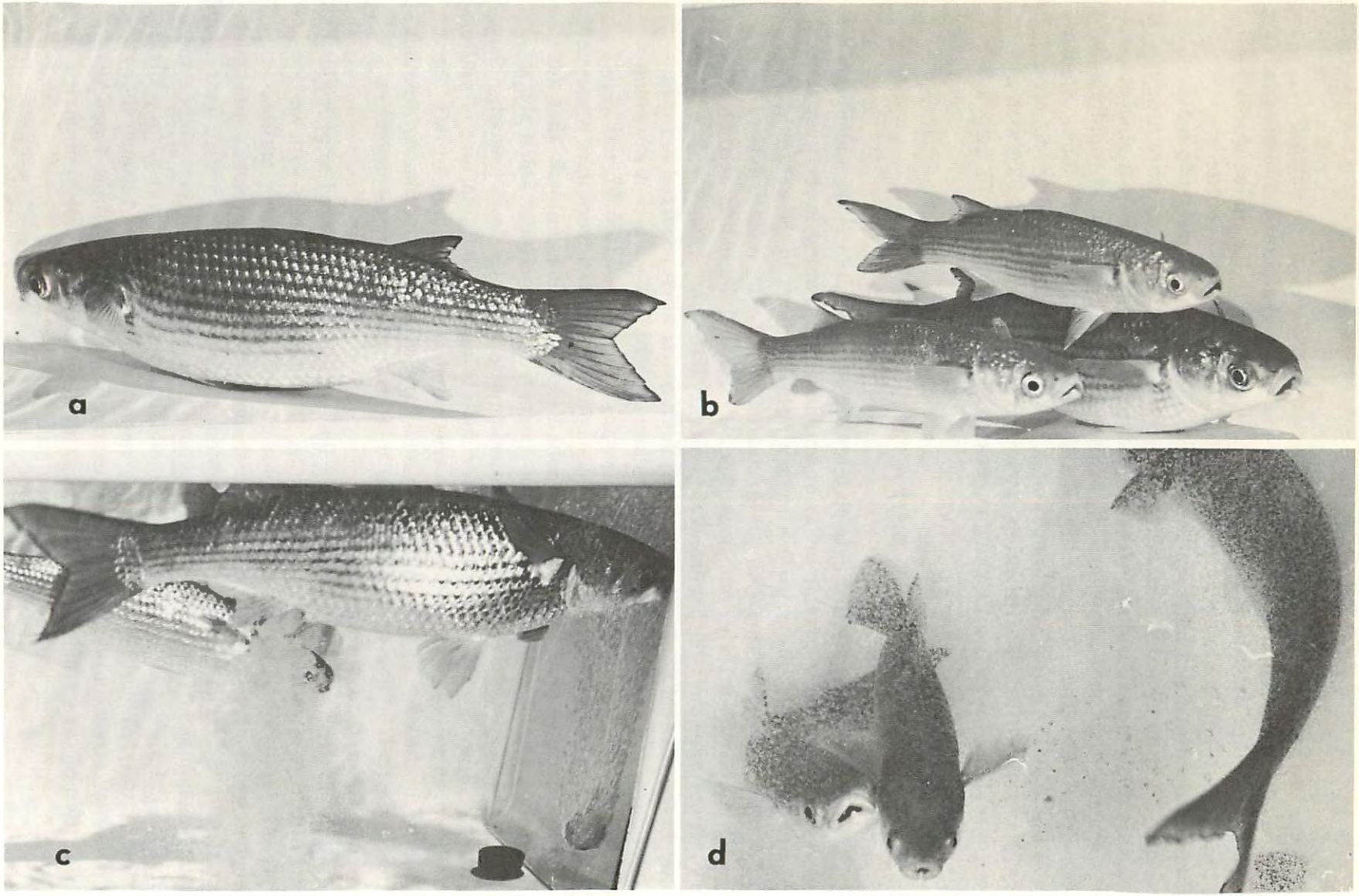



Fig. 3. Mullet spawning sequence. (a) Female mullet a few hours after the "effective" gonadotropin dose. Note distention of abdomen. (b) Males in a typical courtship position with the female. (c) Female mullet in the act of spawning. Male is approaching the egg mass. (d) Two males (left) in the act of fertilization. Note the quick dispersal of the eggs by the action of male caudal fins.

A noteworthy finding is the ability of non-injected milting males to exhibit courtship behavior and fertilize spawned eggs, in perfect coordination with the female, despite the viscous nature of the milt. Lengthy treatment with mammalian androgen or crude pituitary saline extracts was used earlier to induce hydration of such males before their use in artificial fertilization of eggs (Shehadeh and Ellis, 1970; Shehadeh et al., 1972). This is apparently unnecessary. Males seem to be stimulated by the presence of a female undergoing abdominal distention (ovulation). The possible involvement of female pheromones, however, cannot be ruled out.

The results of this study, together with similar results reported for Aristichthys and Hypophthalamichthys (Sinha, 1971), demonstrate the value and practicality of using fractionated teleost pituitary gonadotropin(s) in the induced spawning of important food fishes that do not normally breed in captivity. Sinha (1971) demonstrated that fractions prepared from immature teleosts were equally effective as spawning agents. This removes the limiting factor of collecting pituitaries from gravid fish only and permits a purification "unit" to operate continuously and consequently more economically.

## SUMMARY


1. Fractionated salmon pituitary gonadotropin was found to be a potent spawning agent in Mugil cephalus.
2. The total spawning dose varied inversely with initial mean egg diameters of recipient females and ranged between 11.9 and 20.9 µg/g body wt.
3. The "effective" dose which precipitated spawning was always preceded by a "critical" mean egg diameter of 690 ± 9 µ (mean ± standard deviation).
4. The "latency" period following the "effective" dose ranged between 10 and 15 hours.
5. Females with "critical" egg diameters were spawned with a dose of 11.9-15.3 µg/g body wt applied in two injections, one third of the quantity initially, followed by the remaining two thirds 24 hours later.
6. The initial injection acts as a "priming" dose. This phenomenon cannot be accounted for by changes in mean egg diameters, diameter-frequency distributions or egg hydration.
7. Milting males exhibited courtship behavior and fertilized spawned eggs without gonadotropin injection.
8. Fecundity was estimated to be 648 ± 62 eggs/g body wt.

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VIII.

# A PRELIMINARY REPORT ON THE DEVELOPMENT, GROWTH AND SURVIVAL OF LABORATORY REARED LARVAE OF THE GREY MULLET, Mugil cephalus L.


## Abstract

Larvae from artificially bred grey mullet were reared in the laboratory and survival rates of 0.2%, 5% and 5% were achieved in three of six trials. Food consisted of wild zooplankton and artemia nauplii. Feeding began on the fifth day when the yolk sac was depleted, and intensified on the ninth day. The rate of yolk absorption and feeding intensity were reflected in the growth curve. Larval survival was not affected by withholding food from the larvae till the seventh day from hatching. Two critical periods associated with high larval mortality were apparent on the 2nd-3rd and 8th-11th days after hatching. These were preceded by an increase in specific gravity of larvae followed by passive sinking to the bottom of the rearing tank. Larval length increased from 2.63 mm at hatching to 17.69 mm at the end of the 42-day larval period. The larvae survived on benthic diatoms thereafter. Maximum survival rates were achieved at 22 $^{o}$C.



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## INTRODUCTION

Interest in artificial spawning and rearing of larvae of marine teleosts under laboratory conditions has been intensified during the last decade. Reviews of this field have been presented by Shelbourne (1964) and May (1970).

Information on the controlled rearing of grey mullet (Mugil cephalus L.) larvae is scant (Liao, 1969; Yashouv, 1969; Liao et al., 1970) despite the economic importance of this species in the Pacific, Southeast Asia and the Mediterranean basin. This laboratory has embarked on a long term larval rearing program in association with ongoing work on the artificial breeding of commercially important food fish. The current study is a preliminary report on the laboratory rearing of larvae obtained by artificial breeding of M. cephalus (Shehadeh and Kuo, 1972).

## MATERIALS AND METHODS

### 1. Incubation of Eggs

Fertilized eggs were obtained from laboratory spawned mullet. Females were induced to spawn by means of gonadotropin injection and all eggs were fertilized naturally in the tanks by milting males (for details see Shehadeh and Kuo, 1972). Eggs were incubated in well aerated static sea water (32 $^{o}/_{oo}$) in 140 liter fiberglass tanks. Incubation temperatures were 22 $^{o}$C and 24 $^{o}$C. Egg development, at 24 $^{o}$C and 32 $^{o}/_{oo}$ salinity, was monitored in a plankton chamber under a compound microscope and the various developmental stages timed and photographed.

### 2. Feeding, Growth and Survival

#### a. food uptake

Food uptake and preference tests were carried out during the first 15 days of larval life. A total of 10 larvae were transferred daily to each of four 100 ml beakers containing a dense food mixture consisting of newly hatched artemia nauplii, wild zooplankton and Gymnodinium sp. The type of ingested food and frequency of feeding were determined under the microscope after a feeding period of one hour. All beakers were painted black on the outside to provide contrast and assist the larvae in locating prey. Wild zooplankton used throughout this study was collected from Hawaiian waters by means of a plankton pum and sieved through a 150-$\mu$ screen.

b. larval growth

The relationship of larval growth to yolk sac and oil globule depletion during the first 10 days of larval life was studies in 140-liter tanks at water temperatures of 22 and 24 °C and a salinity of 32 °/oo. A total of 840 larvae was used in each test and a daily sample of 10-30 larvae was removed for yolk sac, oil droplet and total length measurements. Larval length was measured from the tip of the upper jaw to the distal extremity of the caudal fin. The oil globule and yolk sac were measured along the longitudinal axis only. Wild zooplankton (150 μ) was offered as food in both tests beginning two and a half days after hatching. Tanks were cleaned and the water partially exchanged on a daily basis.

Larval growth during the 42-day larval period was monitored at 22 °C and 32 °/oo salinity. A total of 800,000 eggs were incubated in a well aerated, 800-gallon, fiberglass tank, and 500,000 larvae were hatched and used in this study. Feeding and tank maintenance were carried out as described for the preceding tests.

c. relationship of larval survival to temperature and initial feeding

Survival during the 42-day larval period was studied at 19, 22, and 24 °C. The effect of delayed initial feeding on larval survival was determined by running duplicate tests at 22 and 24 °C. Initial feeding was carried out 2-3 days after hatching in one set at each temperature and 7-8 days after hatching in the second set. The study was carried out in 12-liter, circular plexiglass tanks and zooplankton (150 μ) used as food in all experiments. The total number of larvae used in each test is indicated in Figs. 5 and 6. (see Results and Discussion section). Larval mortality was checked twice daily and the dead larvae removed by pipette and counted. All tanks were cleaned daily.

3. Vertical Distribution and Specific Gravity of Larvae

Observations on vertical distribution of larvae during the first 10 days of larval life was carried out in an 80-cm graduated glass cylinder. A total of 30-100 larvae were transferred into the cylinder and allowed to distribute themselves over a period of one hour after which vertical distribution patterns were recorded. Specific gravity of larvae was determined in a density gradient column, made up with 15% calcium nitrate in distilled water, with a specific gravity range of 1.005 to 1.047 g/cc. A daily total of 30 live larvae was introduced into the column and larval specific gravity determined at the neutral buoyancy level.

## RESULTS AND DISCUSSION

### 1. Embryonic Development

The mean egg diameter of fertilized eggs was 930 μ with a range of 880-980 μ. A single large oil globule was present with a uniform diameter of 330 μ. The perivitelline space and first meroblastic cleavage occurred 15 minutes and 50-65 minutes after fertilization respectively. A summary of embryonic development till hatching at 24 °C is presented in Table 1 and Fig. 1.

Hatching was evident 36-38 hours after fertilization at 24 °C and 48-50 hours at 22 °C. Total length of newly hatched larvae was 2.65 ± 0.23 mm (mean ± standard deviation) compared to 2.08-3.40 mm reported by Liao et al. (1971). These data are in agreement with previous reports of hatching time; 34-38 hours at 23-24.5 °C and 49-54 hours at 22.5-23.7 °C in salinities of 30.1-33.8 °/₀₀ (Liao et al., 1970). Hatching time was prolonged further at lower temperatures: 60-65 hours at 21 °C (Liao, 1969) and 60 hours at 18.2-19.5 °C (Yang and Kim, 1962).

Sanzo (1936) reported that all fertilized eggs sank to the bottom of the incubation vessel within the first hour while Yashouv (1969) noted that fertilized eggs floated initially, then began to sink as the embryo took form (22 hours) and later came to rest on the bottom. In the present study, in aerated sea water (32 °/₀₀ , the majority of the eggs collected from the bottom of the incubation tank were found to be either unfertilized (absence of perivitelline space) or undeveloped. When eggs were prevented from settling by strong aeration in small incubation vessels (5-gallon circular tanks) higher hatching rates of 90-95% were obtained and time to hatching reduced by two hours at the same temperature. It is apparent that water temperature as well as water turbulence have a decided effect on incubation time.

Yashouv and Berner-Samsonov (1970) reported that eggs of Mugil cephalus and M. capito have one or more oil globules and that both types of eggs developed normally and hatched. The multiple oil globules merged into one on hatching. The presence of multiple oil globules was also noted in M. saliens (Perceva-Ostroumova, 1951) and M. chelo (Sanzo, 1936). In the present study a single oil globule was present in all naturally spawned M. cephalus eggs. Multiple oil globules were obtained only when eggs were removed from gravid females by manual pressure. The frequency of multiple oil globules increased when manual extrusion of eggs was carried out at earlier time intervals. The immature oocyte usually contains a great number of oil globules which gradually coalesce and decrease in number during the process of maturation. The presence of multiple oil globules

Table 1. Embryonic development at 24 °C.

| Hour | Minutes | Stage | Description |
|---|---|---|---|
| | 50 to | 2-cell | First cleavage meridional near center |
| 1 | 05 | | of germ disc |
| 1 | 05 to | 4-cell | Second cleavage at right angles to first |
| 1 | 10 | | |
| 1 | 25 to | 8-cell | Cleavage at right angles to first division |
| 1 | 30 | | and parallel to second |
| 1 | 35 | 16-cell | 16 blastomeres in a single plane; cleavage parallel to first division |
| 1 | 50 | 32-cell | Arrangement of blastomeres irregular |
| 2 | 25 | 64-cell | Blastomeres reduced in size as division |
| 2 | 40 | 128-cell | continues |
| 3 | 25 | | Blastodisc well formed and berrylike in appearance |
| 5 | 15 to | Blastula | Blastulation in progress |
| 5 | 40 | | |
| 7 | 15 to | | Blastocoele, germ ring and embryonic |
| 7 | 35 | | shield appear |
| 11 | 55 | Gastrula | Head fold extending to vegetal pole, embryonic shield expanded; invagination of blastomeres complete 12 h 45 min after fertilization |
| 13 | 30 | Neurula | Neural groove appears |
| 14 | 30 | | Optic vesicles apparent, tail expanded |
| 15 | 30 | | Somatic segmentation begins, optic vesicles begin to differentiate |

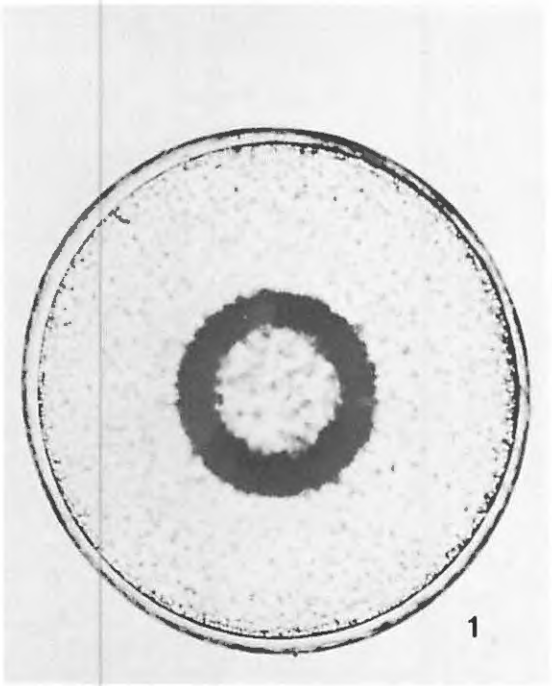
(continued)

(Table 1 - continued)

| | | | |
|---|---|---|---|
| 20<br>21 | 55 to<br>25 | | Differentiation of olfactory lobes begins; melanophores appear in oil globule and dorsal aspect of embryo; tail tapered and bent ventrally |
| 25<br>25 | 00 to<br>10 | | Optic vesicles and olfactory lobes well developed; finfold appears; embryo elongated with tail near head; heart begins to beat |
| 27 | 00 | | Tail free of yolk sac and body movement begins |
| 30<br>31 | 30 to<br>05 | | Tip of tail reaches the head; body movement more frequent and embryo position changing in the egg |
| 35 | 40 | | Wave-like body movement occurring regularly every 3-5 seconds; tail movements very frequent |
| 36<br>38 | 00 to<br>00 | Free larva | Larva breaks egg membrane with tail movement; head emerges first |

Fig. 1. Development series.

(1) fertilized egg (75X)
(2) 2-cell stage (75X)
(3) 4-cell stage (75X)
(4) 8-cell stage (72X)
(5) 16-stell stage (70X)
(6) 32-cell stage (71X)
(7) 64-cell stage (73X)
(8) 128-cell stage (75X)
(9) pre-blastula stage (71X)
(10) blastula (71X)
(11-13) gastrula (74-75X)
(14-15) neurula (72X)
(16-24) developing embryo (67-75X)
(25) hatching (40X)
(26) newly hatched larva (45X)
(27) newly hatched larva - ventral view (45X)
(28) newly hatched larva - lateral view (45X)
(29) 2-day-old larva (59X)
(30) 4-day-old larva (52X)
(31) 8-day-old larva (57X)
(32) 42-day-old juvenile (6.3X)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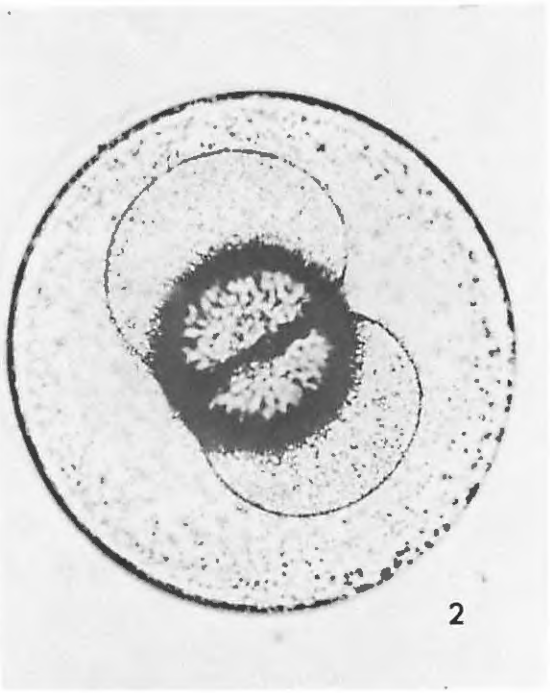
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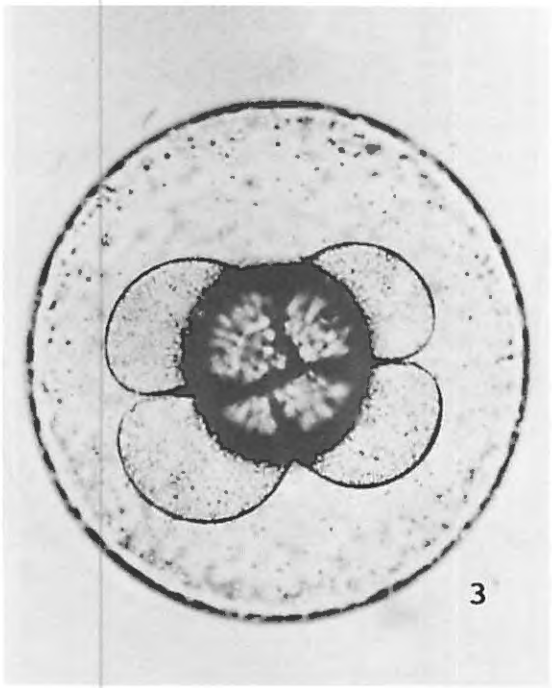
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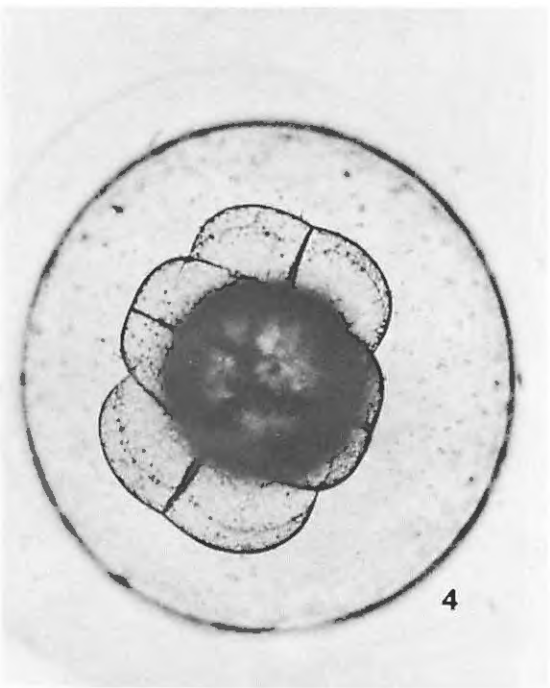
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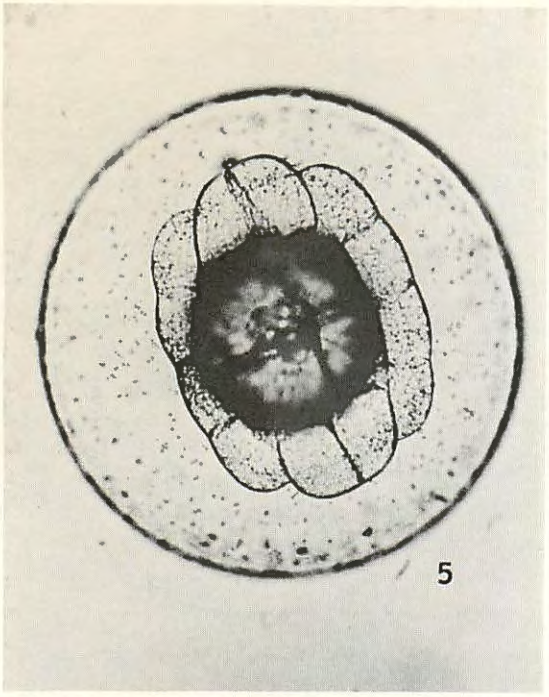
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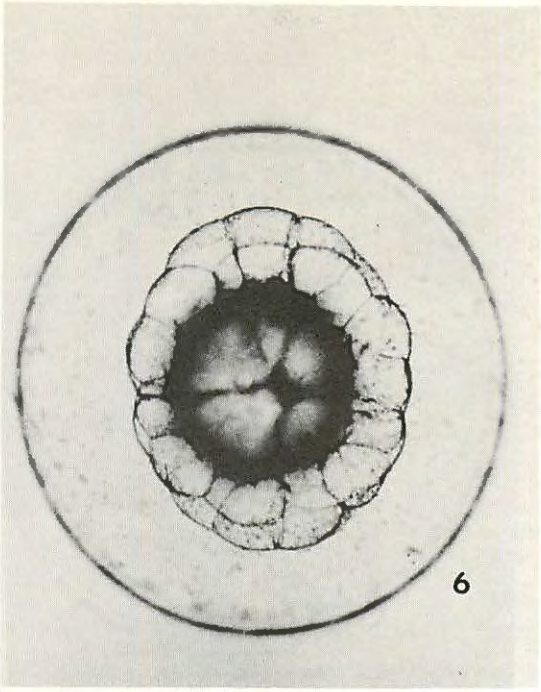
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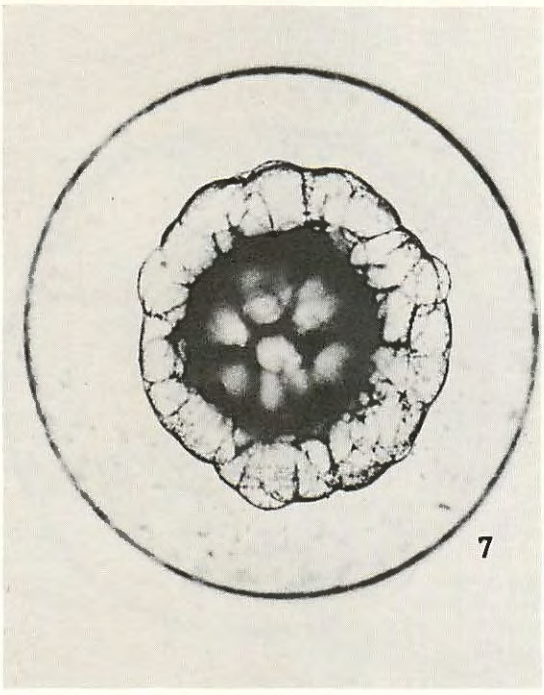
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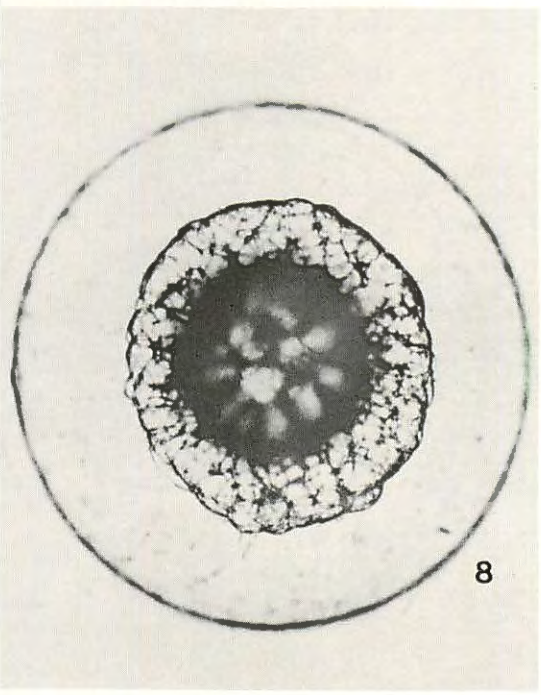
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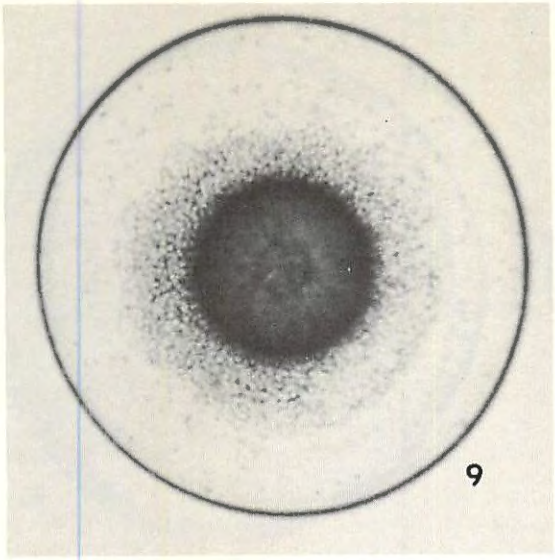
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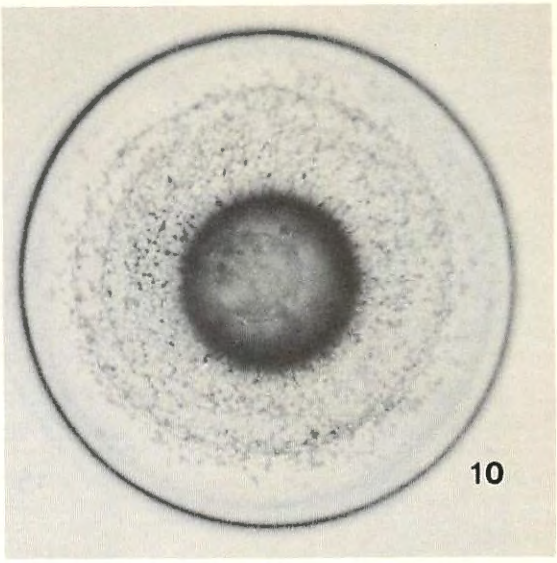
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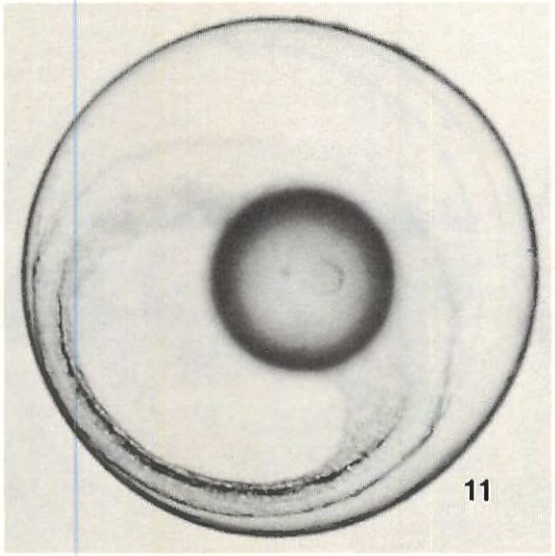
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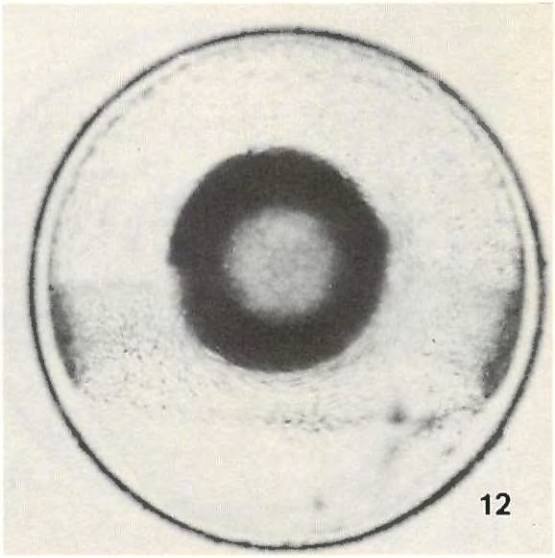
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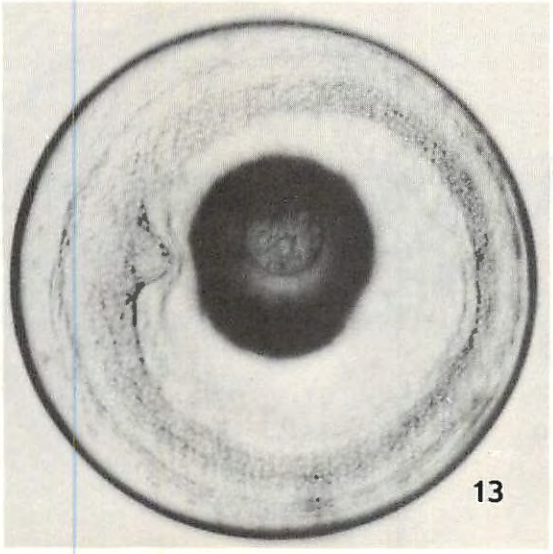
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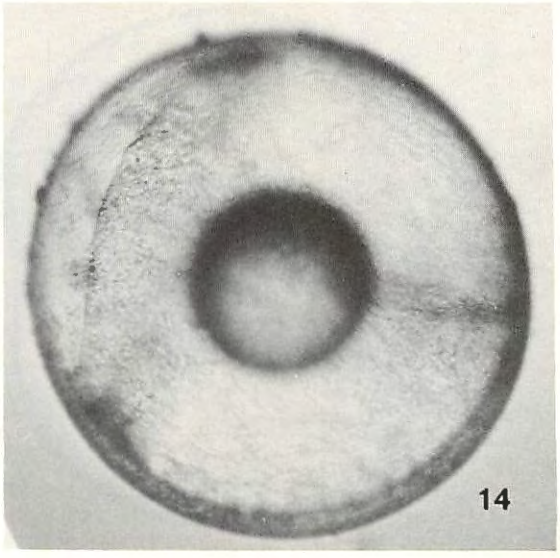
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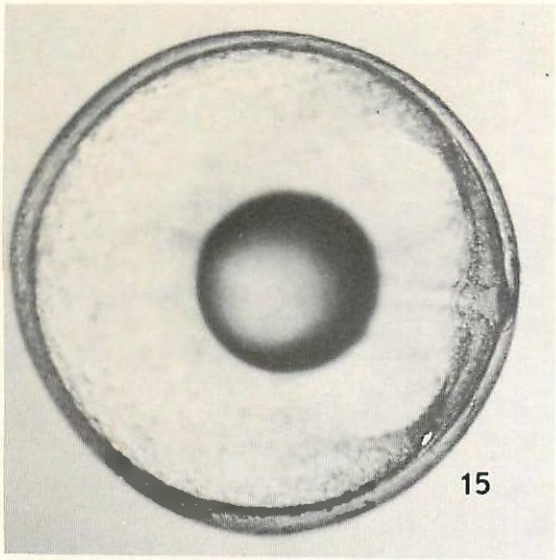
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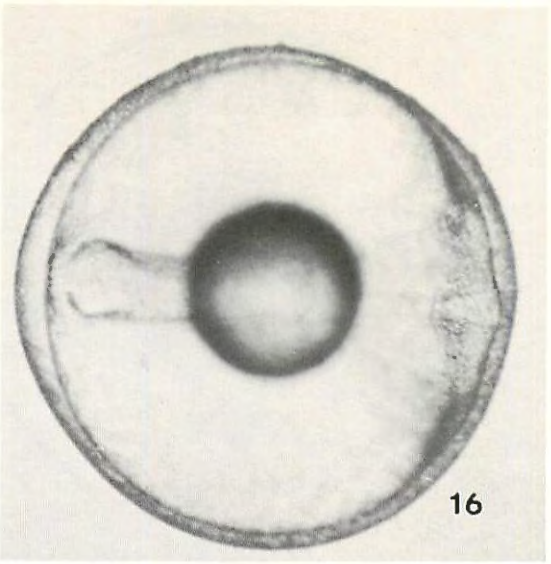
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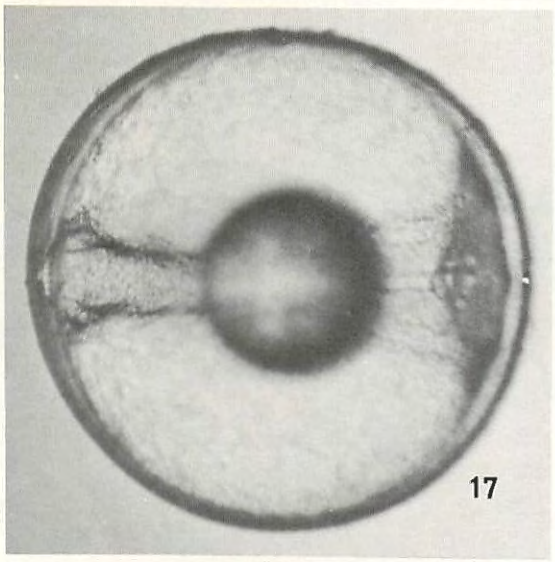
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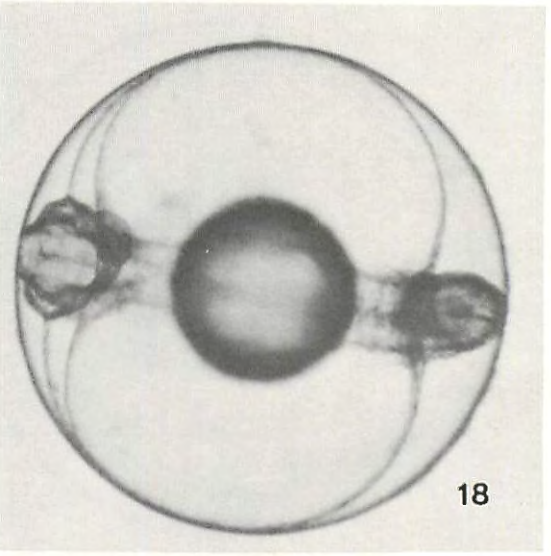
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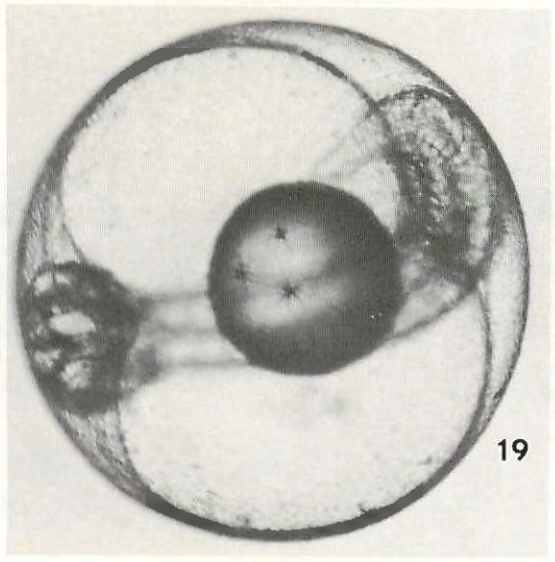
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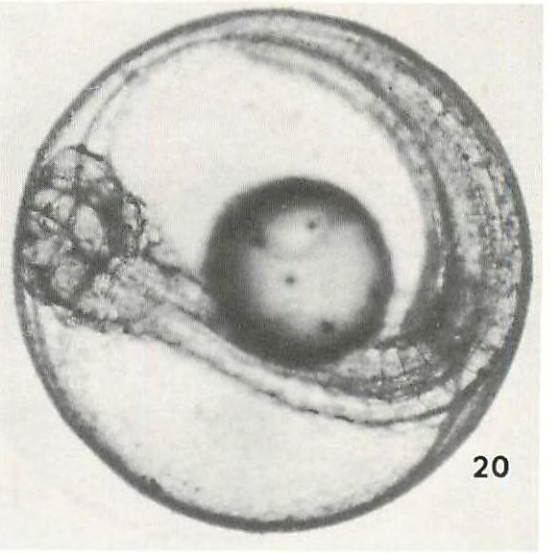
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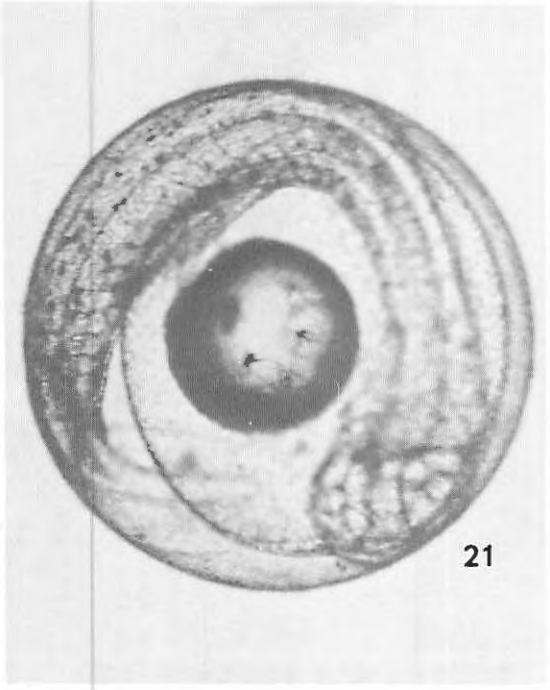

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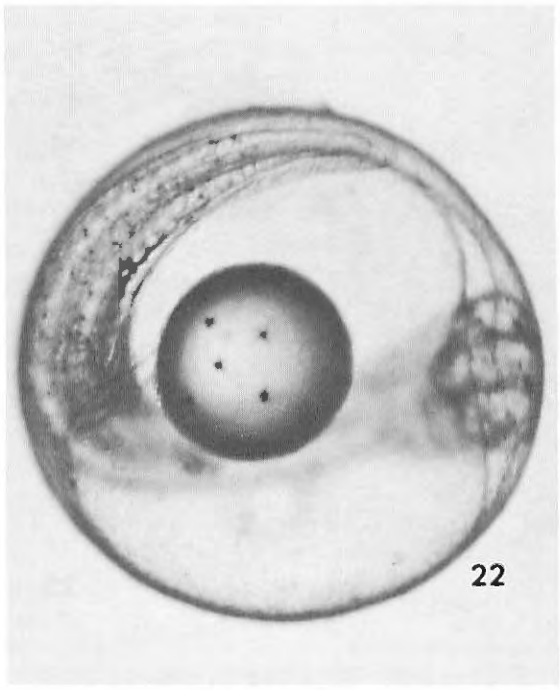

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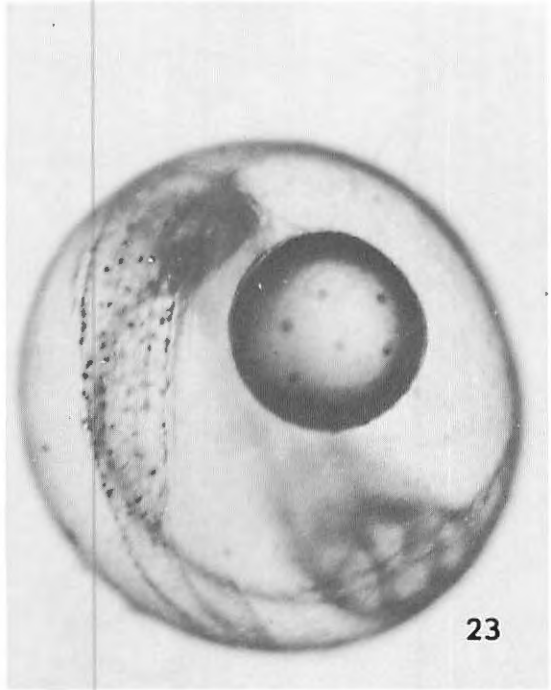

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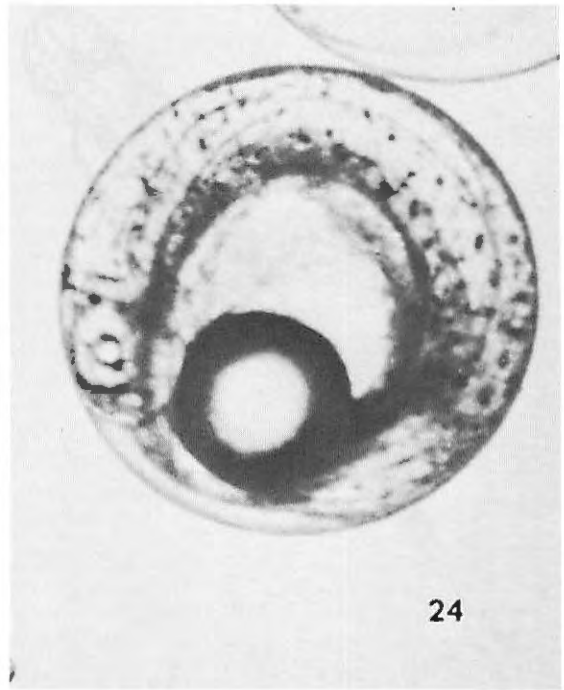

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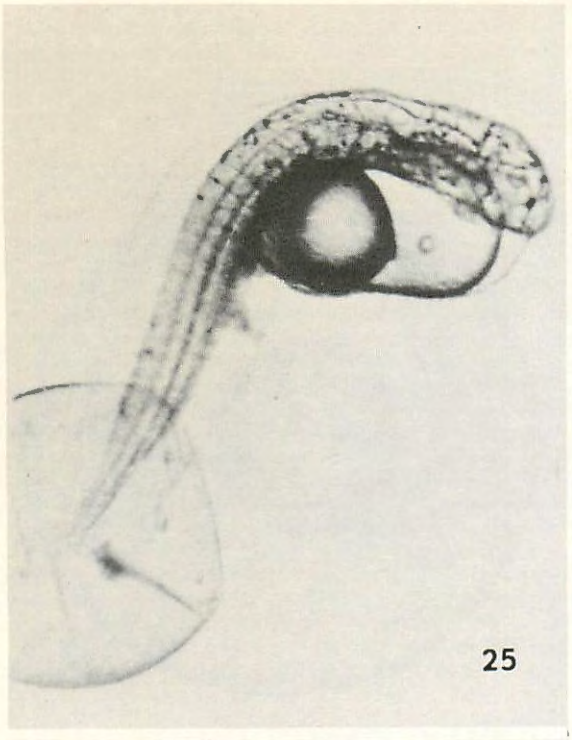

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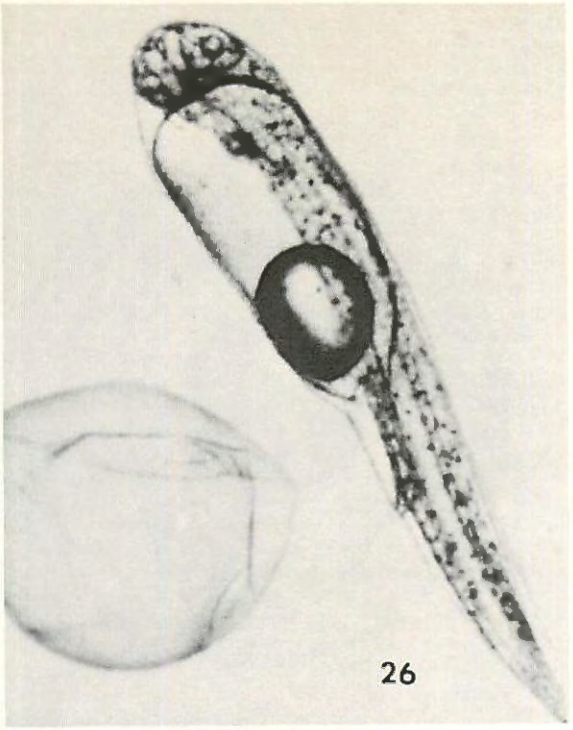

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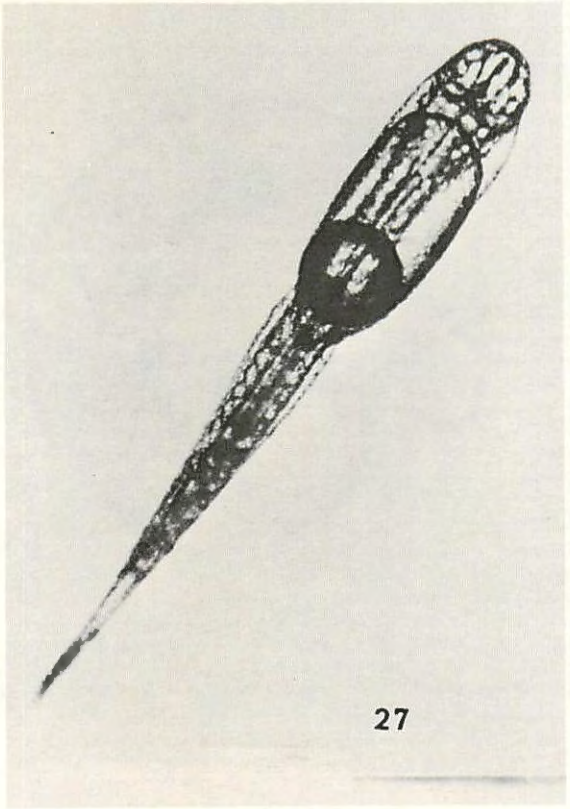

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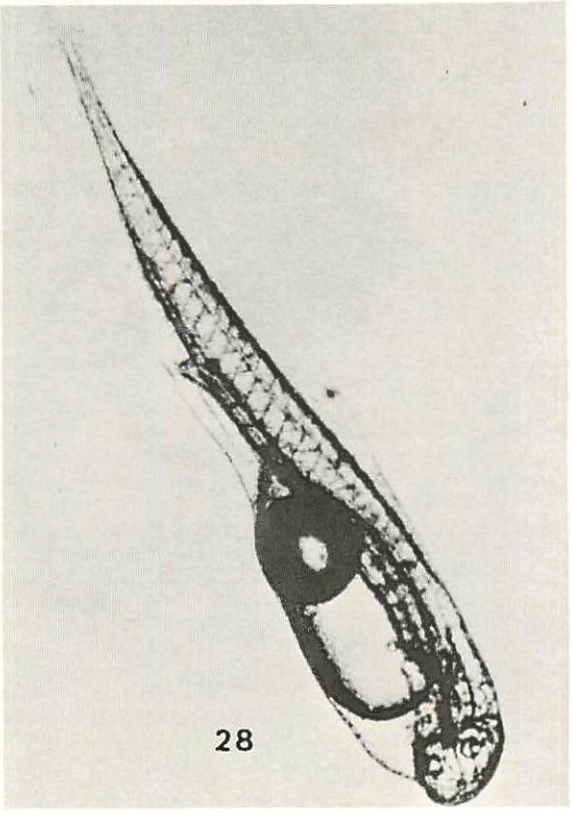

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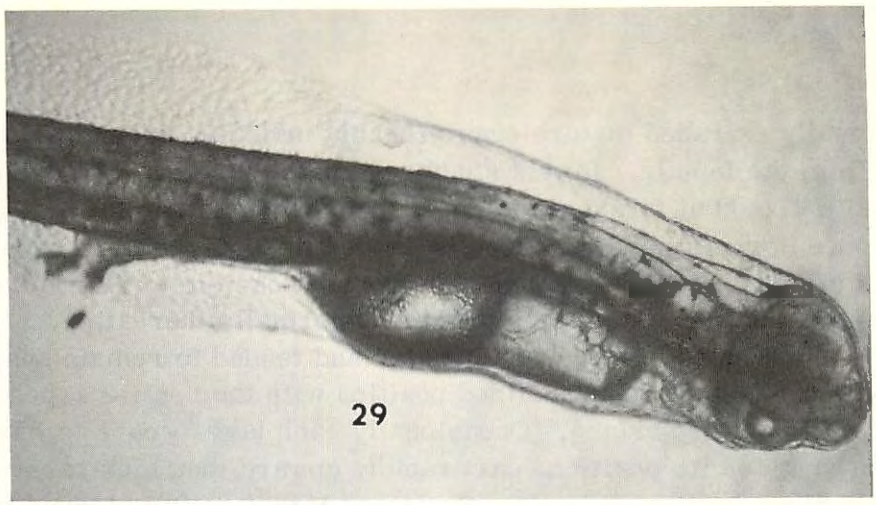

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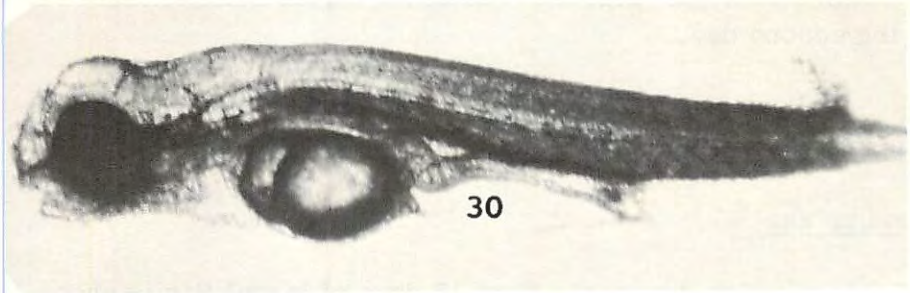

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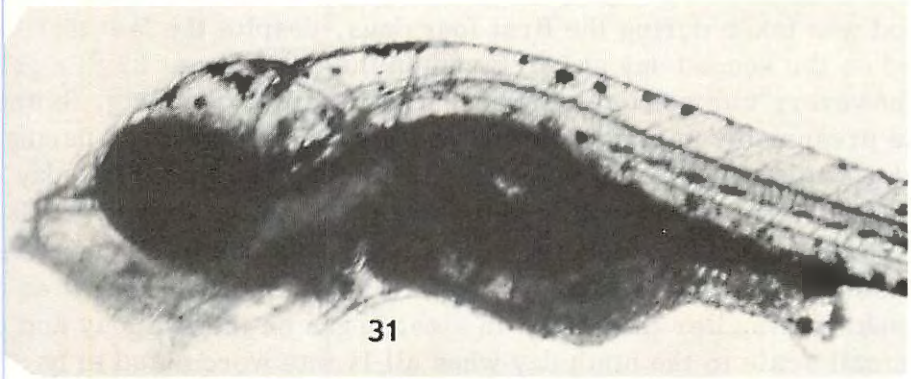

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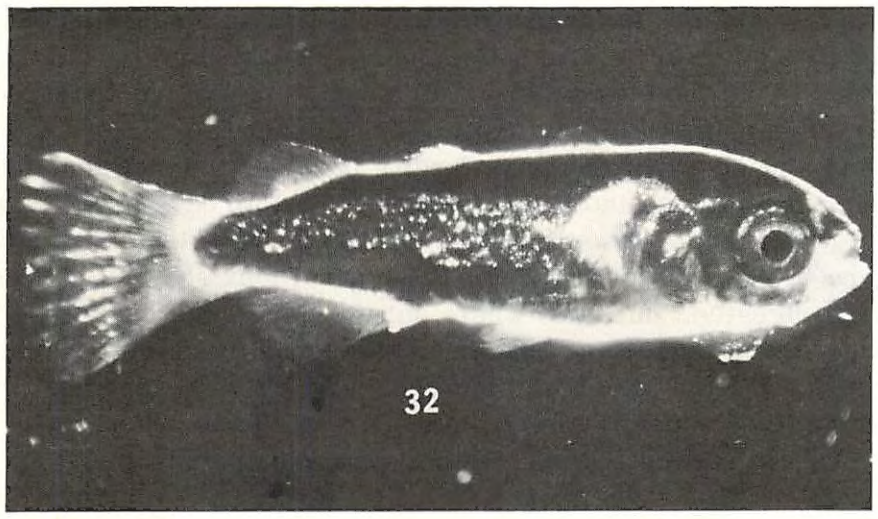

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in manually extruded mature eggs probably indicates premature removal of eggs from the female. In this study eggs with more than four oil globules were fertilized but failed to divide. Eggs with less than four oil globules developed normally and the globules tended to coalesce during the latter stages of embyonic development. It was not possible to determine whether larvae hatched from such eggs developed normally thereafter.

Newly hatched larvae were inactive and tended to remain suspended in the water column in an inclined position with the ventral aspect oriented towards the water surface. Occasionally each larva would go into jerky motion, righten its position, dart rapidly upward then sink passively back to its resting position (Fig. 2). Sustained larval activity tended to increase after the second day.

## 2. Feeding, Growth and Survival

### a. food uptake

Food ingestion during the first 15 days of larval life is shown in Table 2. No food was taken during the first four days, despite the fact that the mouth opened on the second day at 24 °C and on the third day at 22 °C. The yolk sac, however, was not totally absorbed till the fifth day (Fig. 3) and the larvae presumably survived on this endogenous food reserve during the first four days of larval life. Liao et al. (1971) reported that three-day old larvae fed when offered oyster veligers (<70 μ) on the third day. It is possible that the lack of feeding in this study was due to the absence of food organisms of the proper size during the first four days. Ingestion of wild zooplankton, smaller than 150 μ in size, began on the fifth day and continued on a small scale to the ninth day when all larvae were noted to have substantial numbers in the gastrointestinal tract. Moderate uptake of artemia nauplii took place at this time also, in 24 % of the larvae, and increased dramatically by the eleventh day. Gymnodinium was not observed in any of the larvae during the 15-day test.

It is apparent that intensive feeding began on the ninth day from hatching and over-feeding was indicated during the 12-15th day period. The larvae did not cease feeding even after the entire digestive tract was full. Examination of excreted food organisms under these conditions showed the food to be only partially digested. In cases where unhatched artemia eggs were inadvertently introduced into the feeding beakers it was found that larvae which ingested the eggs suffered from intestinal blockage. Larvae with this condition continued to feed and subsequently died from rupture of the abdomen. It is critical therefore that adequate measures be taken to achieve complete separation of

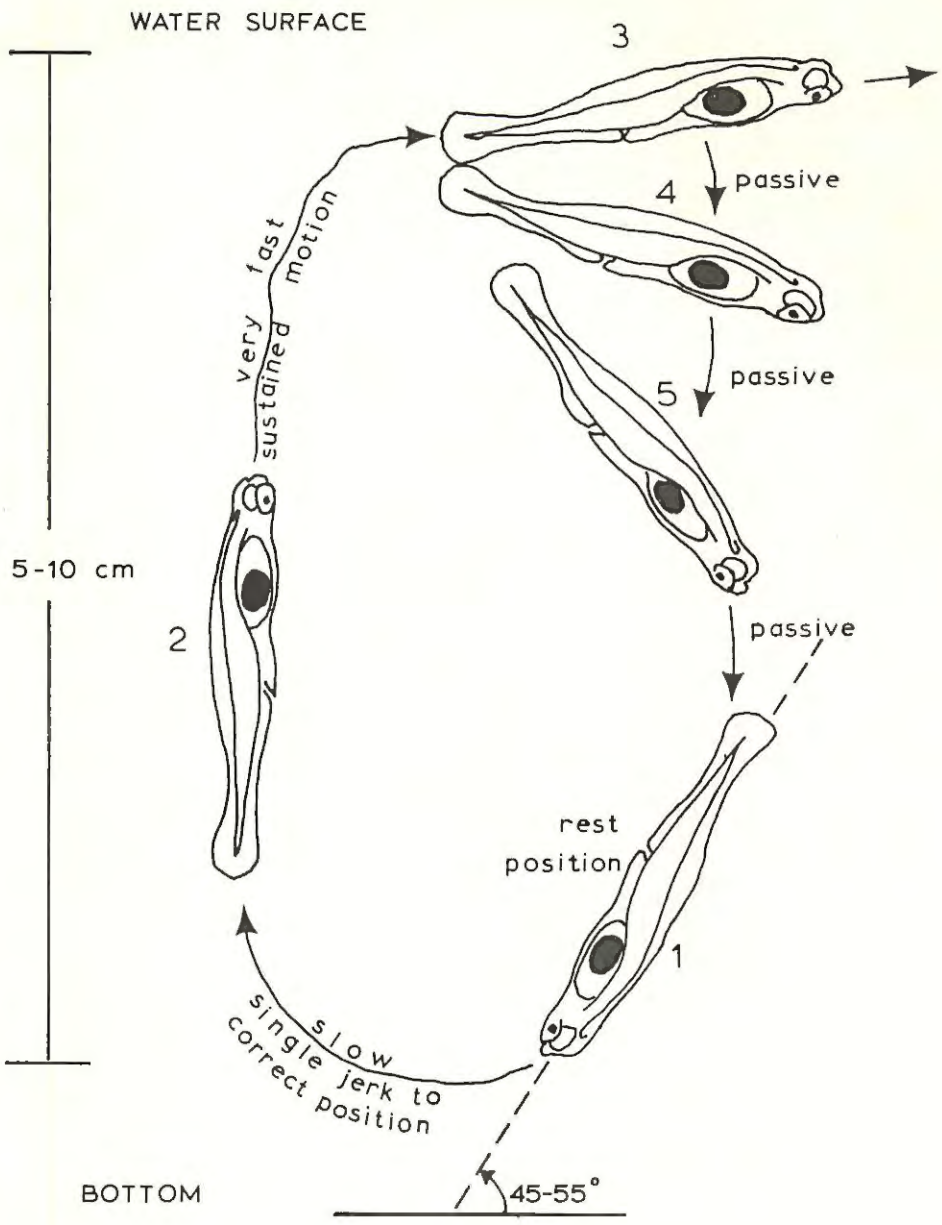


Fig. 2. Resting position and swimming movements of newly hatched larva.

Table 2. Ingestion of life food during the first 15 days of larval life. Numbers in parentheses indicate percent of larvae with the indicated food item.

| Days after hatching | Number of ingested | | |
|---|---|---|---|
| | zooplankton | artemia nauplii | Gymnodinium |
| 1-4 | none | none | none |
| 5-8 | 1-2 (20%) | none | none |
| 9-11 | 3-15 (100%) | 2 (24%) | none |
| 12-15 | 4-14 (100%) | 9-36 (100%) | none |

artemia eggs from hatched nauplii before the latter are offered as food. Equally important is the need to control food density and feeding rates to insure complete digestion of ingested food.

b. larval growth

The relationship of larval growth to yolk sac and oil globule depletion at 22 and 24 °C during the initial 10 days of larval life is shown in Fig. 3. This specific period of larval development was chosen for study, as in the case of the preceding food preference test, because unpublished observations in this laboratory as well as previous reports (Liao et al., 1970) showed that over 90% of larval mortality occurred during this period.

Zooplankton was used in the growth studies to eliminate complications and variables arising from possible non-acceptability of the offered food. Wild zooplankton has proven to be one of the most acceptable foods for larval rearing in the laboratory (see reviews by May, 1970 and 1971). Feeding was commenced two and a half days after hatching to insure the presence of food when the mouth opens and in order to avoid mortality from irreversible starvation (Lasker et al., 1970).

Rapid yolk absorption occurred during the first day and was reflected in the initial steep growth phase at 22 and 24 °C. Yolk absorption was gradual during the following three days with a final steep slope during the fourth day. No significant growth, as reflected by length measurements, was noticeable during this period. A gradual increase in growth began on the seventh day and became prominent on the ninth day at both temperatures. This second growth period coincided with the onset of larval feeding (5-8th day) and intensification of feeding (9-12th day) reported in the previous study (Table 2). These findings are in agreement with earlier reports (Liao et al., 1971). No difference in growth rates was observed between larvae reared at 22 °C and those reared at 24 °C.

Oil absorption was gradual in both larval groups. Absorption of the oil globule was completed on the 10th day at 24 °C and on the 15th day at 22 °C. The reason for the presence of a temperature effect in oil absorption and its absence in yolk absorption is not clear.

Larval growth during the initial 42 days after hatching is plotted in Fig. 4. The data corroborates findings during the first 10 days of larval life (see above). Larval length increased from 2.65 $\pm$ 0.23 mm to 3.36 $\pm$ 0.03 mm during the first day. No obvious growth increment was evident from the second day through the ninth day. Mean larval length at the end of this plateau period was 3.44 mm. Rapid growth, also an increase in length variation, was apparent as the larvae passed the ninth day (beginning of intensive feeding).

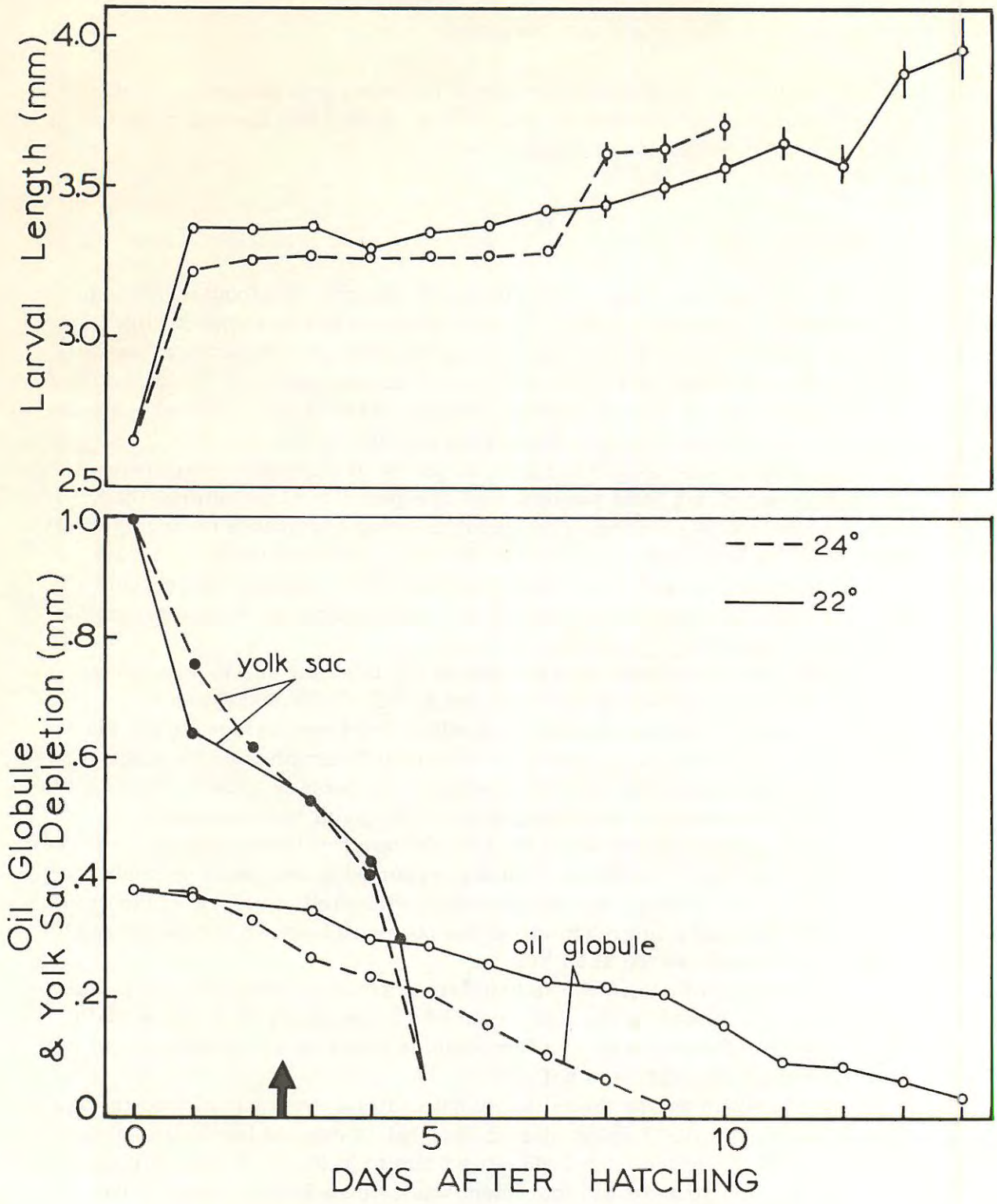


Fig. 3. The relationship between yolk sac and oil globule depletion and larval growth at 22 and 24 °C. Arrow indicates time of mouth opening. Yolk and oil globule measurements indicate length and diameter respectively.

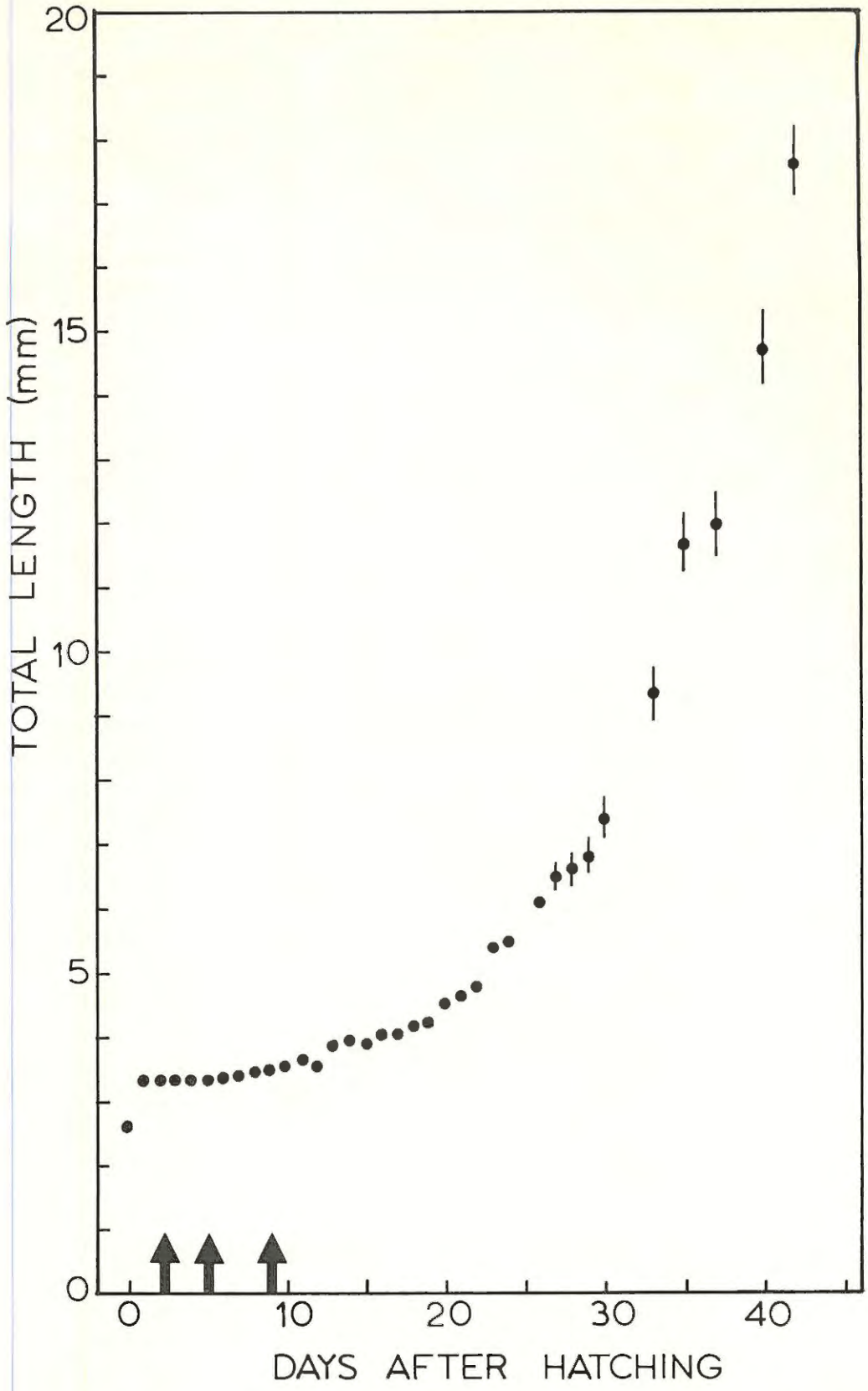


Fig. 4. Growth curve during the 42-day larval period. First arrow indicates opening of mouth, second arrow indicates yolk sac depletion, third arrow indicates onset of intensive feeding.

Two "critical" periods involving very high mortalities were clearly indicated. The first coincided with the opening of the mouth (2nd-3rd day) and the second preceded and overlapped the second growth phase (8th-12th day). The two critical periods accounted for 25% and 75% of total mortalities during the 42-day larval period respectively. Larvae assumed a fish-like shape during the 10th-12th day and were black in color. Silvering began in the abdominal area, spread dorsally and was completed on the 24th day. The larvae were fully scaled and began to feed on benthic diatoms on the 42nd day. Mean length at this time was 17.69 mm. A total of 1000 larvae (0.2%) survived the larval period.

Liao et al. (1971) reported two similar critical periods for M. cephalus larvae on the third day and 11th-13th day respectively. In this case total mortality during the critical periods was 98.5%, with final survival rate of 0.31%. Survivors measured 27.00 mm in length on the 42nd day.

c. relationship of larval survival to temperature and initial feeding

Larval survival and daily mortalities at 19, 22, and 24 °C when initial feeding was carried out on the second and the seventh days from hatching are shown in Figs. 5 and 6. Larval mortality was lowest during the first day; 1.38% at 19 °C, 0.27% at 22 °C and 4% at 24 °C. High mortalities occurred twice during the first 12 days and the timing agreed with the critical periods noted above. The first high mortality took place on the third day and accounted for 22.6%, 7.5% and 35.2% of the larvae at 19, 22 and 24 °C respectively. A second major mortality of 13.5% (19 °C), 15.0% (22 °C) and 32.8% (24 °C) occurred between the eighth and 12th days. All larvae died by the end of the ninth day and the 11th day at 19 ° and 24 °C respectively. Larval survival on the 12th day at 22 °C was 24.4%. Terminal survival at 30 days was 5.0%.

Larval survival was not related to time of initial feeding but an optimal rearing temperature of 22 °C is indicated.

It was noted that the two critical periods were always preceded by the larvae sinking to the bottom of the rearing tank. A study of vertical distribution of larvae relative to changes in specific gravity was therefore carried out.

3. Vertical Distribution and Specific Gravity of Larvae

Larvae gained sustained swimming powers between the 10th and 12th days from hatching. Prior to this time larval movement consisted of short "jerks" or a series of jerks followed by passive sinking when directed motion stopped.

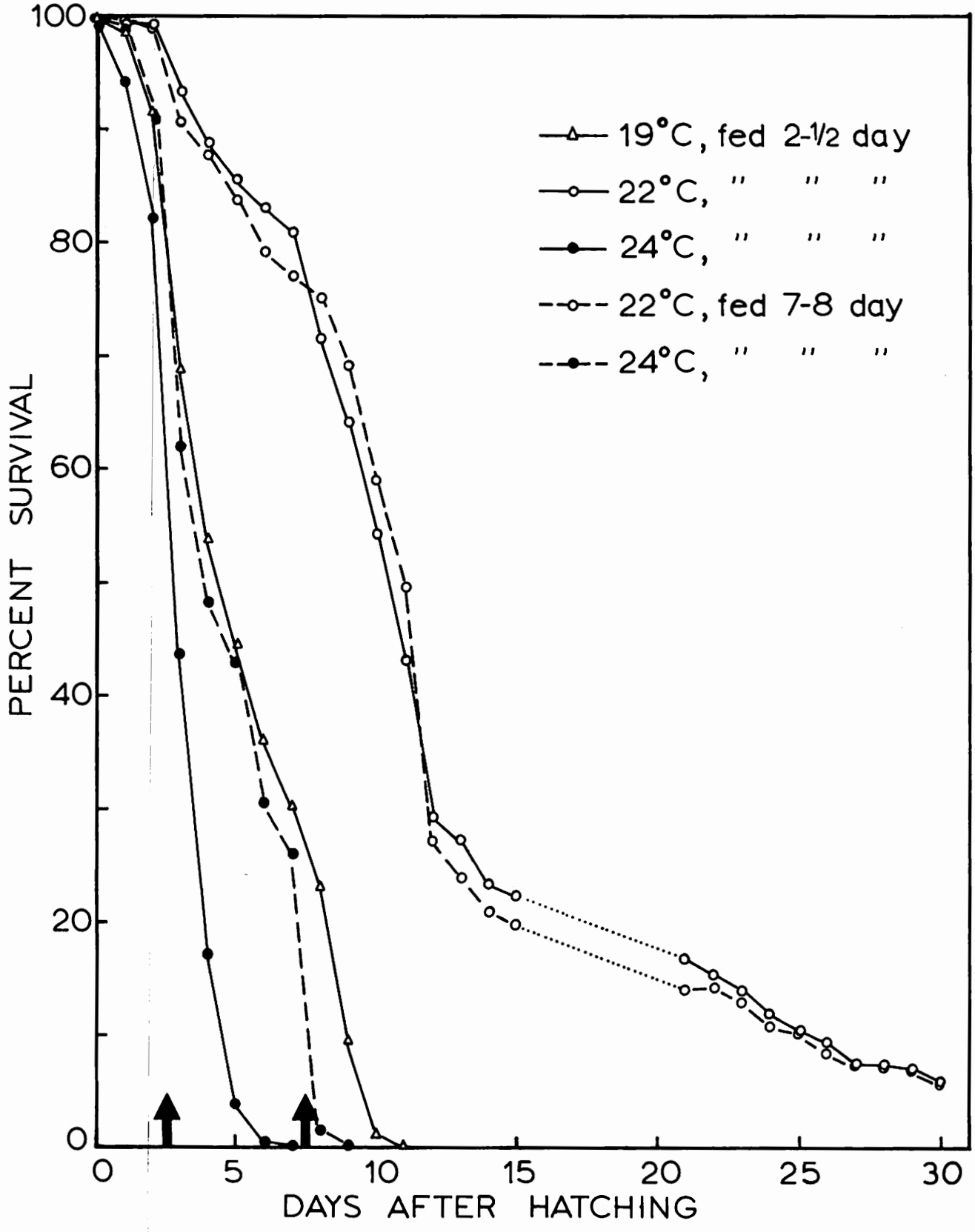


Fig. 5. Larval survival in relation to water temperature and initial feeding time.

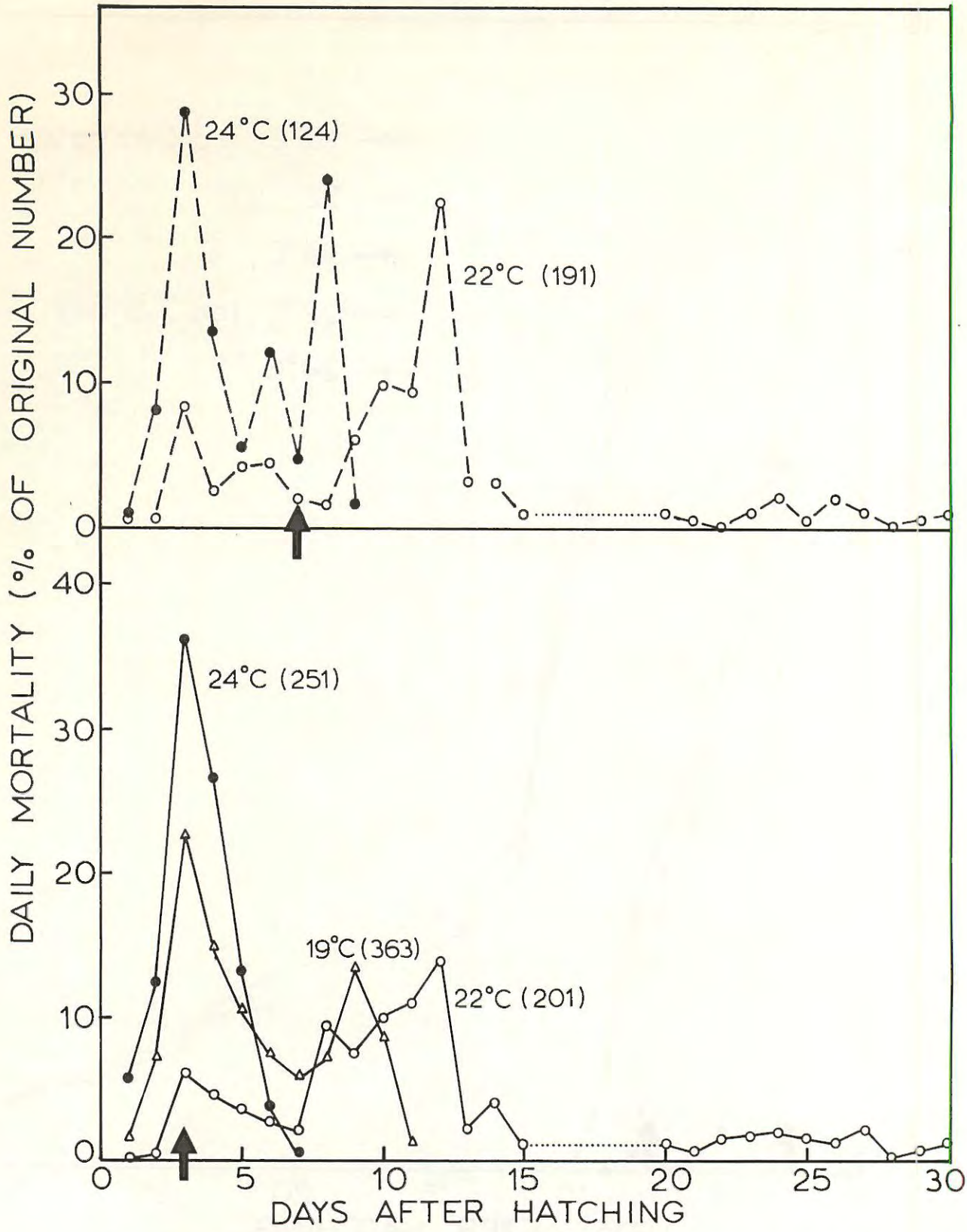


Fig. 6. Daily larval mortality in relation to water temperature and initial feeding time. Arrows indicate time of initial feeding. Numbers in parentheses denote total number of larvae used in each test.

Vertical distribution was consequently documented during the initial 10 days of larval life since changes in larval specific gravity during this period would have a significant effect on their vertical distribution.

Vertical distribution and specific gravity are presented in Table 3. During the first two days the larvae were passively suspended in the water column and tended to become evenly distributed in depth as their specific gravity increased from 1.0263 g/cc twelve hours after hatching to 1.0339 g/cc one and a half days later. One of two transition periods took place between the second and third days. At the end of two and a half days 87.5% of the larvae came to rest on the bottom of the 80-cm column with the balance suspended in the lower 50 cm of the water column. During the third day this pattern was reversed with 85.5% of the larvae aggregated at the surface. During this transition larval specific gravity decreased from 1.031 g/cc at two and a half days to 1.0264 g/cc on the fourth day when 100% of the larvae were at the surface. The larvae remained at the surface till the sixth day. A second transition period took place between the sixth and seventh day as was accompanied by a similar increase in the specific gravity of the larvae on the seventh day. All the larvae were on the bottom on the eighth day with a complete reversal on the ninth day.

The first sinking is probably related to the rapid absorption of the yolk sac and the resulting change in specific gravity. Since the larvae are planktonic at this time they would be expected to sink under these circumstances. The second descent is not as easily explained. Although the larvae were not capable of sustained swimming at this time they were capable of swimming to the surface of the column. Furthermore it is not possible to account for the sudden and transient increase in specific gravity of the larvae on the seventh day. Morphological observation of larval histological sections revealed that the pneumatic duct of the air bladder was occluded between the sixth and seventh days. Whether this has any bearing on larval density is not known at this time. The first and second larval descent occurred irrespective of light or dark conditions.

The importance of this phenomenon is clearly indicated by its relationship to the critical periods described above (Fig. 6). It is noteworthy that the mortalities which followed the second sinking were always greater than those associated with the first descent. The reasons for these high mortalities are not apparent but could possibly be attributed to mechanical damage brought about by prolonged contact with a solid surface (tank bottom). This is supported by our observation (unpublished data) of much lower mortalities when deeper (1.5 m) rearing tanks were used.

Table 3. Vertical distribution and specific gravity of mullet larvae during the initial ten days of the larval period.

| Depth (cm) | Day | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1/2 | 1 | 2 | 2-1/2 | 3 | 4 | 5 | 6 | 7 | 7-1/2 | 8 | 8-1/2 | 9 | 10 |
| surface | | | | | 85.5* | 100.0 | 98.1 | 100.0 | 90.1 | 13.3 | | 30.2 | 90.0 | 100.0 |
| 1-10 | 20.6 | 27.3 | 19.3 | | 9.6 | | 1.9 | | 7.1 | 31.1 | | 27.9 | 10.0 | |
| 11-20 | 5.8 | 24.2 | 16.2 | | 1.2 | | | | 2.8 | 22.3 | | 2.3 | | |
| 21-30 | 29.5 | 12.2 | 13.0 | | 1.2 | | | | | 11.1 | | 4.6 | | |
| 31-40 | | 3.0 | 10.5 | | 1.2 | | | | | 4.4 | | 4.7 | | |
| 41-50 | 2.9 | 6.1 | 8.9 | | 1.2 | | | | | 2.2 | | 4.6 | | |
| 51-60 | 2.9 | | 5.6 | 5.0 | | | | | | 8.8 | | | | |
| 61-70 | 14.7 | 3.0 | 8.1 | 7.5 | | | | | | 6.6 | | 4.7 | | |
| 70 | 23.5 | 24.2 | 18.5 | 87.5 | | | | | | | 100.0 | 20.9 | | |
| N** | 34 | 33 | 134 | 40 | 83 | 97 | 107 | 94 | 112 | 45 | 26 | 43 | 28 | 31 |
| Larval density ($g/cm^3$) | 1.0263 | 1.0294 | 1.0339 | 1.0310 | 1.0292 | 1.0264 | 1.0267 | 1.0267 | 1.0306 | 1.0280 | 1.0277 | 1.0277 | 1.0279 | 1.0279 |

* Numbers denote percent of total larvae

** Total number of larvae

## SUMMARY


1. Naturally fertilized M. cephalus eggs (930 μ mean diameter) hatched in 36-38 hours at 24 °C and 48-50 hours at 22 °C. Length of newly hatched larvae was 2.65 mm.

2. The mouth opened on the second to third day but feeding was first observed on the fifth day. The yolk sac was completely absorbed at that time.

3. The larval growth curve consisted of a steep slope on the first day, which coincided with rapid yolk absorption, a plateau stretching from the second to the ninth day and a sustained rapid growth phase thereafter. The second growth period was concomitant with the beginning of intensive feeding. Larvae grew in length from 2.65 to 17.69 mm during the 42-day larval period.

4. There was no detectable difference in yolk sac absorption rate or larval growth at 22 and 24 °C. The oil globule persisted five days longer at the lower temperature. Larval survival was highest at 22 °C.

5. There was no difference in larval survival when initial feeding was delayed from the second to the seventh day after hatching.

6. Two well defined critical periods, associated with high larval mortalities, was evident on the 2nd-3rd and 8th-11th days. Each of the two periods was preceded by larvae sinking to the bottom of the rearing tanks. The sinking was associated with an increase in the specific gravity of the larvae.

7. Larval survival rates of 0.2% and 5% were obtained.

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IX.

# SUBPOPULATIONS OF THE HAWAIIAN GREY MULLET *Mugil cephalus*: ANALYSIS OF VARIATIONS OF NUCLEAR EYE-LENS PROTEIN ELECTROPHEROGRAMS AND NUCLEAR EYE-LENS WEIGHTS


## Abstract

The method of electrophoretic separation of nuclear eye-lens proteins was applied to a study of the population structure of the grey mullet *Mugil cephalus* L. in Hawaii. Electropherograms produced five pattern types, with most variations due to non-developmental genetic polymorphism. Some variations, however, were associated with size, and an ontogenetic basis was considered. The frequencies of the pattern types were significantly ($p<0.005$) independent among the three geographical subpopulations examined: Kaneohe Bay, Oahu; Diamond Head, Oahu; and Kawaihae Bay, Hawaii. Growth rates of mullet eye-lens nuclei demonstrated significant ($p<0.05$) differences between sexes and localities, except for Kaneohe Bay males versus Diamond Head males, which were nearly significant ($p\sim0.075$). These variations in eye-lens nuclei growth rates are probably regulated by genetic factors. Nearly complete genetic isolation is evident for the three subpopulations of mullet in the Hawaiian Islands. The occurrence of genetic interchange is apparently less frequent between the Oahu and Hawaii populations than between the two Oahu populations, in accordance with their respective geographical distances.



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## INTRODUCTION

The grey mullet Mugil cephalus in Hawaii, as elsewhere (Thomson, 1963), tend to localize nearshore within the vicinity of brackish water. By this habit, geographically separate populations would be expected to have little genetic interchange. Juvenile drift or adult nomadism (Thomson, 1963), however, may permit a limited genetic flow between such populations, particularly where geographical distances are small, such as within the Hawaiian Islands. No geographical variations for the Hawaiian grey mullet are known.

Electrophoretic separation of nuclear eye-lens proteins is a useful method for identifying genetically different populations of various fish species (Smith, 1965, 1966, 1969b, 1970; Smith and Goldstein, 1967). Some advantages of using nuclear eye-lens proteins are ease of collection and storage of lenses, stability of proteins against environmental variables and seasonal change, etc. (Smith, 1966, 1969b, 1970; Smith and Goldstein, 1967; Peterson and Smith, 1969). These advantages will make this method well suited for the present study of mullet populations. This is the first time the method of electrophoretic separation of nuclear eye-lens proteins has been applied specifically to examine population structure. Genetically based population variations in nuclear eye-lens protein electrophoretic patterns (electropherograms) have been reported earlier (Smith, 1966, 1969b; Smith and Goldstein, 1967), but population structure analysis was not attempted in these studies.

Preliminary work revealed variations in the ratio of the weight of the nuclear portion of the mullet eye-lens to the size of the fish, and as no report of such variation is known, a statistical evaluation of the parameter in relation to sex and locality of the fish is undertaken. Friend (1968) has reviewed quite extensively the work on eye-lens weight studies in vertebrates, and reported no investigations on nuclear eye-lens weights. A literature survey, although not exhaustive, revealed no published work on nuclear eye-lens weights or fish eye-lens weights. The analysis of nuclear eye-lens weight variation with respect to size of the mullet is, thus, of novel interest.

## MATERIALS AND METHODS

Eye lenses were collected from a total of 120 grey mullet Mugil cephalus L. caught from four locations in the Hawaiian Islands (Fig. 1, Table 1). Fish from the Kaneohe Bay and Diamond Head localities were brought live to the laboratory and later sacrificed to obtain lenses. Lenses from the Kawaihae Bay and Kaunakakai fish were collected in the field and kept on ice until

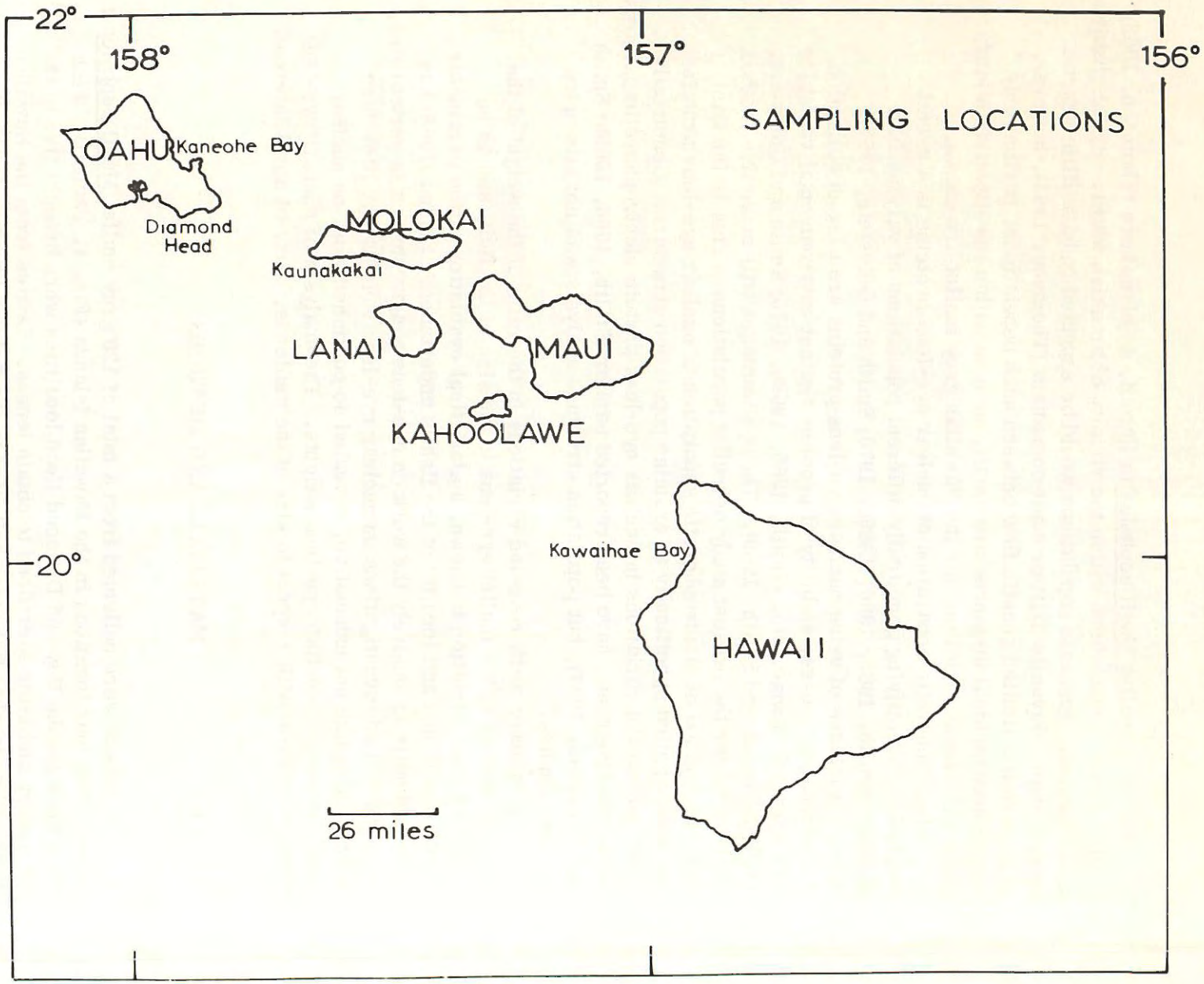



Fig. 1. Sampling locations

delivered to the laboratory. At the laboratory, lens samples were stored frozen until ready for dissection to obtain the nuclear portions.

Table 1. Mugil cephalus. Number, size, locality and dates of collection of mullet from which eye-lens samples were taken

| Number of fish | | Standard length range (cm) | Locality (area, island) | Dates of sample collections |
|---|---|---|---|---|
| Males | Females | | | |
| 41 | 33 | 24.1 - 35.4 | Kaneohe Bay, Oahu | Sept. to early Dec., 1969 |
| 16 | 7 | 25.5 - 41.0 | Diamond Head, Oahu | late Dec., 1969 |
| 3 | 17 | 28.0 - 38.0 | Kawaihae Bay, Hawaii | late Dec., 1969 to late Jan., 1970 |
| | 7[a] | 10.0 - 17.5 | Kaunakakai, Molokai | May, 1970 |

[a] Sex undeterminable

Lens nuclei were weighed to the nearest tenth of a milligran and then macerated in a test tube containing seven times, w/v(ml), of extracting fluid. To reduce the potential problem of low amounts of soluble nuclear eye-lens proteins found in some fish (Smith, 1969a), the saline-urea (0.14 g % NaCl and 0.1 M urea) extracting medium of Smith (1969a) was used. This extraction fluid has been recently verified for increasing the amount of protein extracted from mullet eye-lens nuclei (Peterson, 1970). The extract preparation was allowed to stand on a shaker for 24 h under refrigeration (12° C). Either a 10 µl (small Kaunakakai-lens samples only) or a 20 µl aliquot was then examined by cellulose acetate (1" x 6" Gelman Separaphore III polyacetate strips) electrophoresis at pH 8.6 (sodium barbital buffer, ionic strength 0.05). Electrophoresis was carried out in a Colab Universal Electrophoresis Chamber at 300 V for 20 min. Preparation and densitometric analysis of the electropherograms was carried out as previously described (Peterson and Smith, 1969).

Eye-lens nuclei weights were compared to standard lengths of all fish and to weights of the Kaneohe Bay and Diamond Head-caught fish. Lens nuclei weight-body length regressions were examined by analysis of covariance to determine the significance of variations between sexes (Kaneohe Bay group only) and between localities (Kaneohe Bay males and females,

Diamond Head males and Kawaihae Bay females). Analysis of covariance on the regressions of lens nuclei weight-body weight and of body weight-body length were also examined for Kaneohe Bay males and females. Statistical methods were according to Snedecor and Cochran (1967).

The limited number of lens samples from small mullet, obtained from the Kaunakakai, Molokai area, were used only for an overall evaluation of fish size (standard length) in relation to protein variation and nuclear eye-lens weight.

## RESULTS

Electrophoretic separation of Mugil cephalus nuclear eye-lens proteins produced five different patterns (Fig. 2). Four fractions (Nos. 1 to 4) migrated toward the cathode and one (No. 5) toward the anode. Fractions 2 and 3 varied in relative staining intensity. Patterns were coded A where fraction 2 was more intensely stained than fraction 3, and coded B where it was less intensely stained. The anodal fraction consists of at least two variably staining components, one migrating further and and more intensely stained than the other. Both components were observed together whenever the staining intensity was sufficient. Fraction 5 appeared in both patterns A and B, and was graded subjectively into three categories based on its staining intensity relative to the overall staining intensity of the cathodal fractions. These categories were coded 1 for light to non-detectable or trace amounts, 2 for intermediate staining intensities and 3 for peak intensity roughly equivalent to cathodal fractions.

Table 2 shows the frequencies of the various pattern types of nuclear eye-lens protein from mullet samples in all areas except Kaunakakai. Chi square analysis of the sex ratios in the Kaneohe Bay fish for each of the coded pattern types gave no significant deviation ($P>0.8$) from a 1:1 distribution probability. Similar analysis of the Diamond Head and Kawaihae Bay samples was not possible because of the largely unequal distribution of sexes in these samples (Table 1). In both of these latter samples, the sex of low frequency demonstrated pattern types which were also shown by their opposing sex in the same sample, and these appeared in the most frequent pattern types. Except for the small Kaunakakai fish, which were all of type A1, no significant correlation of the pattern types with size of the fish was evident.

Differences in the staining intensity of fractions 1 and 4 were observed in fish of different sizes for both the A (Fig. 3) and B (Fig. 4) pattern groups. Figures 3 and 4 demonstrate that, with increasing size, there is a progressive decrease in fraction 1 and an increase in fraction 4, particularly in fish greater than 35 cm standard length.

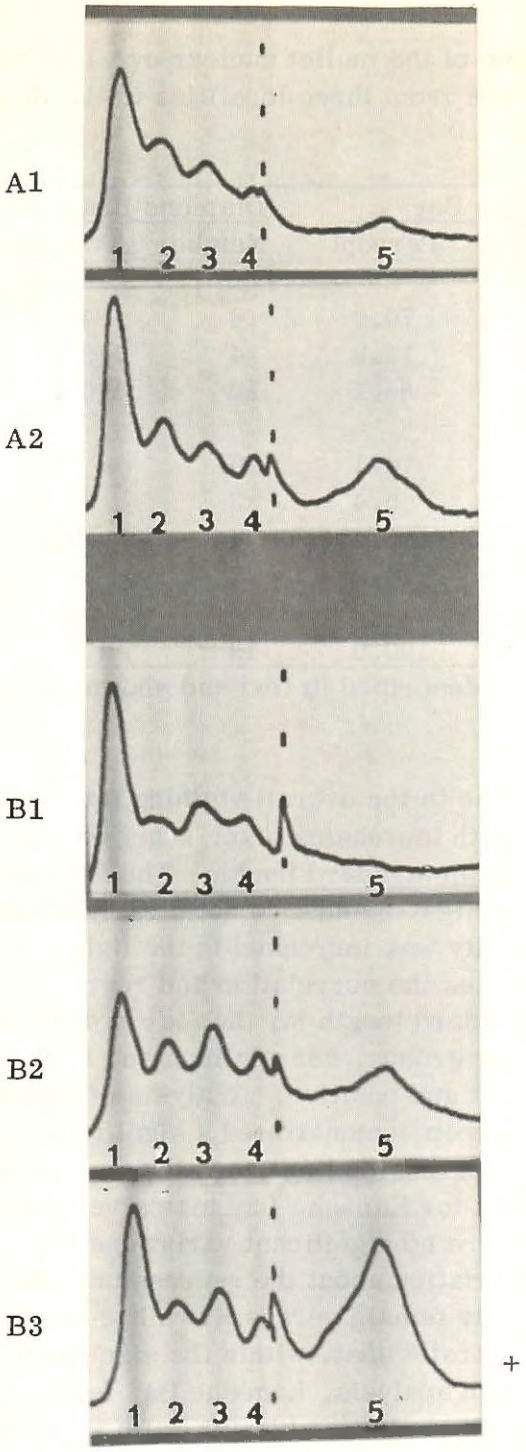



Fig. 2. Mullet nuclear eye-lens protein electropherograms showing the five coded pattern types. Dashed line: point of sample application.

Table 2. Frequencies of the mullet nuclear eye-lens protein electrophoretic pattern types from three localities in the Hawaiian Islands

| Pattern[a] code | Kaneohe Bay | | Diamond Head | | Kawaihae Bay | |
|---|---|---|---|---|---|---|
| | Number | Percent | Number | Percent | Number | Percent |
| A1 | 52 | 70.3 | 8 | 42.1 | 8 | 40.0 |
| A2 | 11 | 14.9 | 4 | 21.1 | 1 | 5.0 |
| Total A | 63 | 85.1 | 12 | 63.2 | 9 | 45.0 |
| B1 | 3 | 4.1 | 0 | 0.0 | 10 | 50.0 |
| B2 | 4 | 5.4 | 3 | 15.8 | 0 | 0.0 |
| B3 | 4 | 5.4 | 4 | 21.1 | 1 | 5.0 |
| Total B | 11 | 14.9 | 7 | 36.8 | 11 | 55.0 |
| Total A + B | 74 | 100.0 | 19 | 100.0 | 20 | 100.0 |

[a] Pattern codes as described in text and shown in Fig. 2

A general decrease in the overall staining intensity of the patterns was observed in all fish with increasing size. This was more evident in fish greater than about 28 cm standard length. The densitometer graphs of the patterns shown in Figs. 3 and 4 do not clearly demonstrate this loss, as the recording sensitivity was increased in the lighter patterns.

Table 3 summarizes the correlation and regression analysis of eye-lens nucleus weight on standard length for the four groups which permitted this evaluation. In all four groups, correlations and linear regressions were statistically significant and positive. Analysis of covariance on the calculated regressions of each group demonstrated a significant ($p \leq 0.05$) difference in the elevations of the regression lines for all possible pair combinations of the sample groups, except for Kaneohe Bay males versus Diamond Head males ($p \sim 0.075$). There were no significant variations in the slopes of the regression lines. Variation about the regressions was significant ($p \leq 0.05$) only in the Kaneohe Bay female versus Kawaihae Bay female comparison.

Figure 5a demonstrates that, within the size range (Table 3) of fish examined by regression analysis, Kaneohe Bay females showed a lower eye-lens nucleus weight to standard length ratio than males from the same area. Similarly, Fig. 5b shows that this ratio was higher in Kaneohe Bay males than in Diamond Head males, and Fig. 5c shows that it was higher in Kaneohe Bay females than Kawaihae Bay females.

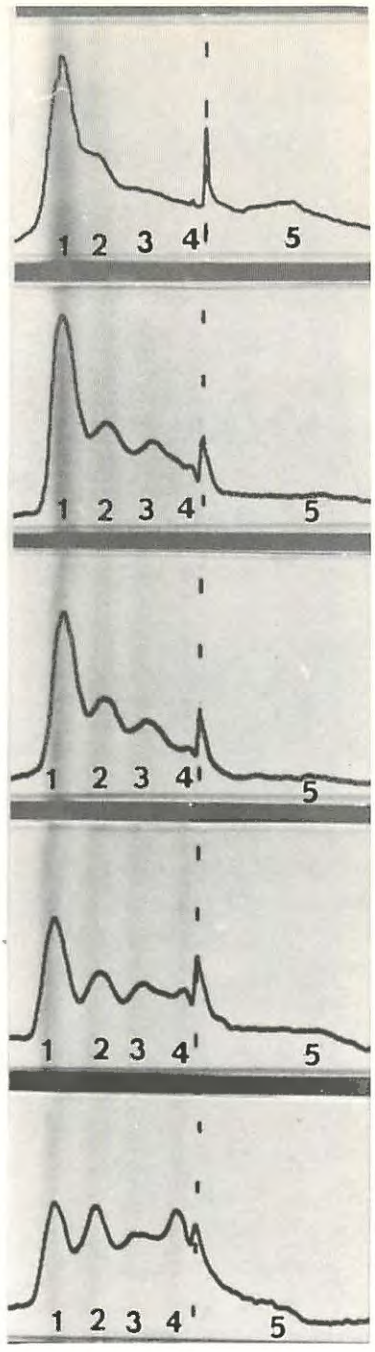
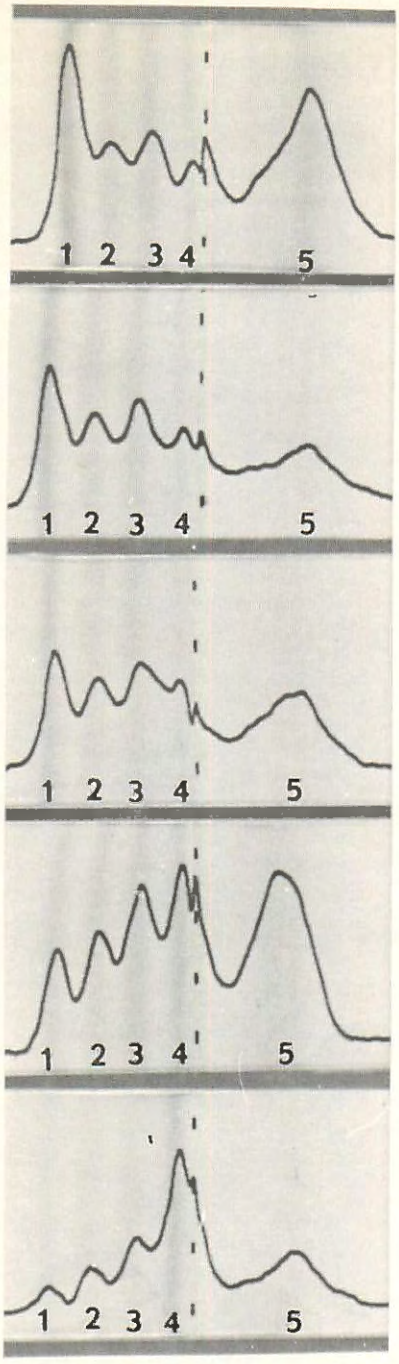



Fig. 3. Pattern group A from mullet of different size as measured by standard length. Note relative progressive decrease of fraction 1 and increase of fraction 4. Dashed line: point of sample application.




26.5 cm

29.5 cm

35.0 cm

38.0 cm

41.0 cm

+

Fig. 4. Pattern group B from mullet of different size. Notations as in Fig. 3

Table 3. Mugil cephalus. Correlation and regression analysis data for mullet eye-lens nucleus weight on standard length

| Sample group | No. of fish | Standard length (cm) Range | Mean | Correlation coefficient | Regression coefficient | Regression equation |
|---|---|---|---|---|---|---|
| Kaneohe Bay females | 22 | 27.0-33.1 | 29.1 | 0.540[a] | 0.00508[a] | Y = 0.00508 X - 0.0100 |
| Kaneohe Bay males | 19 | 25.5-31.8 | 28.2 | 0.467[a] | 0.00311[a] | Y = 0.00311 X - 0.0339 |
| Diamond Head males | 16 | 25.5-32.5 | 29.1 | 0.494[a] | 0.00279[a] | Y = 0.00279 X - 0.0319 |
| Kawaihae Bay females | 16 | 28.0-36.0 | 30.8 | 0.543[a] | 0.00205[a] | Y = 0.00205 X - 0.0302 |

[a] Significance probability $p \leq 0.05$

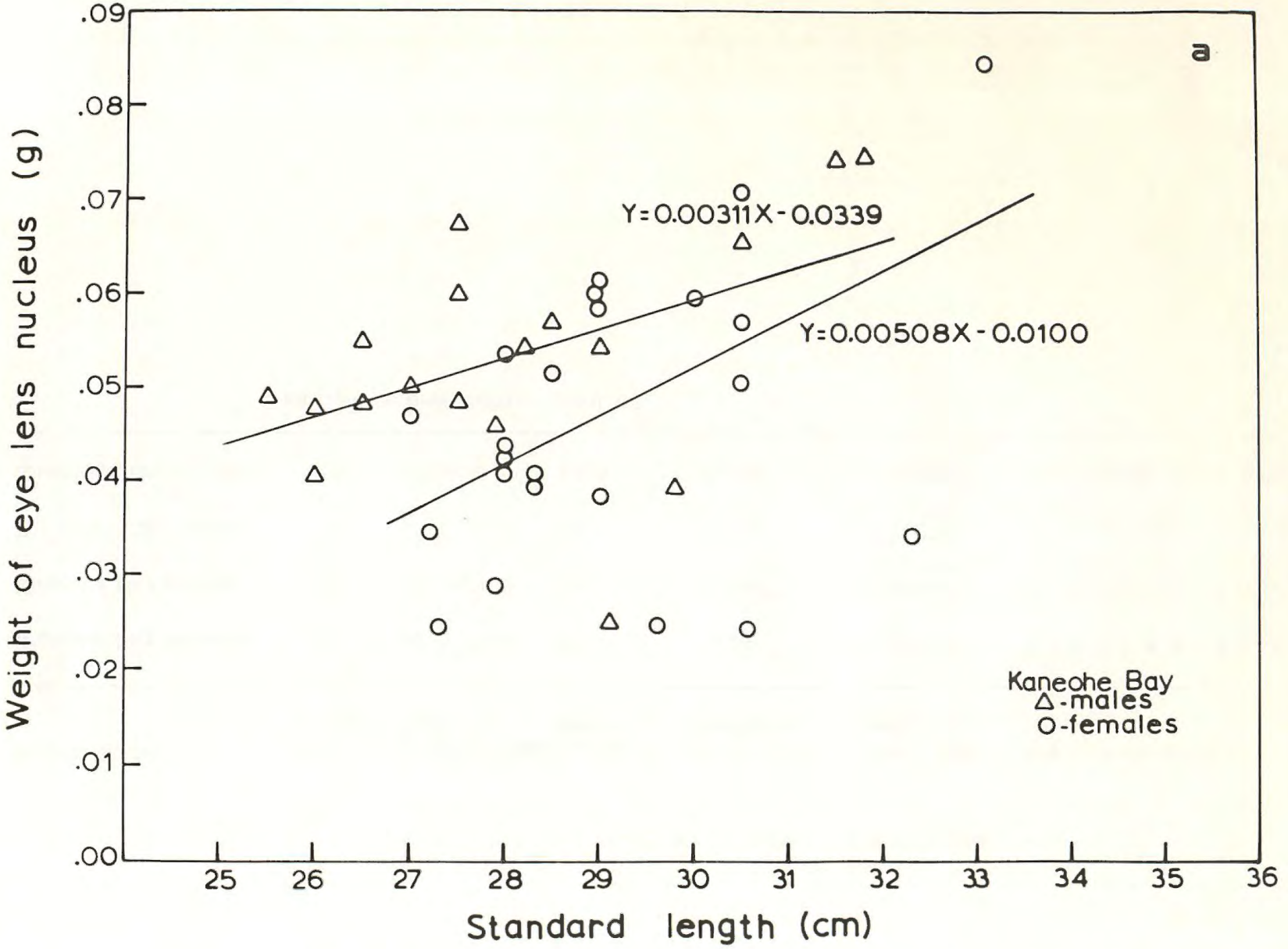



Fig. 5a. Comparative relationships of eye-lens nucleus weight to standard length, with calculated regression lines for mullet sample groups in Table 3. Kaneohe Bay males and females.

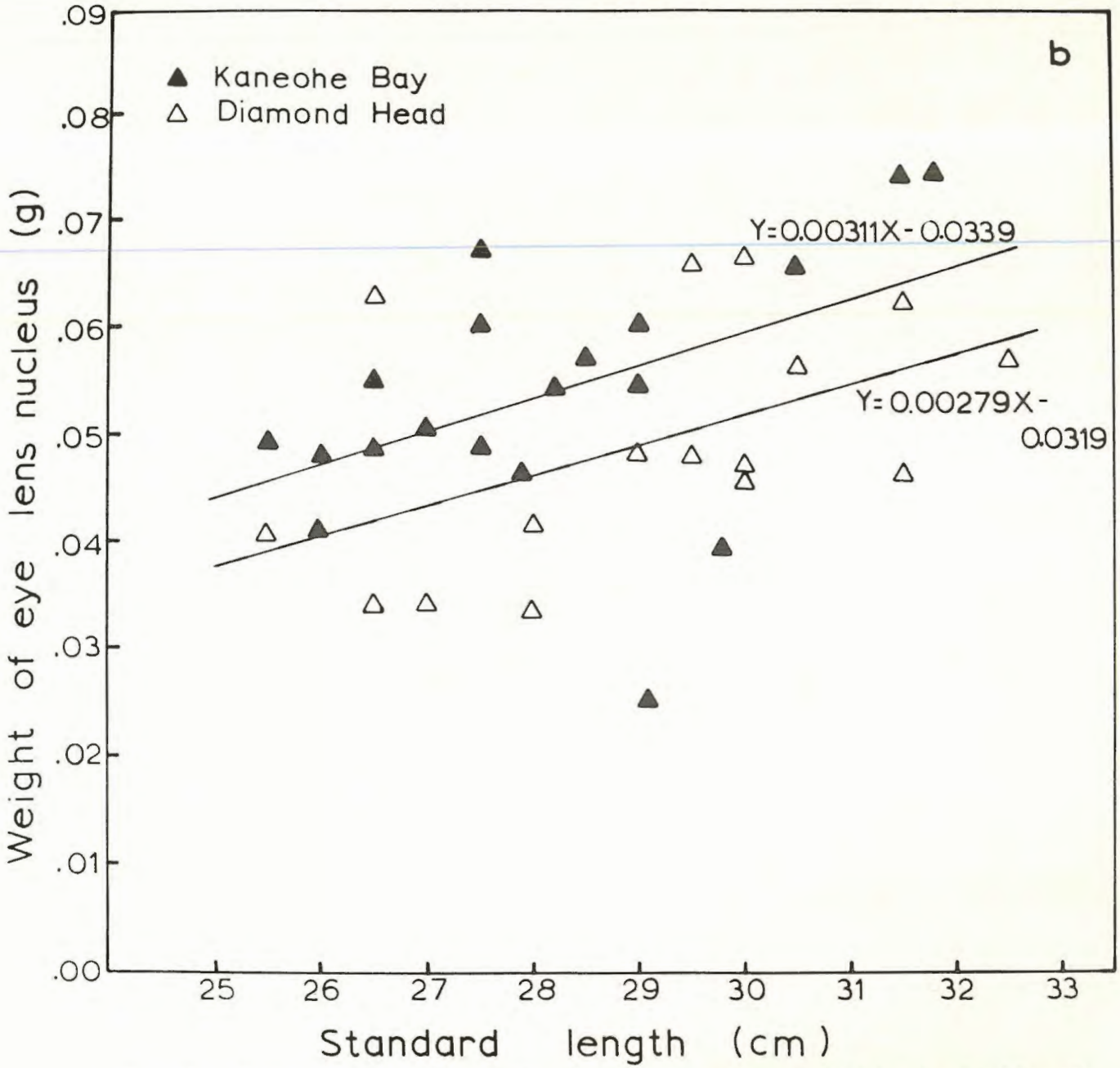



Fig. 5b. Comparative relationships of eye-lens nucleus weight to standard length, with calculated regression lines for mullet sample groups in Table 3. Kaneohe Bay males and Diamond Head males.

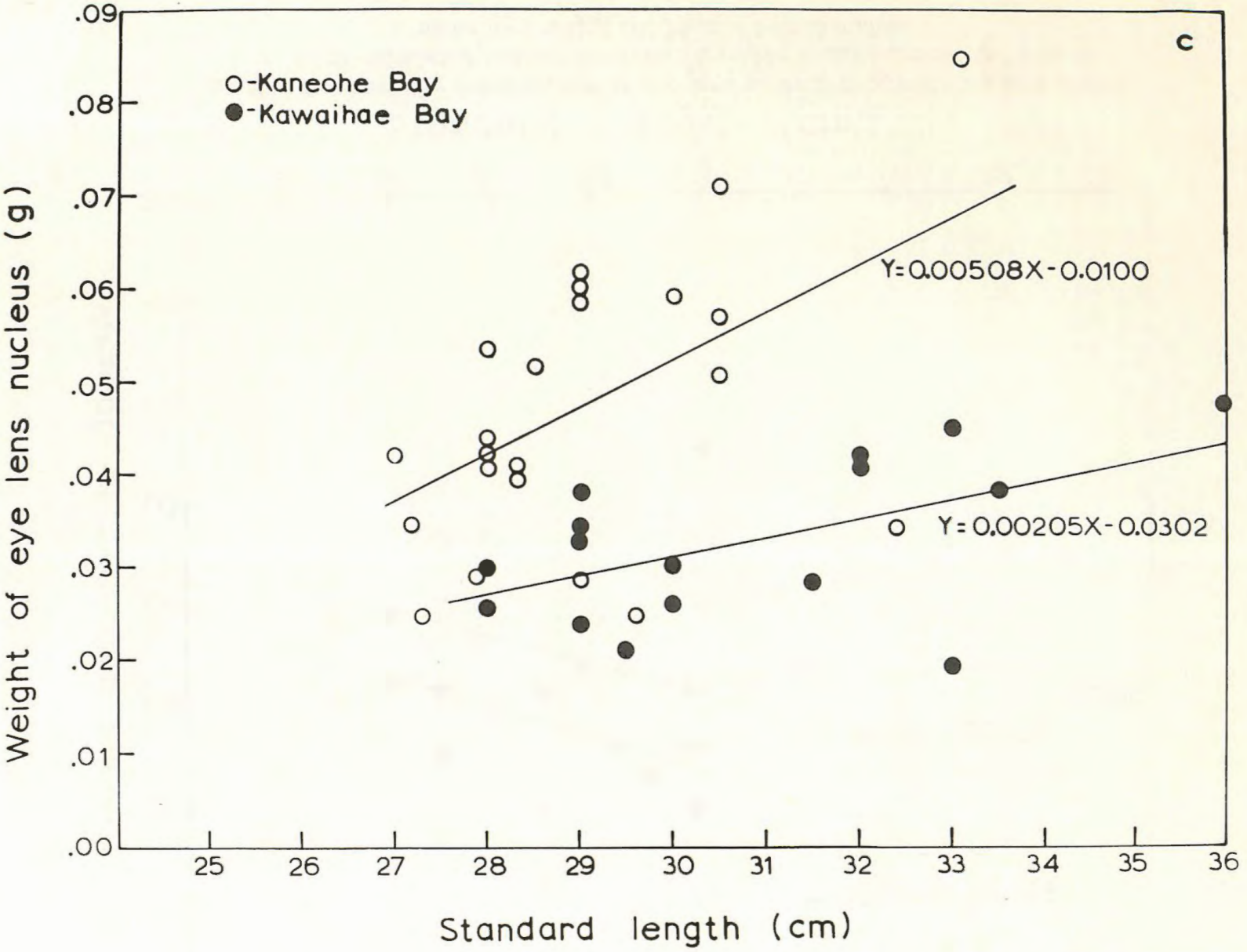



Fig. 5c. Comparative relationships of eye-lens nucleus weight to standard length, with calculated regression lines for mullet sample groups in Table 3. Kaneohe Bay females and Kawaihae Bay females

Figure 6 presents the composite data on eye-lens nucleus weight versus standard length for the four sampling locations irrespective of sex, and includes size samples of fish beyond those of Figs. 5a-c (Table 3). A

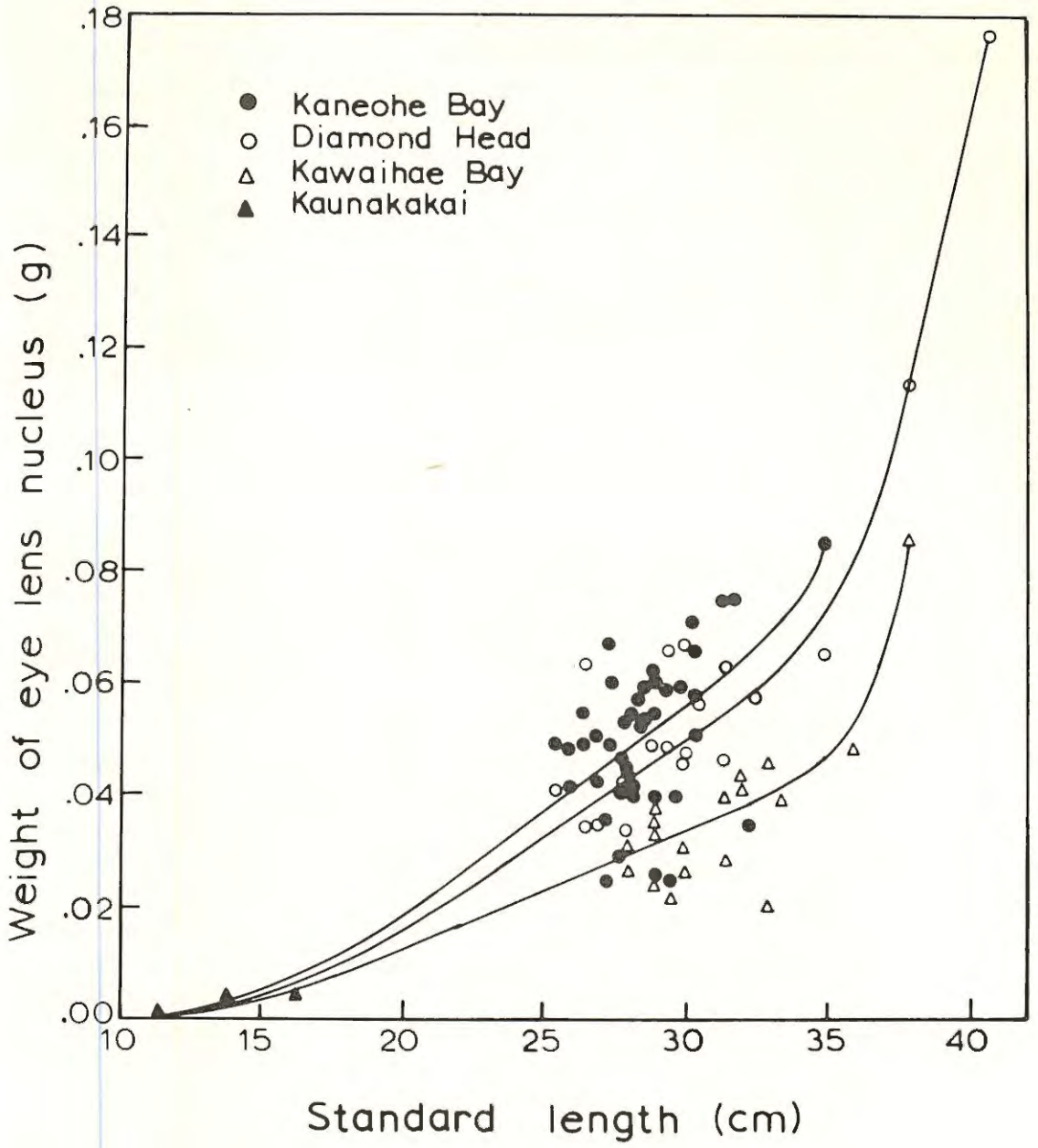



Fig. 6. Allometric relationship of eye-lens nucleus weight to standard length for all mullet sampled. The Kaunakakai mullet (lower left) were used to estimate lower limit of lens nucleus weight. Curves were drawn in part by inspection (far left and right of figure) and in part by calculated regressions (central cluster of points)

pronounced inflection in the ratio of eye-lens nucleus weight increase to the size of the fish was evident with all mullet above about 35 to 36 cm standard length. The allometric nuclear eye-lens growth curves representing the three major sampling areas were of different heights with the following order: Kaneohe Bay>Diamond Head>Kawaihae Bay.

Regression analysis of body weight on standard length for Kaneohe Bay males and females revealed significant ($p \leq 0.01$) correlation and regression coefficients for both sexes, but covariance analysis showed no significant variations in the variance, slopes, or elevations of the regression lines (Fig. 7). Diamond Head mullet had a higher body weight to standard length ratio than Kaneohe Bay mullet. Values for Kawaihae Bay mullet were estimated from field observations (Mr. W. Madden, personal communication) to be similar to Diamond Head mullet. The Diamond Head and Kawaihae Bay mullet were caught during the early period of the spawning season (Shehadeh, unpublished data). Their larger body weight was roughly equivalent to an estimated fat and gonad weight increase for spawning mullet.

## DISCUSSION AND CONCLUSION

Results from electrophoretic separation of mullet nuclear eye-lens proteins demonstrate the occurrence of two general types or sources of variations: those which are not correlated with the size (age) of the fish (Fig. 2) and those which are (Figs. 3 and 4). The former group includes most of the proteins (Fractions 2, 3, and 5), while the latter shows variations in the staining intensities of fractions 1 and 4.

Because of its graded nature, the variations in protein fractions 1 and 4 of the mullet suggest a direct relationship with ontogeny. Ontogenetic effects have been suggested by Barret and Williams (1967) to account for certain polymorphism in bonito-lens proteins, although this interpretation was not supported by the genetic studies of Eckroat and Wright (1969), nor confirmed in the recent studies of bonito by Smith (personal communication). Smith, however, examined whole-lens proteins by starch gel electrophoresis, which largely reduces the comparability of these two studies. A non-developmental explanation for the variations in fractions 1 and 4 may be supported by recognition of the similar, i.e., graded, variation in fraction 5 which was not correlative with size. Nevertheless, the ontogenetic interpretation remains favorable with the evidence in several animals that different complements of proteins are found between embryonic and adult lenses (Francois and Rabaey, 1957; Papaconstantinou, 1965; Genis-Galvez et al., 1968; Mehta and Maisel, 1968b; Rana and Maisel, 1969) and between young and old lenses (Cobb and Koenig, 1968; Genis-Galvez et al., 1968). This interesting facet of lens-protein variation certainly deserves further investigation.

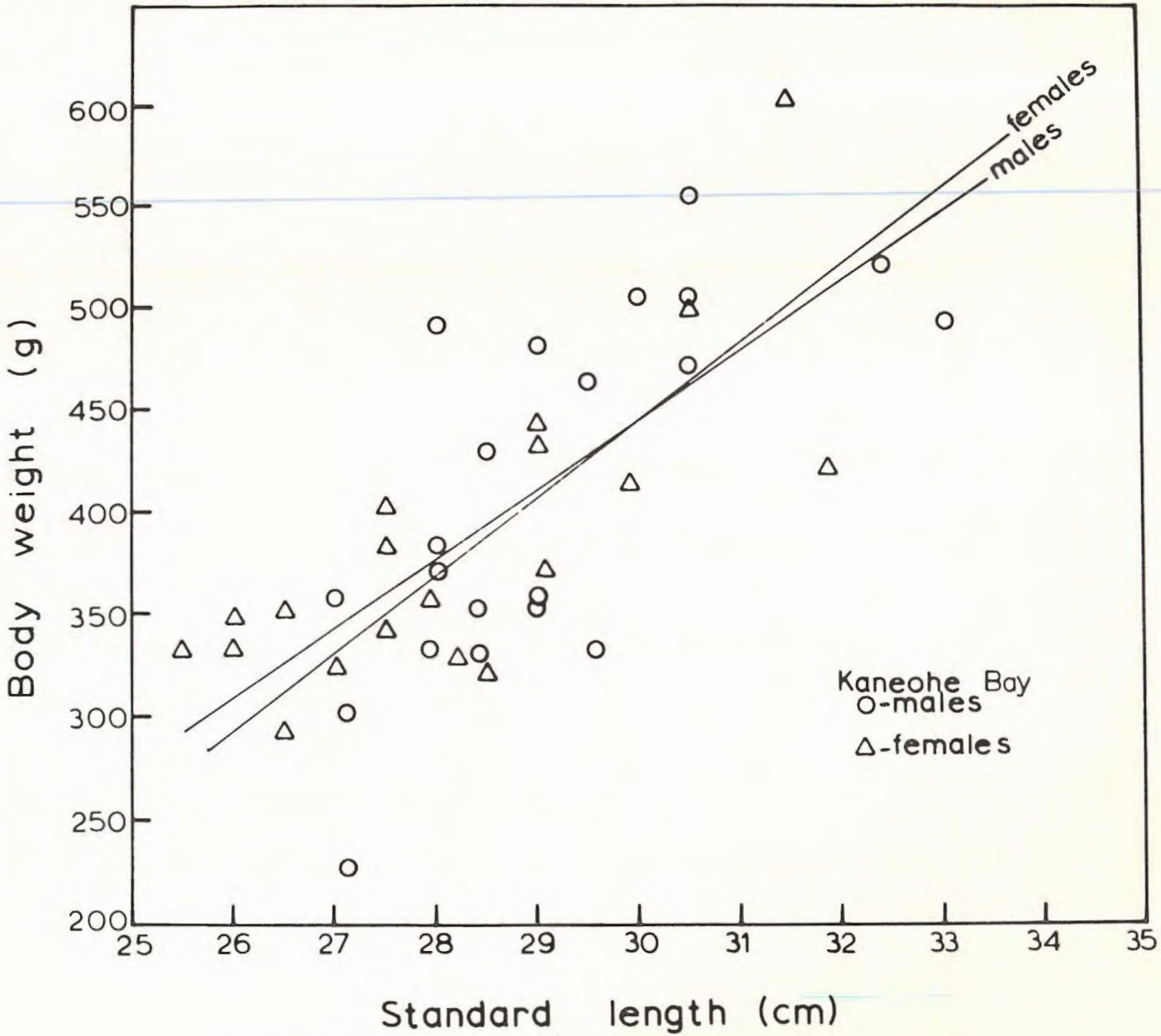



Fig. 7. Relationship of body weight to standard length of mullet, with calculated lines of best fit for males and females of Kaneohe Bay

A general decreased staining of the total pattern of the older mullet agrees with earlier findings in the sandbar shark (Peterson and Smith, 1969; Peterson, unpublished data). This is apparently due to formation of albuminoid (Dische et al., 1956; Lerman and Zigman, 1965; Fulhorst and Young, 1966; Young and Fulhorst, 1966; Lerman et al., 1968; Manski et al., 1968; Mehta and Maisel, 1968a; Bloemendal, 1969), coupled with an age-related decrease in the synthesis of soluble crystallins (Dische et al., 1956; Lerman and Fontain, 1962), as previously suggested (Peterson and Smith, 1969).

The electrophoretic pattern types shown in Fig. 2 indicate genetic polymorphism. Evidence for their genetic basis is shown by the repeatability of the pattern types (Table 2), lack of dependence on size, and lack of environmental influence, as deduced by the appearance of the same patterns from different localities at various times. Similar results have been reported for other fish (Smith, 1965, 1966, 1969a, 1970; Smith and Goldstein, 1967; Gutiérrez, 1969; Peterson and Smith, 1969). Furthermore, the genetic basis of lens-protein polymorphism has been verified in teleosts (Smith, 1966; Eckroat and Wright, 1969) and mammals (Greiffendorf and Beck, 1969). Absence of sex-related eye-lens protein electrophoretic pattern heterogeneity is also in agreement with findings in other fish (Smith, 1966; Eckroat and Wright, 1969; Gutiérrez, 1969; Peterson, unpublished data).

Attempts to fit the subjective categorization of the quantitative variation of fraction 5 to a Hardy-Weinberg equilibrium produced twice the expected frequency for the theoretical heterozygous gene combination (type 2) in all three major localities (Table 2). This, in addition to the total absence of an expected A3 type pattern, and possibly also the absence of type B1 in the Diamond Head population and B2 in the Kawaihae Bay population, suggest that the variations in fraction 5 are complex. This complexity suggests polygenic control in agreement with the evidence and consideration of others (Zwaan, 1968; Greiffendorf and Beck, 1969; Smith, manuscript in preparation), concerning genetic control of lens proteins. Insufficient data preclude a genetic interpretation of the heterogeneity evident in fraction 2 (pattern groups A and B).

In examining the frequencies of the five pattern types (Table 2), the Kawaihae Bay population shows the lowest frequency of group A and the highest of group B. The Kaneohe Bay population is reversed, and the Diamond Head population shows intermediate values. Chi square analysis of the frequencies of total A and total B in each of the three localities set in a 2 x 3 contingency table showed significant ($p<0.005$) pattern-frequency independence of the localities. Further chi square analysis of the A and B pattern group frequencies set in 2 x 2 tables with corrections for continuity, was used to compare Kaneohe Bay versus Diamond Head and Kaneohe Bay versus Kawaihae Bay. Nearly significant ($p\sim0.07$) independence was found

for the former comparison, and clearly significant ($p<0.005$) independence for the latter. Small sample size prevented similar analysis of the Diamond Head versus Kawaihae Bay comparison. Nevertheless, the uniquely high frequency of pattern type B1 in the Kawaihae Bay population distinguishes this group. These results provide strong evidence for genetic isolation of these three populations of mullet.

Since the mullet from Kaunakakai were small (<14 cm) and perhaps only a few months old, they may be the progeny of a restricted sample of the adult population. In this case, the production of only the A1 type pattern by the mullet from this locale would reflect the molecular-genetic composition of part, but not necessarily the entire population.

Results of the analysis of eye-lens nucleus weight in relation to size (standard length) of the fish demonstrate statistically significant sexual dimorphism (Fig. 5a) and population variation (Figs. 5b, c). The lower rate of growth of eye-lens nuclei from Kaneohe Bay females compared to Kaneohe Bay males cannot be due to overall growth differences (body weight to standard length ratio) between the sexes, as these are essentially the same in non-spawning mullet (Fig. 7; Broadhead, 1953). As seasonal and environmental variations are the same (caught over the same period of time at the same locality), this sexual dimorphism is assumed to have a genetic basis. Friend (1968) points out in his review that sexual variation in eye-lens weight is a species phenomenon. Thus, the present demonstration of its existence in the Hawaiian Mugil cephalus must be considered a characteristic of this fish.

The larger body weight to standard length ratio of Diamond Head males to Kaneohe Bay males and of Kawaihae Bay females to Kaneohe Bay females is probably due to spawning condition effects, as described by Broadhead (1953). Nevertheless, in contrast to body weight, the eye-lens nucleus weight to standard length ratios for the Diamond Head males and Kawaihae Bay females were lower than their respective counterparts in the Kaneohe Bay population. The variation of the sample points around the regression lines was, in addition, significantly lower for Kawaihae Bay females than for Kaneohe Bay females. The composite data on lens nucleus weight versus standard length shown in Fig. 6, indicate that the Diamond Head mullet have a nuclear lens to standard length ratio below that of the Kaneohe Bay mullet and above that of the Kawaihae Bay mullet. These results suggest the presence of different populations of mullet in Hawaii, but the source of nuclear-lens weight variation needs further examination.

Factors which may provide non-biological sources of lens weight variation include postmortem changes prior to death, effects of freezing on the lens, and inaccurate dissection of lens nuclei. The former was suggested by Hockwin et al. (1963, as cited by Mehta and Maisel, 1968a) to result in increased lens weight due to water absorption. However, according to

sample collections, this would have given the Kawaihae Bay samples the largest lens weight to standard length ratio, which was not the case. According to Friend (1968), the effects of freezing the lens are weight losses due to decomposition, which does not account for the lower lens weights found in the Diamond Head samples compared to the Kaneohe Bay samples. Furthermore, under the sampling and storage conditions, nuclear eye-lens protein decomposition does not occur (Smith, 1966; Peterson, unpublished data). Inaccurate, or inconsistent, dissection of lens nuclei, together with natural individual variation in lens-nuclei weights, represent the variation evident within each sample group. Nevertheless, this latter variation was insufficient to preclude determination of a statistically significant ($p<0.05$) regression or correlation on body length (Table 3).

The question which now arises is whether the population differences in nuclear eye-lens weights are genetically related or merely due to environmental nutritional influences. The latter is suggested not to be the case, since nutritional restriction or dietary deficiencies which reduce the growth of the lens, also reduce overall growth of the animal (Dische and Zelminis, 1959; Friend, 1968); a situation not evident in the mullet populations. Furthermore, despite lens growth being largely due to protein synthesis (Young and Fulhorst, 1966; Waley, 1969), with protein comprising about 95% of the total solid matter (Lerman, 1967), restriction or even complete absence of dietary protein resulting in general growth impairment may have no effect on lens growth (Kauffman and Norton, 1966). The more favorable hypothesis of population variation in nuclear eye-lens weights due to genetically related factors gains support from others (Donaldson and King, 1936; Mehta and Maisel, 1968a), and may reflect differences in lens metabolism (Klethi and Mandel, 1965; Hockwin and Korte, 1968). It is also suggested that due to the nature of its formation and growth (Papaconstantinou, 1967), the nuclear region may be more "sensitive" to racial or population variation in weight than the whole lens.

The nuclear eye-lens protein electropherograms and the nuclear eye-lens growth rates clearly distinguish geographic populations of Hawaiian grey mullet Mugil cephalus. The Kawaihae Bay population, which is farthest from the Kaneohe Bay population, shows the greatest differences. Variations in the electropherograms and nuclear eye-lens growth rates for the Diamond Head population show closer resemblances to the Kaneohe Bay population than to the Kawaihae Bay population, in agreement with their respective geographic distances. It is, therefore, concluded that these three populations of mullet are, for the most part, genetically isolated, with genetic interchange occurring at low frequencies in direct relationship to geographical proximity.

## SUMMARY

1. A total of 120 grey mullet, Mugil cephalus L., obtained from four localities in the Hawaiian Islands, were examined for variations in their nuclear eye-lens proteins by cellulose acetate electrophoresis.

2. Statistical evaluation of nuclear eye-lens weight to fish size was made in relation to sex of the fish and locality where caught.

3. Five different types of nuclear eye-lens protein electrophoretic patterns were produced from the fish caught at the four localities. The pattern variations are attributable to genetic polymorphism.

4. Differences in the frequencies of the five genetic pattern types were significant ($p<0.005$) for mullet from three major sampling locations, viz., Kaneohe Bay, Diamond Head, and Kawaihae Bay. Samples from the Kaunakakai area were insufficient for population analysis.

5. Size-associated variations in the farthest and least cathodal migrating fractions were common to all pattern types. Both ontogenetic and non-ontogenetic mechanisms for these variations were considered.

6. Covariance analysis showed significant ($p<0.05$) differences in the allometric eye-lens nuclei growth rates between sexes (Kaneohe Bay population) and between localities (Kaneohe Bay, Diamond Head and Kawaihae Bay populations). The one exception was the Diamond Head males versus Kaneohe Bay males, in which the comparison was nearly significant ($p\sim0.075$).

7. Variations in eye-lens nuclei growth rates were suggested to be due to genetic factors.

8. It is concluded that the three mullet populations (Kaneohe Bay, Diamond Head and Kawaihae Bay) in the Hawaiian Islands are almost completely isolated genetically, with infrequent gene flow or interchange related to geographic proximity.

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X.

# PRELIMINARY ANALYSIS OF THE MULLET MARKET IN HAWAII


## Abstract

This study describes the drastic changes in the mullet industry in Hawaii during this century, and attempts to identify the most significant factors contributing to major declines in both sea and pond operations. The future marketing potential of the species is also projected.

## INTRODUCTION

The economics of the mullet industry in Hawaii have changed dramatically during this century. Sea caught mullet declined 79.5% during the 1928-69 period and pond production decreased 99.1% over the same time interval. During the decade of 1960-69, sea catch increased while pond production continued to decline at an accelerated rate. In conclusion, the empirical evidence presented indicated that various economic factors were affecting mullet production enough to cause major declines in the respective yields of both sea and pond operations. The purpose of this study is to investigate the general economics of supply and demand in detail, as relating to the mullet industry. Specifically, the study will attempt to identify those forces affecting the industry and project the future marketing potential of the species.

## INDUSTRY CORRELATIONS

Comparison of growth rates of sea and pond caught mullet (Fig. 1) indicates that the decline of sea caught mullet appeared relatively stable when compared to the decline of the entire fishing industry during the 1960-69 decade. On the other hand, the pond yield of mullet was declining at a much faster rate, relative to the total pond production and the industry as a whole. In order to determine the validity of this observation, a trend analysis was applied to the data for the 1950-69 and 1960-69 periods.

The results of the analysis are presented in Table 1 and Figure 2 with one major variation being made. Due to the fact that the mullet yield

Table 1. Comparative trend analysis of mullet production

| | SEA | | POND | |
|---|---|---|---|---|
| Period | All Species | Mullet | Net All Species | Mullet |
| 1959-69 | | | | |
| a | -0.288 | -1.122 | -1.838 | -2.407 |
| r | -39.6 | -46.4 | -57.4 | -97.5 |
| 1960-69 | | | | |
| a | -0.105 | 0.458 | -1.377 | -5.196 |
| r | -12.4 | -15.6 | -19.7 | -96.5 |

a = slope of the trend line
r = total change in percent

Data from Hawaii Fish and Game Commercial Fish Catch Bulletin

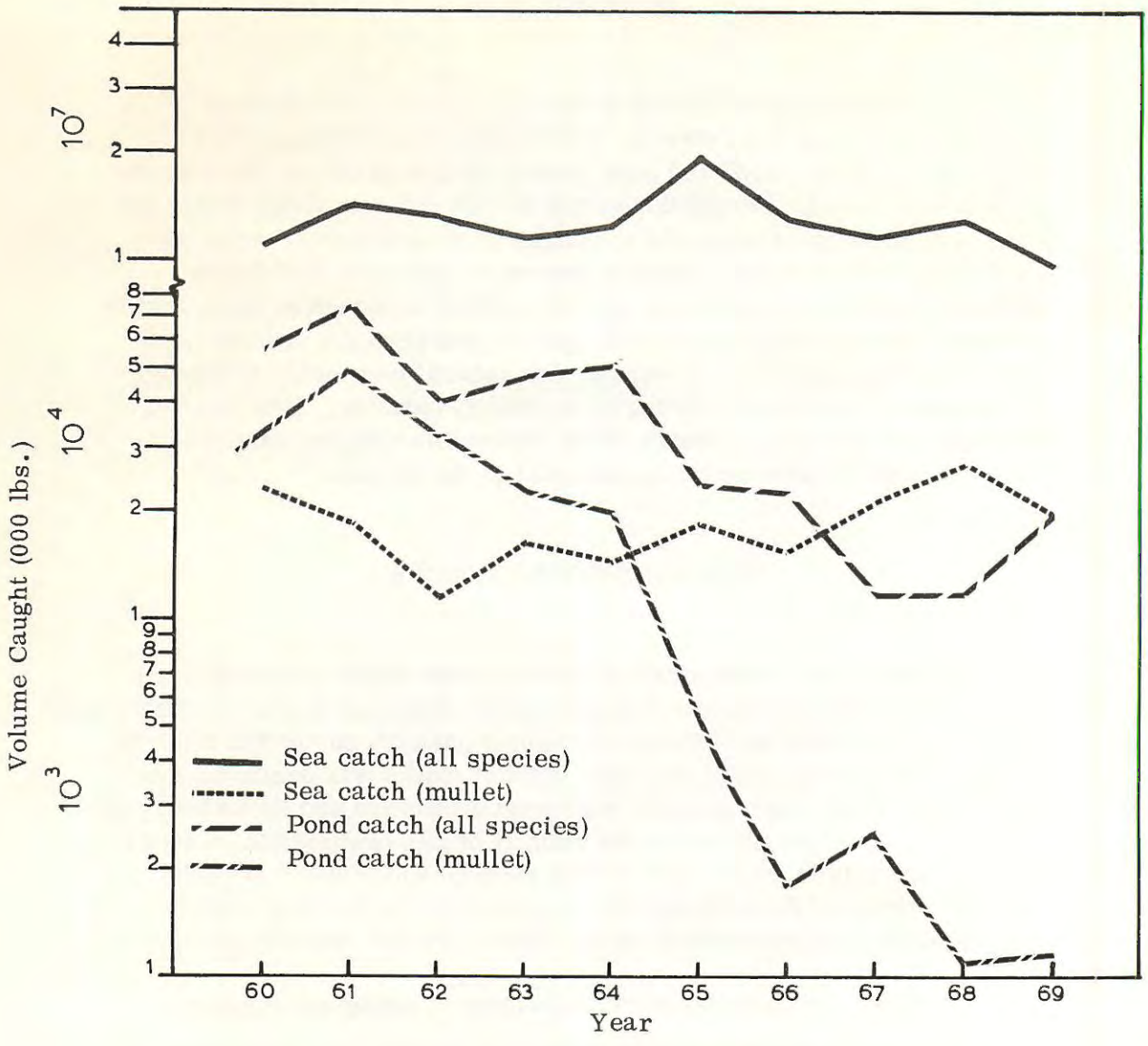



Fig. 1 Comparison of Growth Rates of Sea and Pond Caught Mullet with Industry Growth Rates

from ponds composed such a high percentage of the total volume, it has been netted out of the total pond production figure in order to determine the rate of growth of other species relative to the rate of growth for mullet.

Based on the data, it can be seen that sea caught mullet has declined at a faster rate than the industry as a whole. Specifically, sea catch has decreased an average of 1,122 pounds per year compared to the 288,000 pound

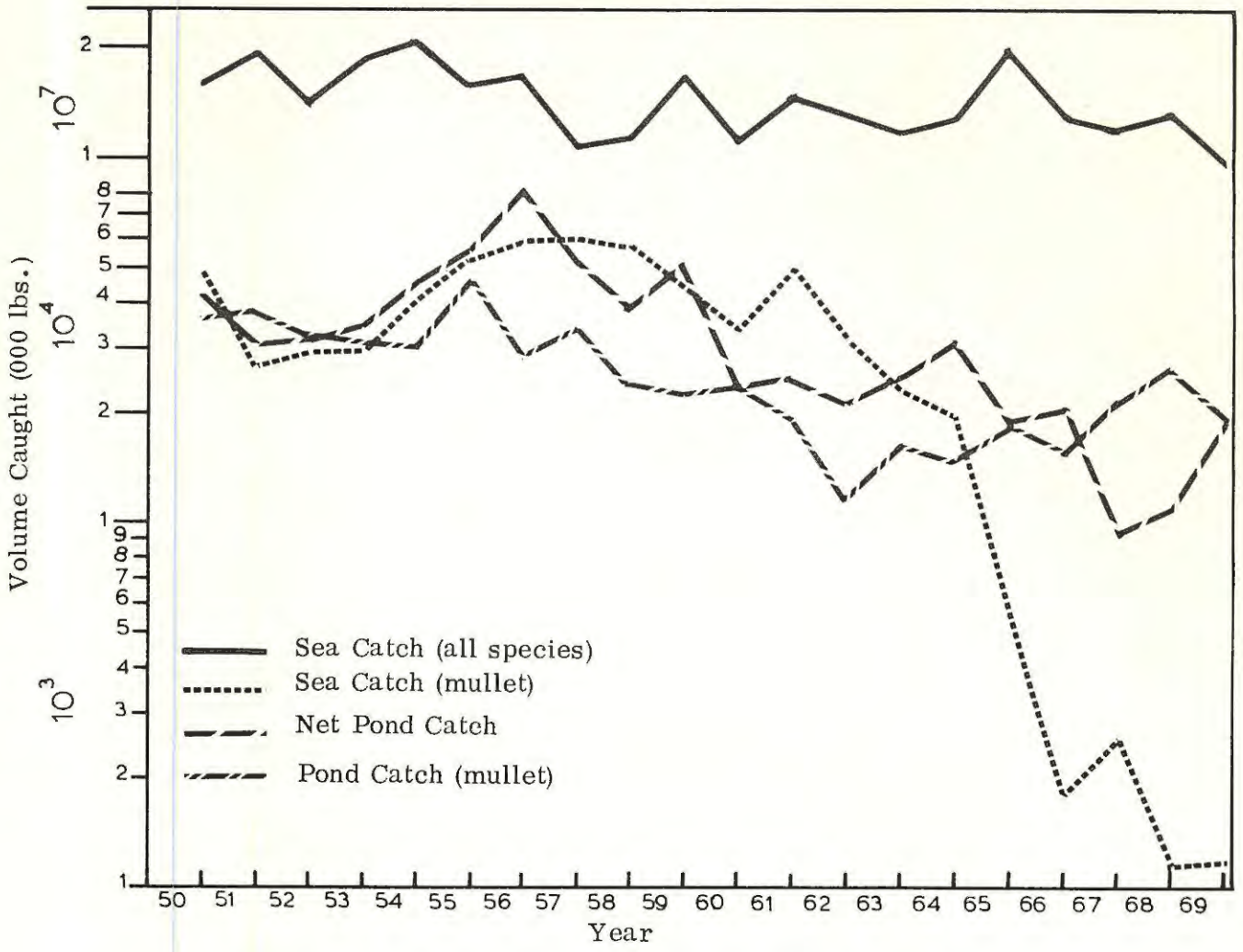



Fig. 2. Trend analysis for pond and sea-caught mullet relative to the total industry.

per annum reduction to the total catch of all species. On the other hand, net pond production has decreased at a slower rate (1,838 pounds per year) than the mullet pond yield (2,407 pounds per year) indicating the possible existence of economic or other factors relating to the production of mullet and not affecting the industry as a whole. As the shorter time interval of 1960-69 is analyzed, it becomes more apparent that there are certain economic factors present that are specifically affecting the pond production of mullet. For example, during the shorter period, sea catch shows

improvement with the rate of decrease being reduced to 105,000 pounds annually. In addition, the sea catch of mullet is actually increasing at the rate of 458 pounds per year. In the case of pond production, the net yield of all other species shows improvement with the rate of decline decreasing to 1,377 pounds per year. Mullet production shows a continued decrease at more than twice the rate of the longer time interval or 5,196 pounds per year. The total percentage decline illustrates the same basic results. In all cases, except the pond mullet, production has shown marked improvement during the 1960-69 decade. The pond yield of mullet continues to decline.

In conclusion, the empirical evidence indicates that the pond production of mullet has suffered possibly from economic forces that have had little effect on the capture industry (or other aquaculture operations). It is reasonable to assume that if the increasing value of land, i.e. the loss of pond facilities, were the major cause of the decline in mullet production, then it would also affect the production of other species. Although this may be true, at least to some extent, it is obvious from the trend analysis that the effect has been much less detrimental in the case of other species. Therefore, the economic forces causing the decline in the production of mullet appear to be more directly associated with the operational and marketing aspects of the species than with the economics of the industry. Among the forces which could cause the decline are (1) high operational costs with respect to profit margins, (2) lack of a constant supply of juveniles, (3) the lack of strong preferential demand, i.e. a high degree of substitution, and (4) the absence of an organized aquaculture effort involving proper facilities and management practices.

Examination of mullet culture techniques and personal interviews of pond operators revealed that: (1) the supply of juveniles has been on the decline for the past decade, and (2) specific mullet husbandry methods do not exist. The ponds in use are aboriginal coastal Hawaiian ponds that are unmanageable. The walls of such ponds consist of uncemented lava rocks which are pervious and permit free entry of predators, most notably Barracuda and Elops. The ponds cannot be drained and are badly silted (as much as 4-8 ft on the bottom). Also, (3) little or no regular feeding is practiced in these ponds.

The decline in pond yield of mullet therefore is more directly related to the lack of an adequate and consistent supply of juveniles and the deterioration, by siltation and storm damage, of the physico-chemical characteristics of the ponds.

## PRICE STRUCTURE

The price structure was analyzed through the use of a wholesale price index with the December 1963 prices representing the base period or 100. All other wholesale prices were then compared to the base period thus developing a price index and eliminating the effects of inflation. The results of the computation are illustrated in Figure 3.

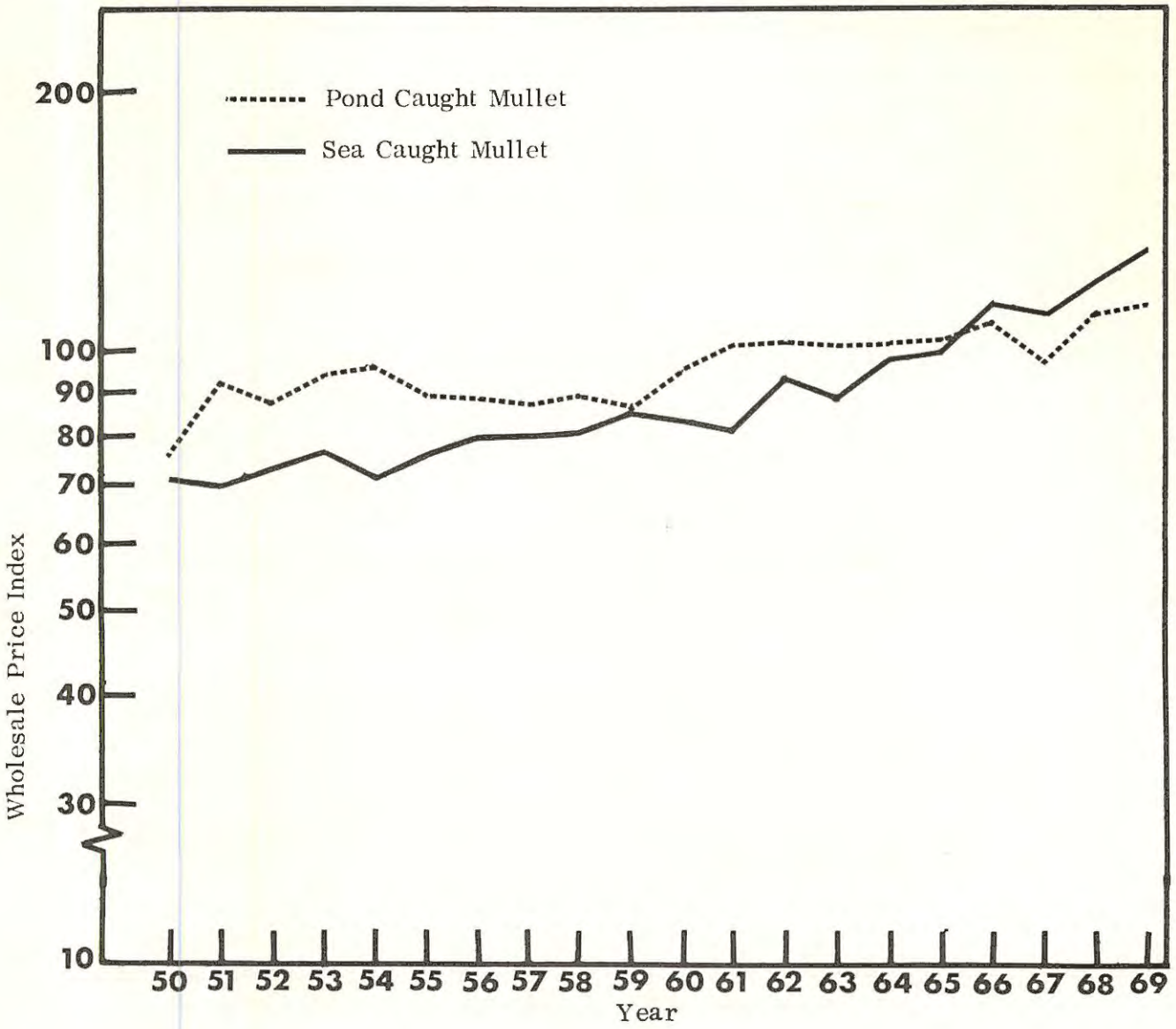



Fig. 3 Comparative Growth Rates of the Wholesale Price Index

Although pond mullet has sold at a higher average wholesale price, at least until 1965, sea caught mullet prices have shown a faster rate of growth. Empirically, as indicated in Table 2, the price of sea caught mullet has increased at the rate of 2.7 cents per year while pond prices increased at the rate of 1.3 cents per annum for the 1950-69 period, i.e. the price for sea caught mullet increased twice as fast the price for pond produced mullet. Over the 1960-69 decade, the average price per pound for sea caught mullet increased its rate of growth to 5.2 cents per year while pond production of mullet showed no change in the price growth rate.

Table 2. Trend analysis of price growth rates

| Period | Sea Mullet | Pond Mullet |
|---|---|---|
| 1959-60 | | |
| a | 0.027 | 0.013 |
| r | 84.5 | 47.5 |
| 1960-69 | | |
| a | 0.052 | 0.013 |
| r | 57.9 | 15.9 |

a = the slope of the trend line
r = total change in percent

In conclusion, when compared to the volume trends, it does not appear that a strong correlation exists between the average price per pound of pond produced mullet and the total volume, i.e. although there are significant decreases in the volume of pond mullet over the 1960-69 period, the corresponding price movement does not suggest strong market demand. Based on the price theory of demand, the price should show an increase due to the increasing competition for the product, assuming a free market and no substitution. In the case of mullet production, the price increase doesn't reflect the enormous drop in volume that has occurred. Sea caught mullet, on the other hand, shows a somewhat different situation with the price increasing faster than the rate of volume decrease during the 1950-69 period. During the 1960-69 decade, both price and volume increased.

## SEASONAL VARIATIONS

In general, the seasonal variations revolve around the three month breeding season of December, January and February which constitute the closed season of the capture fishery. As illustrated in Figure 4, the average monthly production of pond mullet is highest during this period and drops off substantially during the remainder of the year. Sea caught mullet, on the other hand, shows its highest volume during March, or immediately following the breeding season. It remains stable during the mid-year months and then increases again during the three months prior to the closed season. Over the three intervals illustrated, it is evident that the average production of pond mullet has declined to an insignificant level. Although the average volume of sea caught mullet for March declined also, the remaining months have maintained relatively stable positions when compared to pond volume. The fact that the average pond production is highest only when natural sea mullet is not available indicates that a preference may exist for the mullet caught from natural stocks as opposed to the pond produced fish. It is also interesting to note that during the three months of breeding, the average price for pond mullet has been higher than the average prices for the remaining months of the year, as indicated in Figure 5. This would seem to verify the conclusion that pond production is most economical during the period when competitive products are not available. However, further investigation along these lines revealed that pond cultured mullet are intentionally held till the closed season since market price is higher at that time. The closed season also coincides with Chinese New Year festivities during which fresh mullet is a traditional dish and often fetches a wholesale price of $2.00/lb.

In order to eliminate the effects of the large decreases which occurred in the 1960-69 decade to pond production, the same data can be analyzed for the 1950-59 period. Although the averages are higher for each month, as illustrated in Figure 6, the same conclusions are derived in that pond production is highest in the periods of prohibition on ocean stocks. During the mid-year months, sea mullet averages a higher volume than the pond production with the exception of July. The reason for this discrepancy is that in 1950, over 22,800 pounds of pond mullet were sold in July thus raising the ten year average by 2280 pounds. Without this large input, pond production would have remained below sea production for all mid-year months.

As each month is analyzed separately (Figs. 7-18), it can be seen that, with the exception of October, the trend of sea caught mullet has shown improvement during the last decade; whereas pond production has declined faster in every month except July. It is also significant to notice that prior to 1960, pond mullet production was relatively competitive with the sea

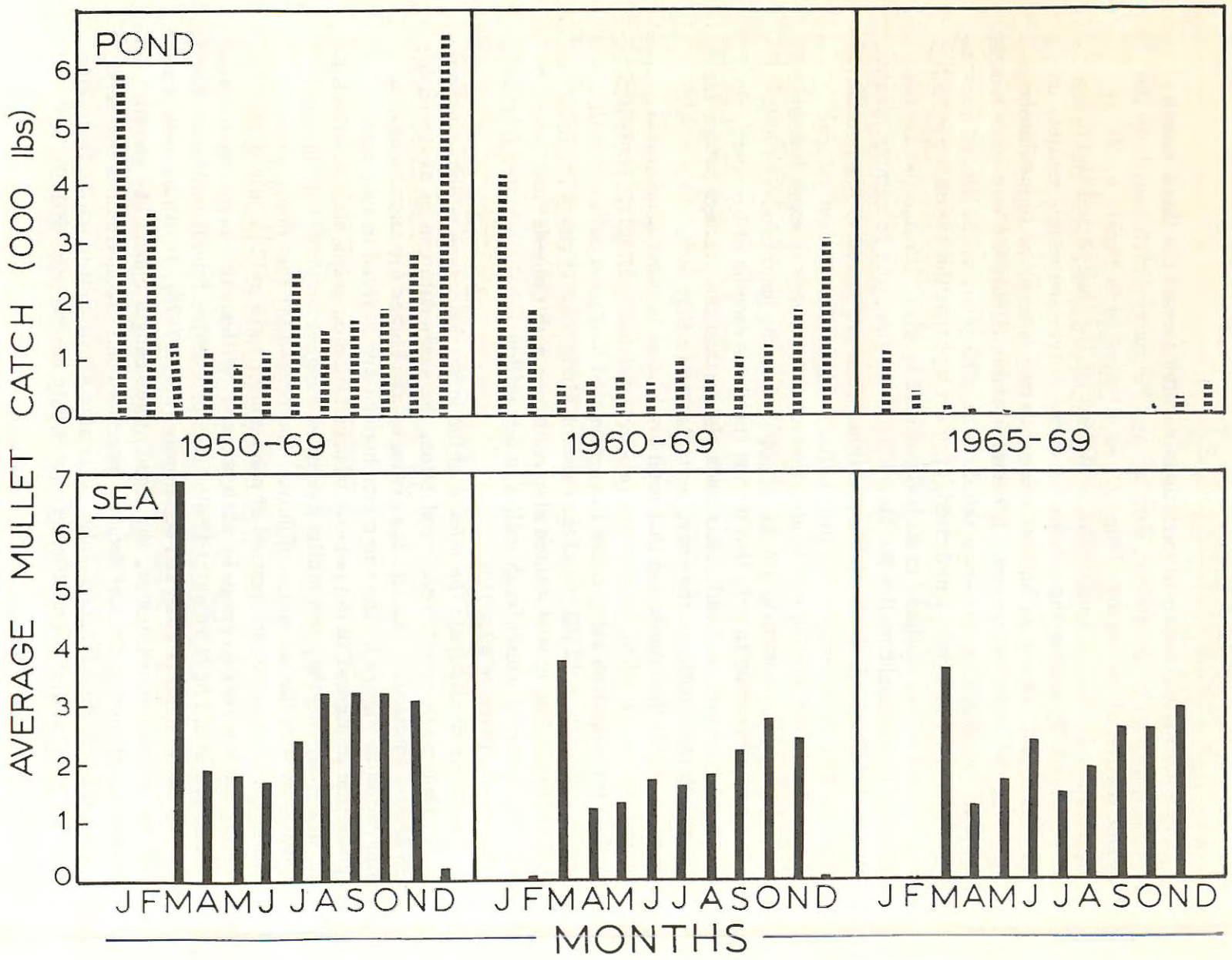



Fig. 4. Average mullet catch by month

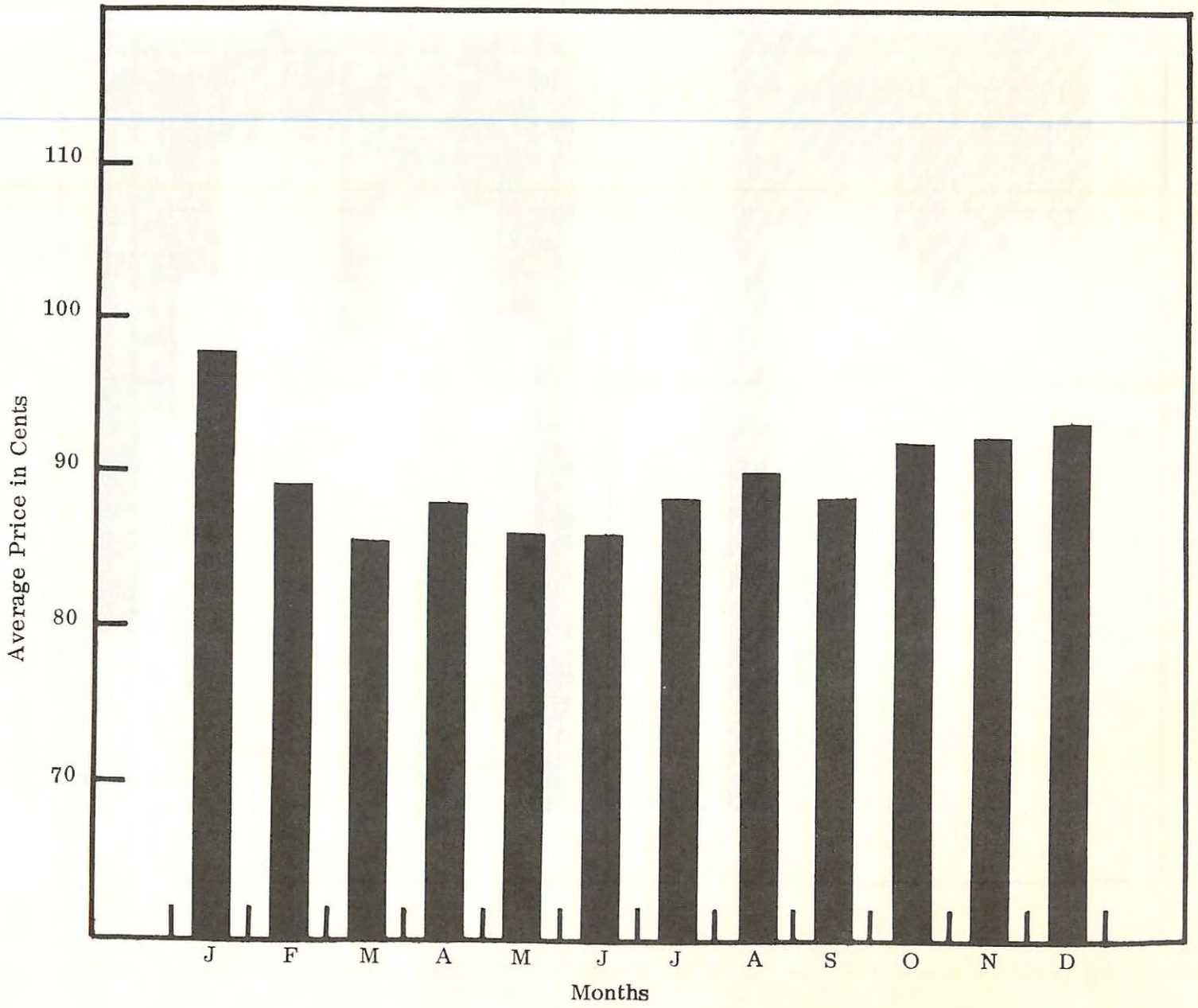



Fig. 5 Average Monthly Prices for Pond Mullet 1950-1969

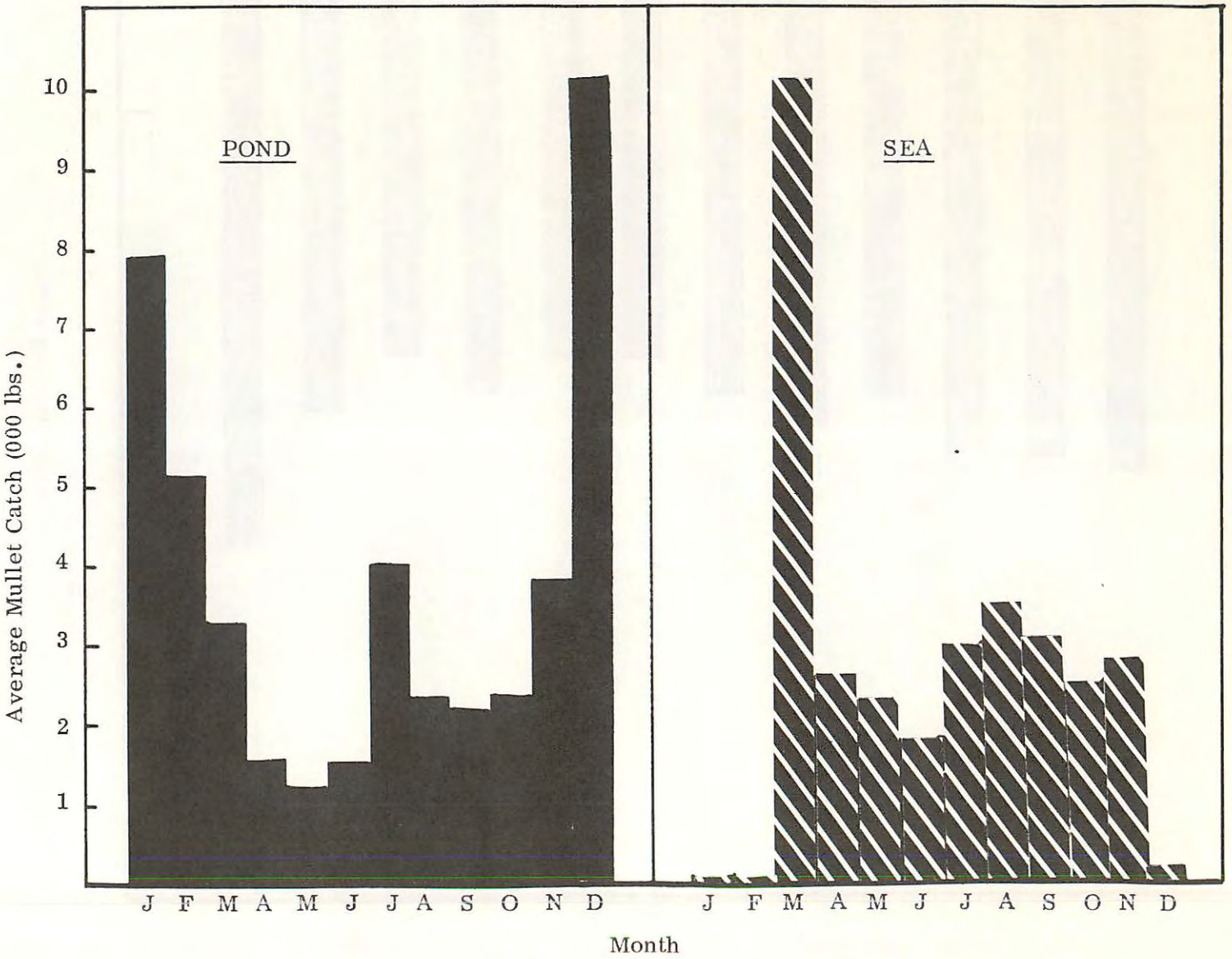



Fig. 6 Average Mullet Catch by Month (1950-59)

production of mullet. Prices for pond production during the 1950-59 period are generally higher than the prices for sea mullet and the volume relatively high although exhibiting wide fluctuations. It should also be noted that there appears to be some correlation between the point in time when the average price for sea mullet overtakes the price for pond mullet and the point in time at which pond volume decreases very rapidly.

## CONCLUSION

Empirically, the evidence presented indicates that sea caught mullet has some advantage over the pond produced fish. When compared to the whole fishing industry, sea mullet has shown definite improvement in its production levels during the 1960-69 period. This trend continued into 1970, as shown in Table 3, with a total of 19,322 pounds being sold on the wholesale market at a price of $1.19 (138 on the price index) per pound. Conversely, pond production of mullet has decreased at a much faster rate than other pond species with this decline continuing through 1970, i.e. only 475 lbs were produced for an average price of $1.13 (121 on the price index) per pound.

Prior to the decade of the 60's, pond production of mullet was relatively competitive with sea caught mullet. The tidal wave that hit Hawaii in 1964 was, by and large, responsible for the sudden decline in pond production. The seaward retaining walls of many aboriginal ponds were destroyed and the prohibitive cost of skilled labor in the Islands precluded repair work. Continuous siltation further reduced mullet production in these ponds due to increased turbidity and decreased photosynthetic activity. Heavy intake of silt by fish also reduced their market value due to the resulting "muddy" taste of the flesh.

Consequently, the major shift in mullet aquaculture economics has occurred in the 1960-69 period. Even though reductions in the yield of pond mullet have occurred in all but one of these years, the most severe decline occurred in 1965 when production levels dropped from 19,823 lbs to 5,426 lbs, as illustrated in Table 3. Based on a conversation with Mr. George Uyemura, one of the few remaining pond operators on Oahu, this decline was primarily due to storm damage to the ponds in late 1964 and early 1965. As a result, the capital costs associated with repairing the damage have prevented him from returning to the production of mullet in his 130 acre pond. Two other factors, which Mr. Uyemura indicated as being of importance, were the lack of juvenile fish supplies for stocking the ponds and the problems of pollution in Kaneohe Bay, where his pond is located. It was also his opinion

Table 3. Total production of the Hawaii fishing industry (1950-1970)

| | Sea Production (pounds) | | Pond Production (pounds) | |
|---|---|---|---|---|
| | All Species | Mullet | Other Species | Mullet |
| 1950 | 16,059,446 | 36,585 | 44,092 | 47,941 |
| 1951 | 19,292,736 | 37,893 | 30,416 | 27,167 |
| 1952 | 13,855,189 | 31,612 | 31,740 | 29,734 |
| 1953 | 18,798,536 | 30,569 | 34,014 | 29,855 |
| 1954 | 20,523,375 | 30,667 | 45,358 | 40,887 |
| 1955 | 15,350,576 | 46,429 | 54,971 | 51,897 |
| 1956 | 16,420,015 | 28,566 | 81,326 | 58,411 |
| 1957 | 10,616,197 | 34,453 | 51,457 | 59,925 |
| 1958 | 11,265,730 | 24,189 | 38,125 | 56,712 |
| 1959 | 16,484,173 | 22,743 | 51,176 | 44,860 |
| 1960 | 11,078,628 | 23,151 | 23,431 | 33,967 |
| 1961 | 14,377,074 | 18,935 | 25,014 | 49,242 |
| 1962 | 13,116,338 | 11,410 | 21,346 | 32,033 |
| 1963 | 11,708,440 | 16,385 | 25,113 | 23,092 |
| 1964 | 12,651,623 | 14,621 | 31,250 | 19,823 |
| 1965 | 19,577,871 | 18,501 | 18,408 | 5,426 |
| 1966 | 13,015,187 | 15,581 | 20,490 | 1,802 |
| 1967 | 11,847,294 | 21,468 | 9,292 | 2,558 |
| 1968 | 13,002,016 | 26,999 | 10,194 | 1,132 |
| 1969 | 9,708,146 | 19,537 | 18,809 | 1,179 |
| 1970 | 11,282,282 | 19,322 | 19,595 | 475 |

that the increasing land values in Hawaii would eventually inhibit pond production and cause land owners to shift to more productive uses of the land available for aquaculture. A good example of this is the Kuapa pond which has been developed into the residential area of Hawaii Kai.

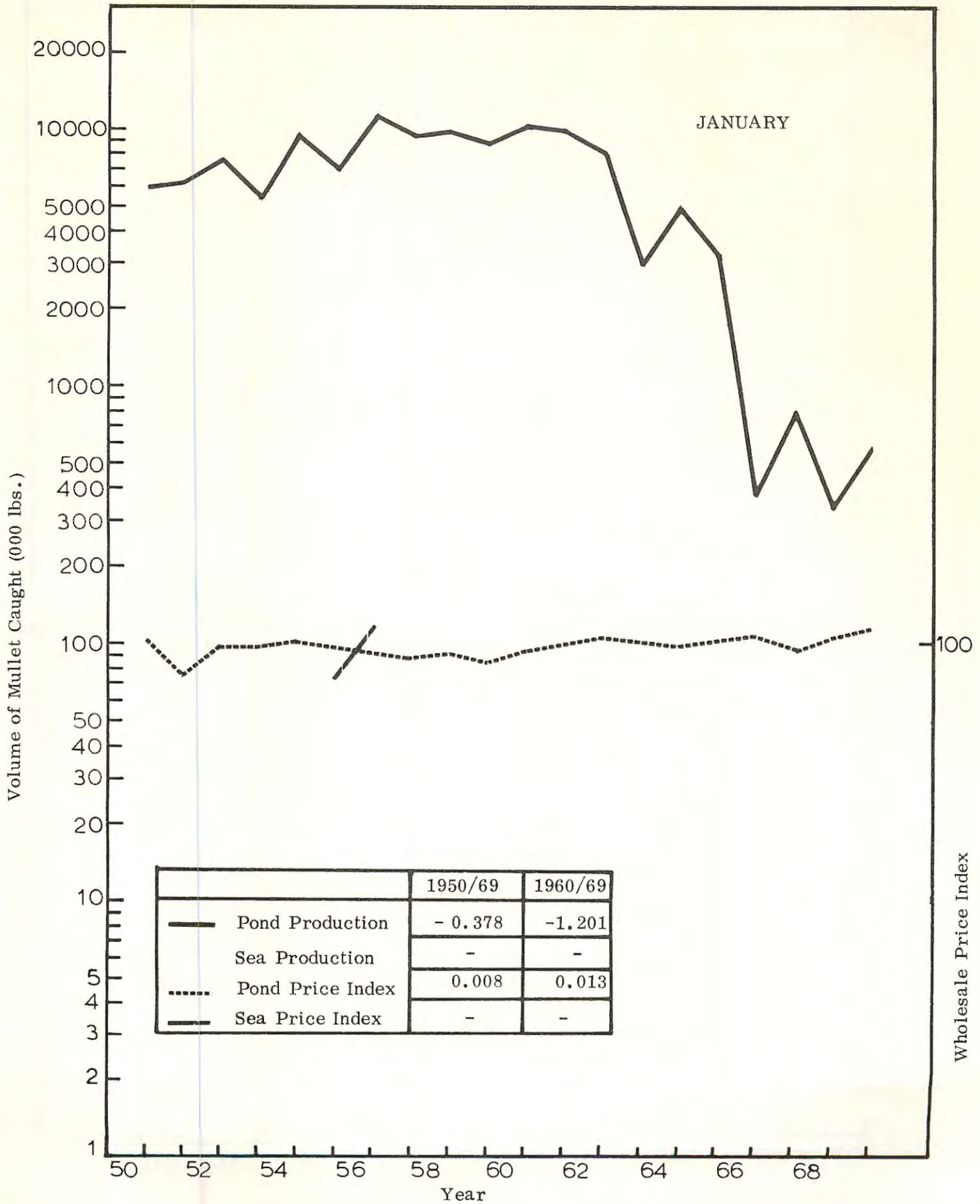
The same general reaction was given by Mr. Kenneth Chow of Oahu. In this case, the pond was operated on a part-time basis and as a result of the increasing time and expense involved, the operation was discontinued in 1966.

Both Mr. Uyemura and Mr. Chow were of the opinion that an adequate market existed for mullet in Hawaii. This assumption is supported by the fact that Miada Fish Markets and Fishland, Inc. import and estimated 120,000 to 150,000 lbs of frozen mullet a year from California and Mexico. It should also be noted that neither company began importing mullet until after the 1965 decline in local pond production. Mr. Larry Konishi, Assistant Manager of the Tamashiro Fish Market in Honolulu, indicated that they import approximately 800 lbs of mullet a month due to the inadequate local supply. It was also Mr. Konishi's opinion that 30,000 to 50,000 lbs of good quality pond mullet could be sold per year in Hawaii.

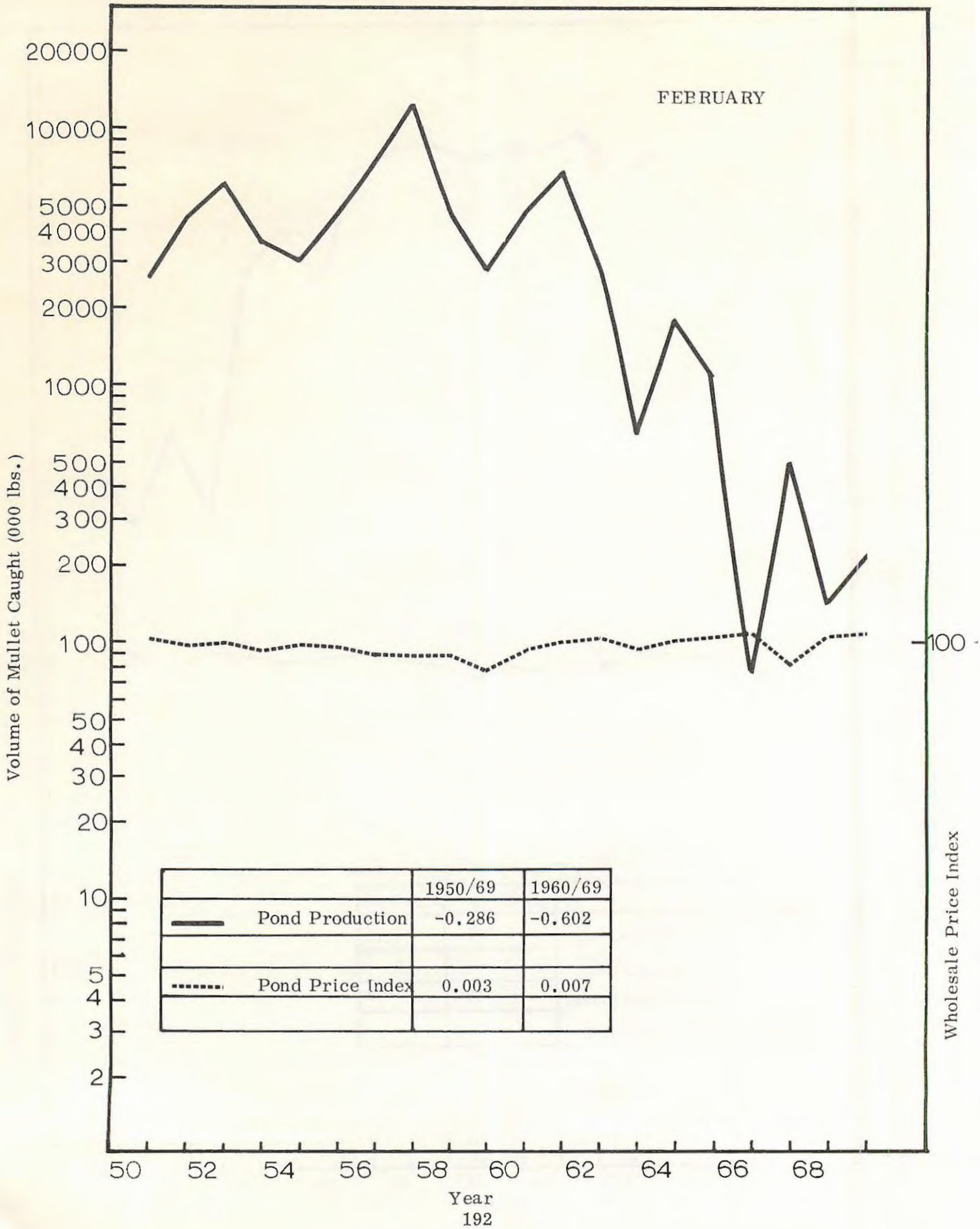
Our research, however, indicates that a local mullet aquaculture operation can replace all imports without meeting the competitive lower price (46¢/lb) of frozen imports. This is due to consumer preference for fresh fish. This preference is related to the fact that the fat content of mullet is of crucial importance, due to the way this fish is prepared by both Hawaiians and Chinese. Frozen imports either not fatty enough or, and more important, freezing causes qualitative changes in body fat which change the taste of the prepared product. Mr. Konishi's remarks are not quite valid. Our estimate of the pond cultured mullet market approximates 300,000-500,000 lbs/yr.

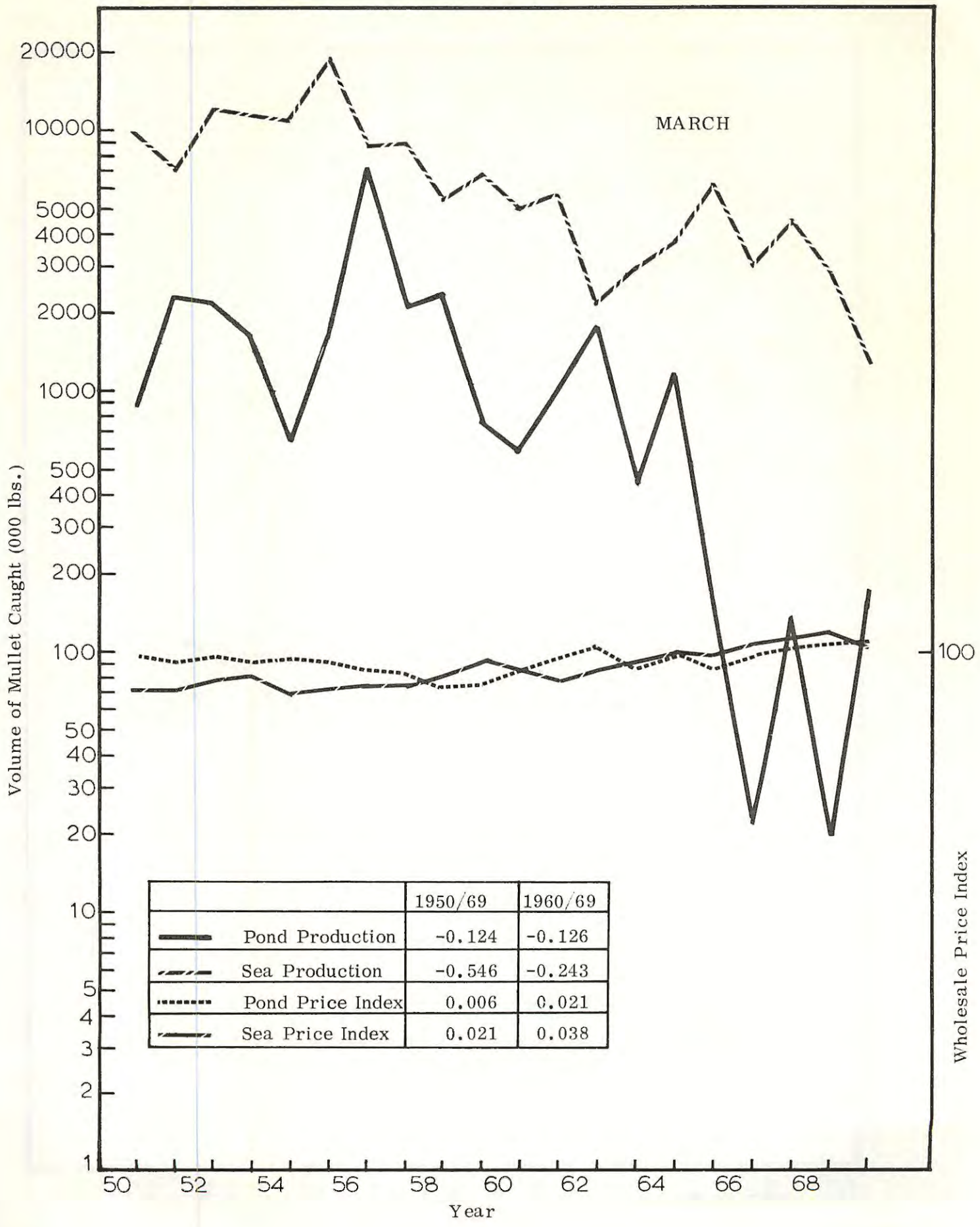
In summary, it appears that a local market does exist and that it is large enough to support a commercial operation. The major obstacles in accomplishing this objective are the operational costs associated with production, and a reliable supply of juvenile fish for stocking. Within the concept of operational costs, is the problem of ever increasing land values, but this could be partially solved by locating the pond operation on the outer islands where the commercial value of land is not rising as fast as on Oahu. The problem of fish quality can be reduced by careful pond construction and the final obstacle of juvenile supply can be eliminated through the use of induced spawning technology now being perfected at the Oceanic Institute.

Fig. 7 - 18. Volume of catch and price index for pond and sea mullet (1950-1969) by month.

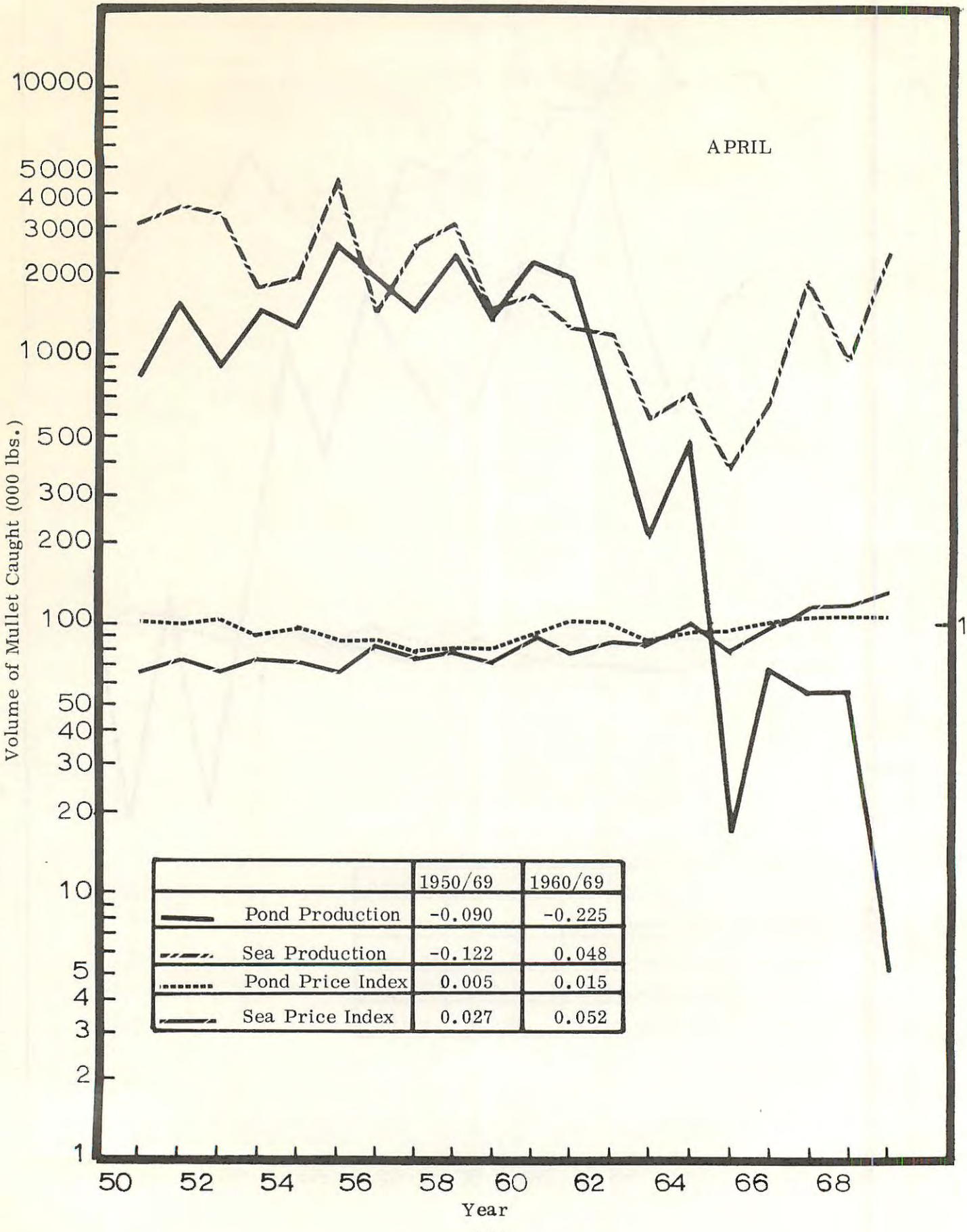



| | 1950/69 | 1960/69 |
|---|---|---|
| Pond Production | - 0.378 | -1.201 |
| Sea Production | - | - |
| Pond Price Index | 0.008 | 0.013 |
| Sea Price Index | - | - |

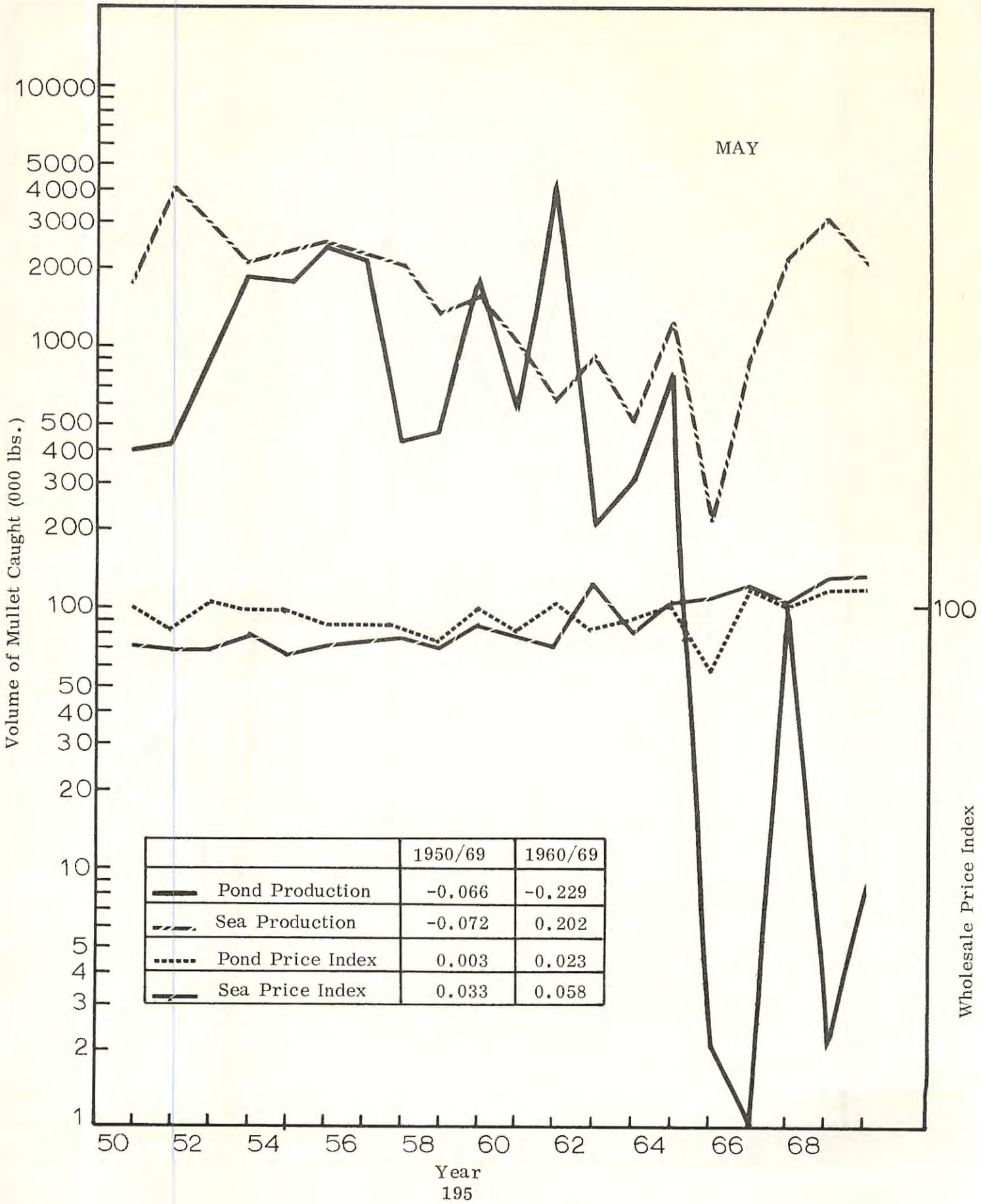

FEBRUARY
20000
10000
5000
4000
3000
2000
1000
500
400
300
200
100
50
40
30
20
10
5
4
3
2
Volume of Mullet Caught (000 lbs.)
100
Wholesale Price Index
50
52
54
56
58
60
62
64
66
68
Year
1950/69
1960/69
Pond Production
-0.286
-0.602
Pond Price Index
0.003
0.007

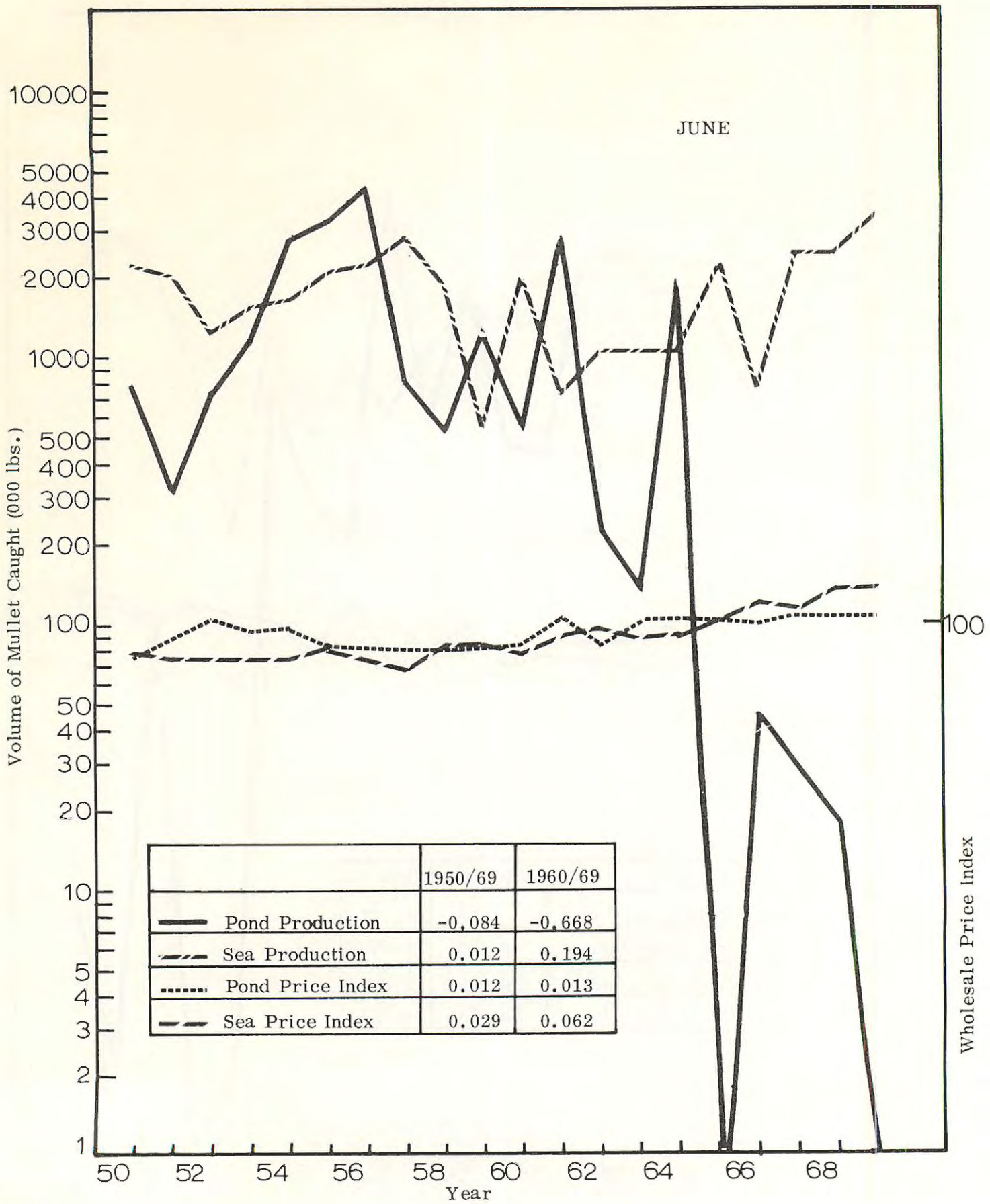


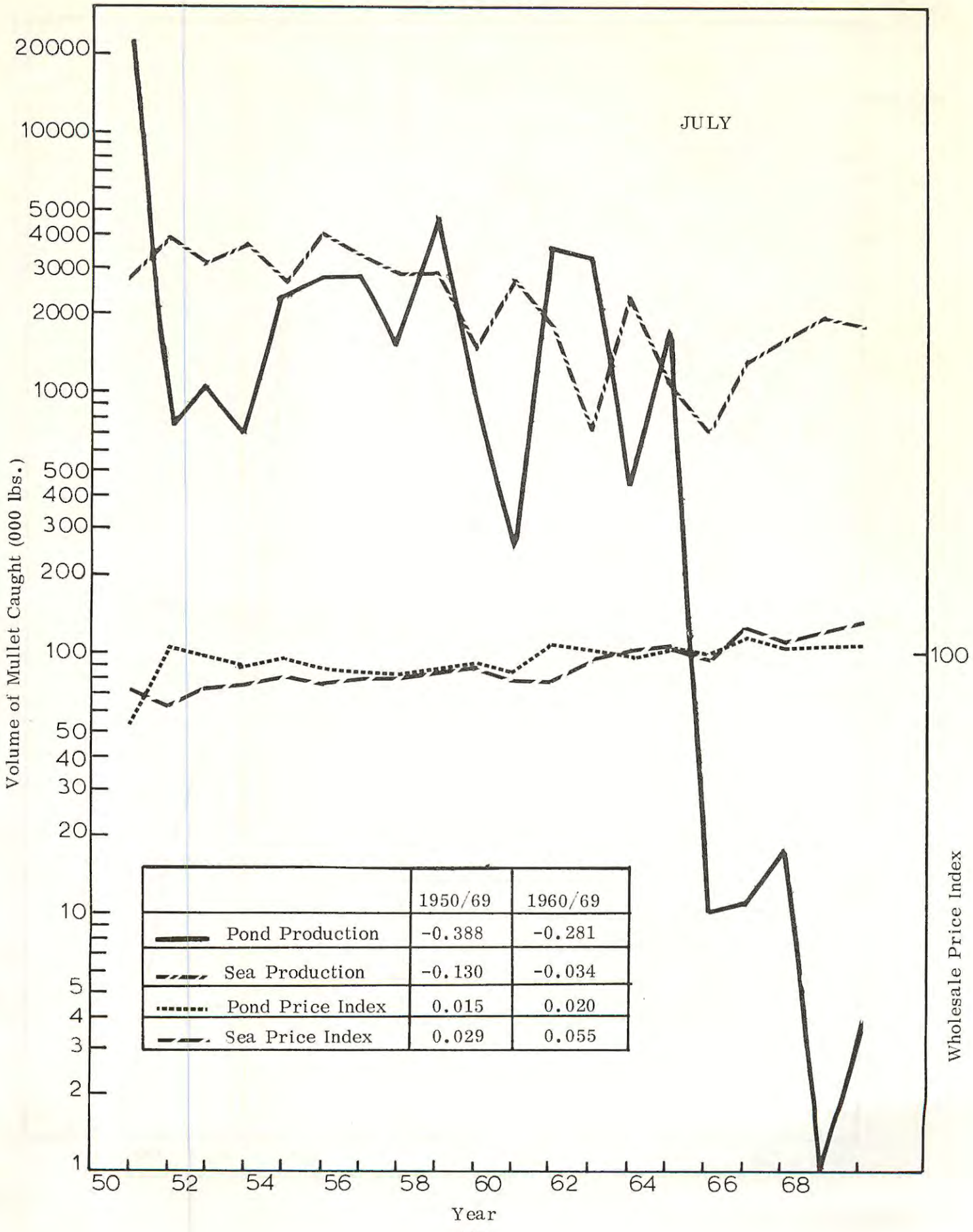

| | 1950/69 | 1960/69 |
|---|---|---|
| Pond Production | -0.124 | -0.126 |
| Sea Production | -0.546 | -0.243 |
| Pond Price Index | 0.006 | 0.021 |
| Sea Price Index | 0.021 | 0.038 |

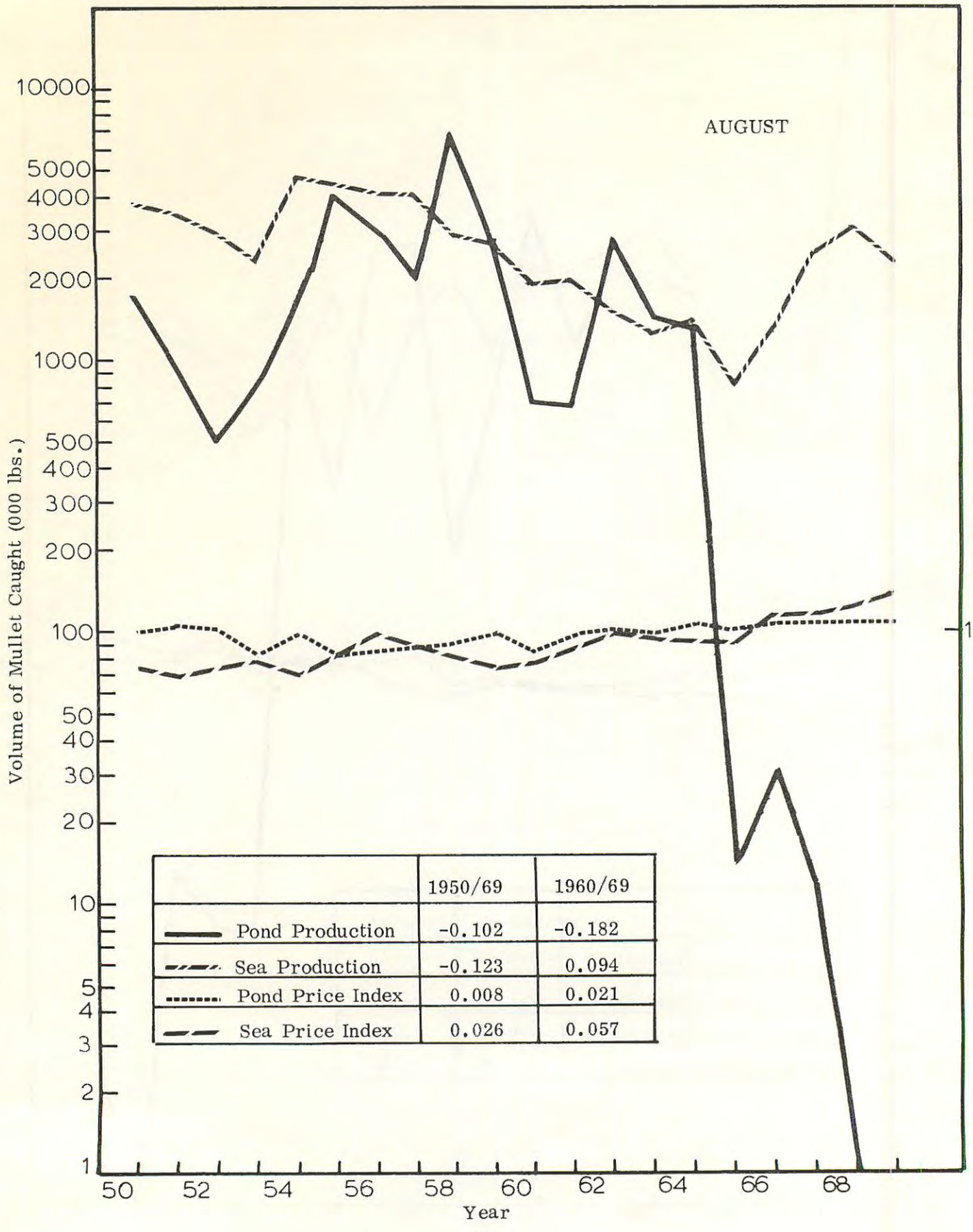


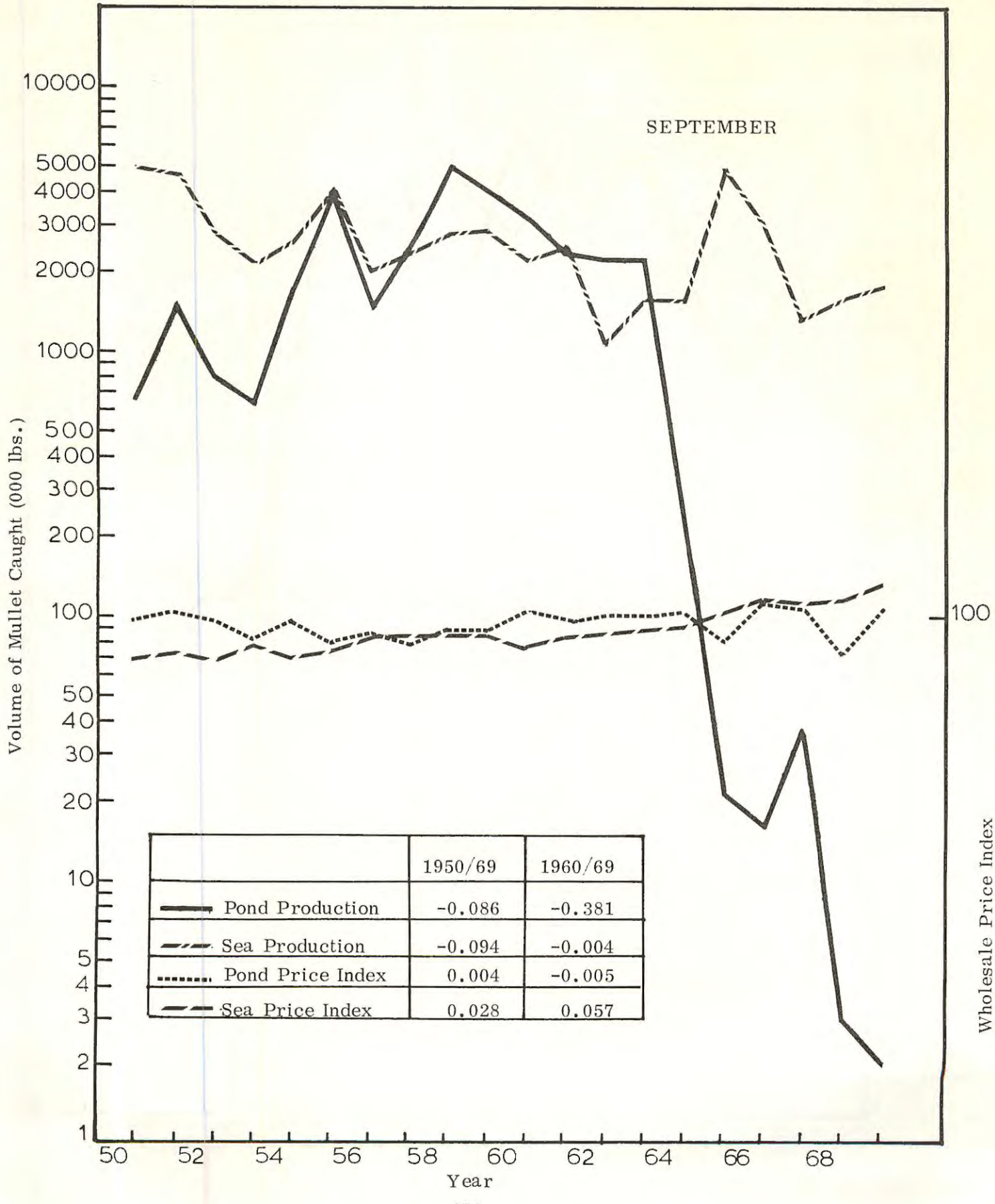

| | 1950/69 | 1960/69 |
|---|---|---|
| Pond Production | -0.090 | -0.225 |
| Sea Production | -0.122 | 0.048 |
| Pond Price Index | 0.005 | 0.015 |
| Sea Price Index | 0.027 | 0.052 |

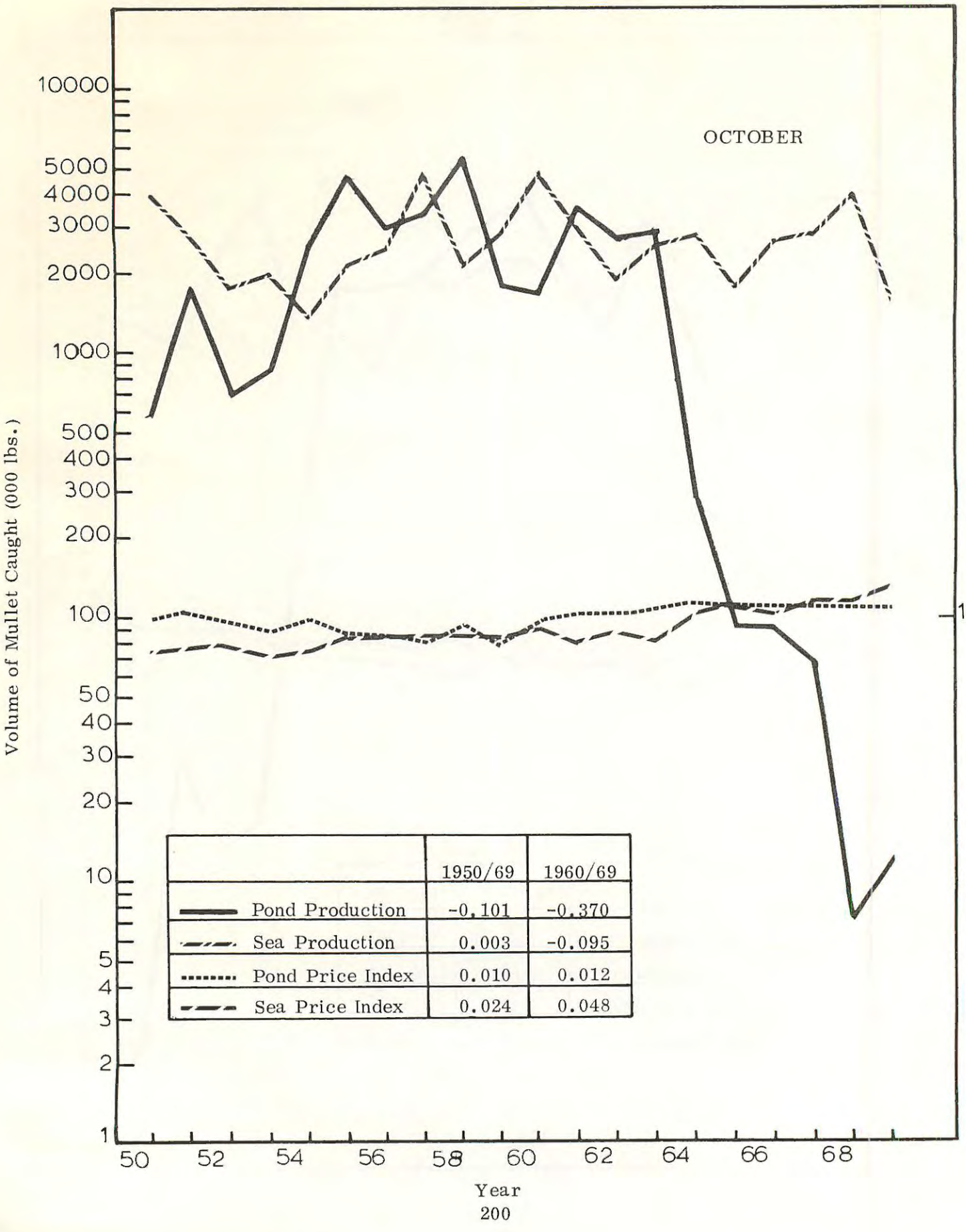

MAY
10000
5000
4000
3000
2000
1000
500
400
300
200
100
50
40
30
20
10
5
4
3
2
1
Volume of Mullet Caught (000 lbs.)
100
Wholesale Price Index
50
52
54
56
58
60
62
64
66
68
Year
1950/69
1960/69
Pond Production
-0.066
-0.229
Sea Production
-0.072
0.202
Pond Price Index
0.003
0.023
Sea Price Index
0.033
0.058

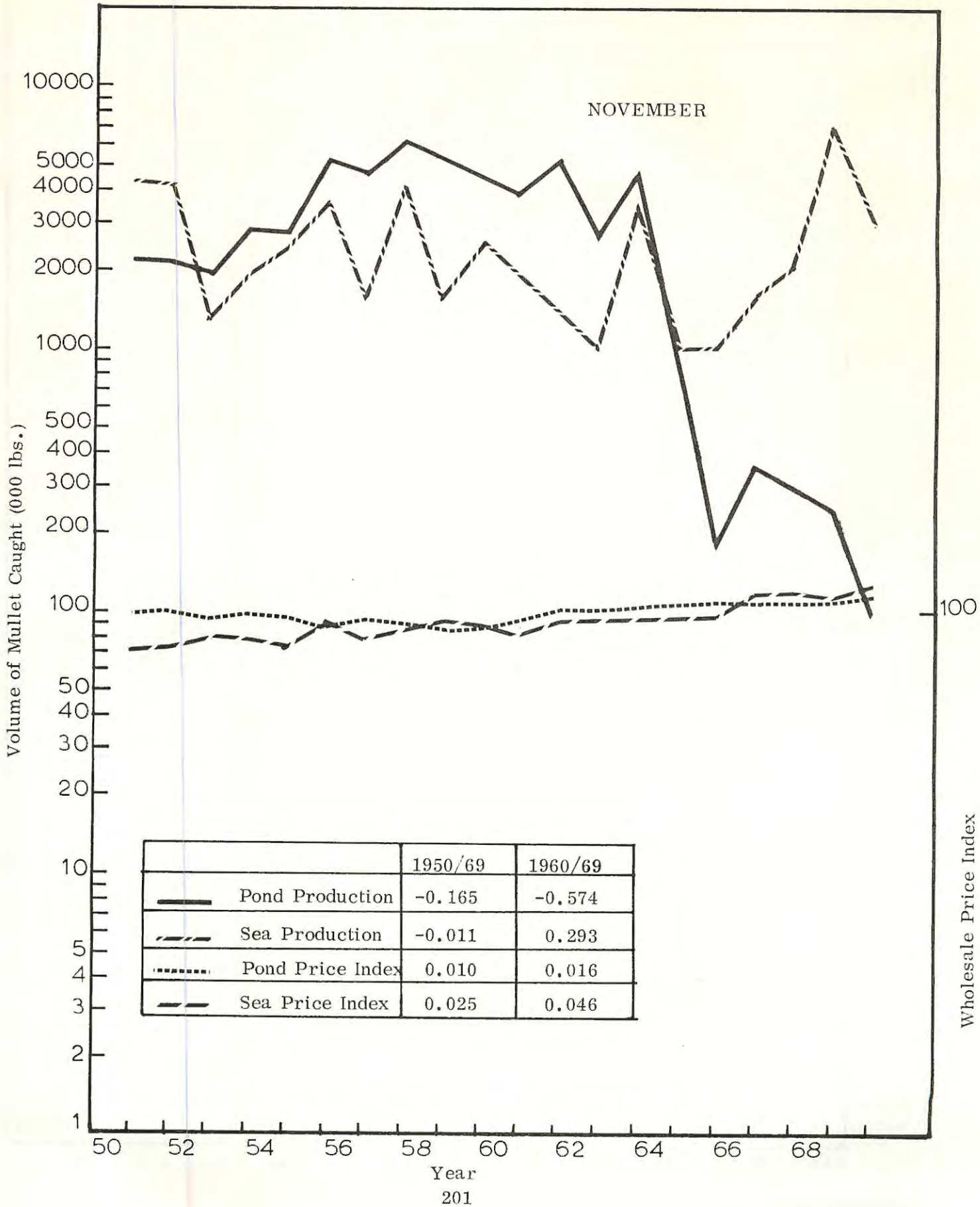

JUNE
Volume of Mullet Caught (000 lbs.)
10000
5000
4000
3000
2000
1000
500
400
300
200
100
50
40
30
20
10
5
4
3
2
1
100
Wholesale Price Index
50
52
54
56
58
60
62
64
66
68
Year
1950/69
1960/69
Pond Production
-0,084
-0,668
Sea Production
0.012
0.194
Pond Price Index
0.012
0.013
Sea Price Index
0.029
0.062


JULY
Volume of Mullet Caught (000 lbs.)
20000
10000
5000
4000
3000
2000
1000
500
400
300
200
100
50
40
30
20
10
5
4
3
2
1
100
Wholesale Price Index
50
52
54
56
58
60
62
64
66
68
Year
1950/69
1960/69
Pond Production
-0.388
-0.281
Sea Production
-0.130
-0.034
Pond Price Index
0.015
0.020
Sea Price Index
0.029
0.055

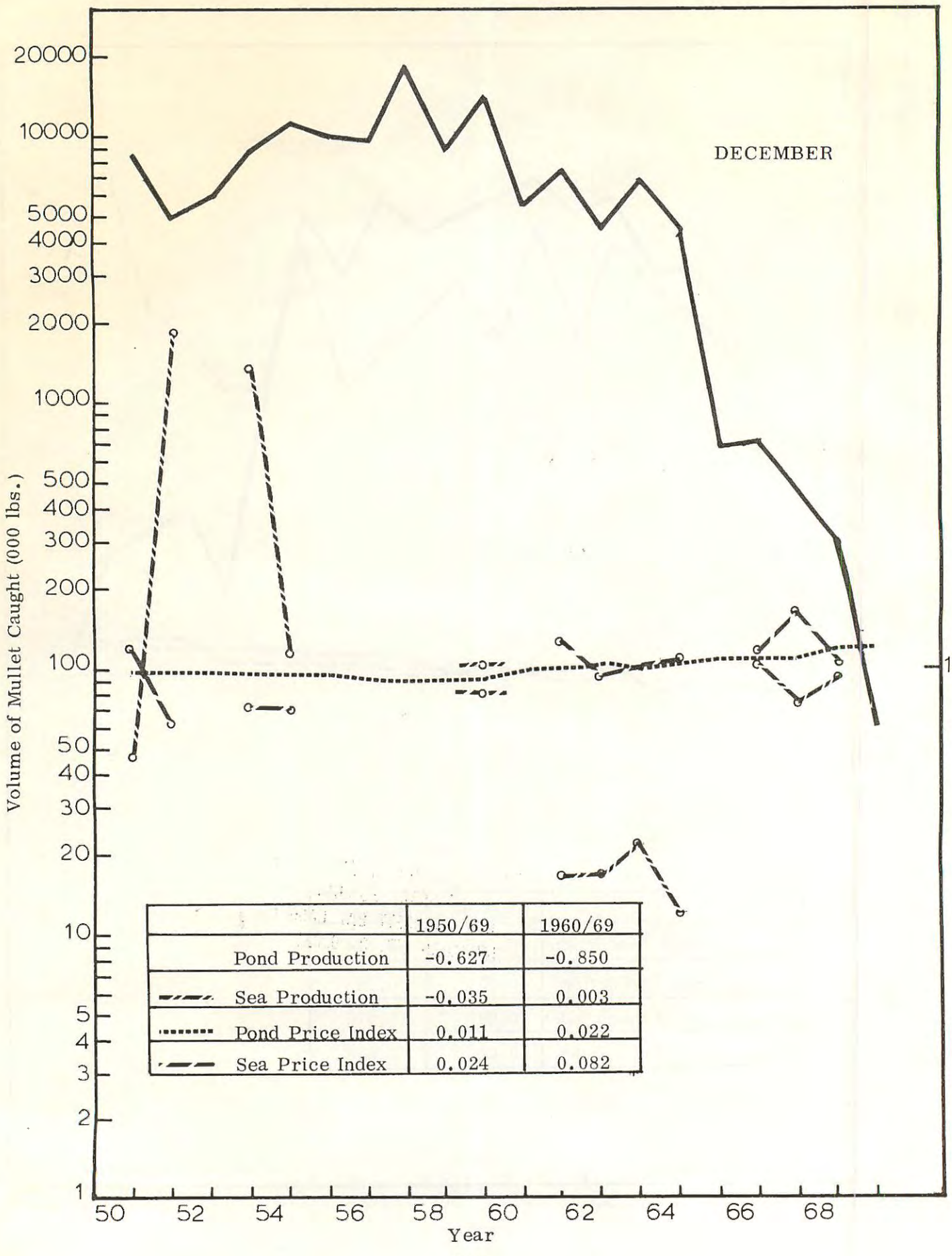

AUGUST
Volume of Mullet Caught (000 lbs.)
Wholesale Price Index
Year
10000
5000
4000
3000
2000
1000
500
400
300
200
100
50
40
30
20
10
5
4
3
2
1
100
50
52
54
56
58
60
62
64
66
68
1950/69
1960/69
Pond Production
-0.102
-0.182
Sea Production
-0.123
0.094
Pond Price Index
0.008
0.021
Sea Price Index
0.026
0.057


SEPTEMBER
10000
5000
4000
3000
2000
1000
500
400
300
200
100
50
40
30
20
10
5
4
3
2
1
Volume of Mullet Caught (000 lbs.)
100
Wholesale Price Index
50
52
54
56
58
60
62
64
66
68
Year
1950/69
1960/69
Pond Production
-0.086
-0.381
Sea Production
-0.094
-0.004
Pond Price Index
0.004
-0.005
Sea Price Index
0.028
0.057


OCTOBER
Volume of Mullet Caught (000 lbs.)
Wholesale Price Index
10000
5000
4000
3000
2000
1000
500
400
300
200
100
50
40
30
20
10
5
4
3
2
1
100
50
52
54
56
58
60
62
64
66
68
Year
200
1950/69
1960/69
Pond Production
-0.101
-0.370
Sea Production
0.003
-0.095
Pond Price Index
0.010
0.012
Sea Price Index
0.024
0.048




| | 1950/69 | 1960/69 |
|---|---|---|
| Pond Production | -0.165 | -0.574 |
| Sea Production | -0.011 | 0.293 |
| Pond Price Index | 0.010 | 0.016 |
| Sea Price Index | 0.025 | 0.046 |




| | 1950/69 | 1960/69 |
|---|---|---|
| Pond Production | -0.627 | -0.850 |
| Sea Production | -0.035 | 0.003 |
| Pond Price Index | 0.011 | 0.022 |
| Sea Price Index | 0.024 | 0.082 |