OPTIONAL FORM NO. 10
JULY 1973 EDITION
GSA FPMR (41 CFR) 101-11.6

UNITED STATES GOVERNMENT

Memorandum

PD-NAO-885

TO : ~~DAA~~/DS/~~FH~~, Tony Babb (Acting)

DATE: NOV 13 1978

THRU : DS/PO, Robert Simpson

FROM : DS/AGR, Dean Peterson

SUBJECT: Small Research Proposal "Swimbladder Inflation Physoclistous Fish" - University of California

DS/AGR staff have carefully reviewed the above referenced research proposal. We believe this is a well conceived research activity and a useful one in terms of advancing knowledge about larval culture of marine fish which are important food products in LDCs. The proposed research does not pertain to milkfish since Chanos sp have the swimbladder-gut connection; however, it is very relevant for mullet and other tropical species, and will address a problem which is likely to be of importance to the aquaculture research - hatchery aspect of the project being implemented by USAID/Cairo.

The proposal is well planned, realistic, and scientifically sound. The proposed research will provide a useful contribution to the body of knowledge generally and as indicated above is justified in terms of its relationship to LDC needs and other AID activities.

The budget level which is proposed is adequate and realistic. The principal investigator is fully competent to direct the research and the University of California has adequate laboratory, support facilities, and supervisory staff to insure that the project will be carried out as planned.

The proposal as presented is an adequate statement of work and we have no suggestions or requirements for amendments.

The Fisheries Division is prepared to assume management responsibility for the project if it is approved for funding. We estimate that about three or four days of staff time would be required to look in on the research work at the University of California from time to time coinciding with other travel commitments. Dr. Heal from the Fisheries Division staff would be assigned as project manager.

5010-110

This proposal so far as this office is concerned is unsolicited and our staff has had no previous discussions relating to this line of research with the principal investigator or with University of California staff.

I recommend that you approve this activity as a small research project at a funding level of $78,057 as indicated in the proposal.

APPROVED: [signature]

DISAPPROVED: _____

DATE: 1/ 28 · 78

DS/PO OFFICIAL FILE

v

YELLOW

AID 1350-1X (1-78)

PIO/T

DEPARTMENT OF STATE
AGENCY FOR
INTERNATIONAL DEVELOPMENT

PROJECT IMPLEMENTATION ORDER/TECHNICAL SERVICES

1. Cooperating Country: DS Bureau

Page 1 of Pages 1

2. PIO/T No.: 931-1157.11-369[illegible]2

3. ☒ Original or Amendment No. ____

4. Project/Activity No. and Title: 931-1157
Swimbladder Inflation
Physoclistous Fish (Univ. Calif., Davis)
(Small Research Grant)

DISTRIBUTION

FILE COPY

5. Appropriation Symbol: 72-1191021.3

6. Allotment Symbol and Charge: 943-36-099-00-22-91

7. Obligation Status: ☒ Administrative Reservation ☐ Implementing Document

8. Project Assistance Completion Date (Mo., Day, Yr.): January 31, 1980

9. Authorized Agent: AID/W

10. This PIO/T is in full conformance with PRO/AG: N/A Date ____

11a. Type Governing AID Handbook: ☐ AID (HB) ☐ PASA/RSSA (HB 12) ☒ AID Grant (HB 13) ☐ Other

11b. Contract/Grant/PASA/RSSA Reference Number (If this is an Amendment)

12. Estimated Financing (A detailed budget in support of column (2) is attached as attachment no. I)

		(1) Previous Total	(2) Increase	(3) Decrease	(4) Total to Date
Maximum AID Financing	A. Dollars		$28,057		$28,057
	B. U.S.-Owned Local Currency				

13. Mission References: DS/AGR to AA/DS (Acting) Memo dtd 13 Nov. 1978

14a. Instructions to Authorized Agent: CM/COD/AN is authorized to prepare a Grant Agreement between AID and the Univ. of Calif., Davis for the Small Research Activity to be entitled Swimbladder Inflation Physoclistous Fish. The total cost of the activity is estimated at $38,242; the University is to provide the difference of $10,185. The period of the Grant should be February 1, 1979, through January 31, 1970.
Attachment II: Research Proposal and Grant Application for the proposed research to be entitled Swimbladder Inflation in Physoclistous Fish, dated 17 November 1978;
Attachment III: Scope of Work; and
Attachment IV: Report Provisions.
Vouchers: Address SF 1034 submissions as shown in block 14b and include grant nc., project no., and DS/AGR/Fisheries.

14b. Address of Voucher Paying Office: SER/FM/PAD, Rm. 601 SA-12
Agency for International Development
Washington, D.C., 20523

15. Clearances—Include typed name, office symbol, telephone number and date for all clearances.

A. The project officer certifies that the specifications in the statement of work are technically adequate
DS/AGR/F, DJones — Phone No. — Date 1/5/79

B. The statement of work lies within the purview of the initiating and approved agency programs
DS/PO/FN, PGage — Date [illegible]
DS/PO/FN, RARogers

C. DS/AGR, CSMcClusky — Date 05 Jan 79
DS/AGR, MMozynski

D. Funds for the services requested are available
FUNDS RESERVED BY
Richard C. Egan
Jan 11, 1979
DS/PO/PPA, MEganS[illegible]

E. DS/AGR, DFPeterson — Date 1/8/79
DS/PO/RES, MRechcigl — 1/10/79

16. For the cooperating country: The terms and conditions set forth herein are hereby agreed to

Signature N/A Date ____

Title

17. For the Agency for International Development

Signature Kenneth A. Milow Date 1/10/79

Title Kenneth A. Milow
Chief, DS PO PPA

ATTACHMENT I

ESTIMATED BUDGET

	AID	UNIVERSITY
A. SALARIES		
Serge I. Doroshov (20%)		$4,920
Wallis H. Clark, Jr. (5%)		1,450
Graduate Research Asst. (50%) (John Cornacchia)	$6,608	
Employee benefits	83	1,405
Total	$6,691	$7,775
B. EQUIPMENT		
Tanks	5,800	
Water chillers	2,800	
Thermoregulators with temperature recorders	2,700	
Total	$11,300	
C. EXPENDABLE SUPPLIES		
Glassware	$1,500	
Electron microscopy (filament time)	800	
Chemicals	700	
Photographic materials	1,200	
Fish feed	500	
Total	$4,700	
D. MISCELLANEOUS		
Publication costs	$ 500	
Travel	900	
Total	$1,400	
Total Direct Costs	$24,091	$7,775
Total Indirect Costs (31%, less equipment)	3,966	2,410
	$28,057	$10,185

ATTACHMENT II

GRANT APPLICATION TO: Agency for International Development
U. S. State Department
Washington, D. C. 20523

I. NAME AND ADDRESS OF INSTITUTION: The Regents of the University of California
University of California
Davis, Ca. 95616

II. TITLE OF PROPOSED RESEARCH: Swimbladder inflation in Physoclistous fish

III. PROPOSED STARTING DATE OF RESEARCH: November 1, 1978

IV. TOTAL PERIOD FOR WHICH SUPPORT IS REQUESTED: One year
11/1/78 - 10/31/79

V. TOTAL SUPPORT REQUESTED: $28,057.00

VI. CHECKS SHOULD BE MADE PAYABLE TO: The Regents of the University of California
c/o Accounting Officer
University of California
Davis, Ca. 95616

APPROVALS:

PRINCIPAL INVESTIGATOR
Serge I. Doroshov, Animal Science Department
Office phone: (916) 752-7603
[redacted]

CO-INVESTIGATOR
Wallis H. Clark, Jr., Animal Science Department
Office phone: (916) 752-7600
[redacted]

CHAIRMAN, ANIMAL SCIENCE DEPARTMENT
R. L. Baldwin

NOV 17 1978

THE REGENTS OF THE UNIVERSITY OF CALIFORNIA
for Allen G. Marr, Dean
Research Development
University of California
Davis, Ca. 95616

INTRODUCTION

Existing laboratory and hatchery techniques for the larval rearing of many marine fish (mullets, tubot, croakers, sea-basses) and some anadromous fish (striped bass) have resulted in low survival rates prior to metamorphosis. Most of these fish belong to the Physoclistous (fish not having a connection between their swimbladder and intestine which have to regulate bladder gas pressure and neutral buoyancy by the secretion of gas) which are very important to commercial aquaculture in both developed and underdeveloped countries.

Ideally, the larvae of pelagic fish develop a functioning swimbladder during the initiation of external feeding. However, under culture conditions large larval mortalities, due to improper filling of the swimbladder, have been reported in striped bass (Doroshov, 1970); turbot (Spectorova et al., 1974; Spectorova and Doroshov, 1976); grey mullet (Nash et al., 1974, 1977); silver mullet (Houde et al., 1976); and polar cod (Aronovich et al., 1975). As a rule, the survival rate of culture pelagic marine Physoclistous fish, at best, is only 30%. In contrast, the survival rate for some bottom fish lacking a swimbladder (Shelbourne, 1964, plaice; and Fluchter, 1965, sole) is 50 to 80%.

Unfortunately, the mechanisms of swimbladder inflation and the abnormalities associated with this phenomenon are poorly understood. This lack of understanding must be rectified before significant advances, in terms of larval survival rates, will be realized in the culture of Physoclistous fish.

WORK TO DATE

Numerous reports have been published on the structure and function of the swimbladder in adult fish (for reviews see: Jones and Marshall, 1953, and Fange, 1966). The theory of the countercurrent mechanism of gas exchange, based on anatomical and physiological studies of the retia mirabile and the gas gland, is normally used as an explanation of swimbladder function in the adult Physoclistous fishes (Scholander, 1954; Scholander and van Dam, 1954; Scholander, 1958; Fange, 1953, 1966; Kunn et al., 1963; Wittenberg, 1961; Wittenberg et al., 1964; Steen, 1963). However, the mechanism of gas formation and the pathway by which the gas reaches the swimbladder lumen is still unclear.

Copeland (1969) has suggested that secretory cells of the swimbladder epithelium are responsible for the transport of molecular oxygen provided by the countercurrent exchange in the retia. He further suggests that this exchange is mediated by an iron-protein compound present in the cytoplasm of the epithelial cells. This explanation is largely based on morphological studies.

Data on swimbladder development and function in larval stages are very scarce and often contradictory. Physostomatous fish larvae undergo swimbladder inflation with apparent ease under culture conditions (Hoar, 1937, salmon; Hunter and Sanchez, 1976, anchovy). These fish regulate the inflation of their swimbladder by simply gulping and releasing atmospheric air which is easily explained since a pneumatic duct exists between the gut and the swimbladder.

It is generally believed by fish culturists and ichthyologists that a primordial pneumatic duct exists in the larvae of Physoclist fishes which becomes occluded and degenerates after the initial inflation of the swimbladder. This initial inflation has been explained by the gulping of atmospheric air (Ledebur, 1928; Ledebur and Wunder, 1937) or the swallowing of microscopic gas bubbles produced by aquatic plants or algae (Kryzanovsky, 1946). The "gulping" theory

for the initial inflation of the swimbladder in Physoclist larvae is generally accepted by fish culturists and ichthyologists. This theory, however, has been questioned by Marshall (1960) and McEwen (1940) who, working with Hemichromis bimaculata, demonstrated the complete lack of a connection between the lumen of the swimbladder and the intestine during larval development.

Preliminary data obtained in our laboratory with Tilapia mossambica and Morone saxatilis has substantiated the results of McEwen (1940). We have not found a duct between the swimbladder lumen and foregut in Tilapia larvae prior to swimbladder inflation, though we have identified columnar epithelial cells in both species which appear secretory. These cells are apparent along the bladder lumen and exhibit a pronounced "secretory" morphology just prior to inflation (which occurs in Tilapia larvae on days 11-12 after hatching at temperatures 24-26° and in striped bass on days 6-8 at 18°). During inflation the columnar cells become cuboidal and lose their secretory appearance.

Further experiments have shown that T. mossambica larvae in low density can successfully fill their swimbladder if placed in containers with a thick film of oil on the water surface so as to deny the larvae surface access. In similar experiments where the larval density was greatly increased or hypoxic conditions were created, the larvae did not exhibit swimbladder filling. Though these experiments are preliminary, they strongly suggest that the gas content of the water may be the determining factor during the critical period of swimbladder inflation.

While our preliminary work has been done with freshwater Physoclistous larvae only, one would expect similar results with marine species. It is interesting to note that Spectorova and Doroshov (1976) found extreme differences in the rate of swimbladder inflation of larval turbot held in the tanks with different algae blooms and oxygen saturations.

OBJECTIVES

The overall goal of the proposed project is to improve techniques for larval rearing of commercially important marine and anadromous fishes by better understanding the mecnanisms of the initial swimbladder inflation and the environmental factors controlling this phenomenon. Specifically, the following will be examined:

1. a careful correlation will be made between environmental factors (gas content and temperature) and swimbladder inflation;
2. morphological studies will be conducted on swimbladder development (at both the light and electron microscopic level) prior to, during and after inflation (both in normal and abnormal conditions) from animals selected from the above (1) environmental studies.

A careful correlation between (1) and (2) should illucidate the influence of environmental factors on swimbladder inflation as well as providing a means of monitoring normal inflation in larval culture technology.

PROCEDURES

1. ANIMALS

Experimental animals will include a freshwater, anadromous and marine Physoclistous fish. The species to be used will be:

a. Tilapia mossambica, a freshwater tropical mouth-brooder, will be used throughout the year. A breeding population is maintained on the Davis campus. Progeny will be obtained by natural spawning of fish in aquaria and newly-emerged larvae or fertilized eggs will be removed from the female's mouth.

b. Morone saxatilis eggs and larvae are routinely obtained from the California Fish and Game Elk Grove Hatchery. Spawning is seasonal and larvae are available during the months of April and May

c. Mugil cephalus and/or one of the species of Sciaenidae will be obtained in Southern California and a stock maintained at the Bodega Marine Laboratory. Maturation and spawning will be induced by photoperiod, temperature and hormonal injections.

2. EXPERIMENTAL DESIGN

Combinations of three temperatures and three oxygen saturation levels will be used with the larvae of each species. These environmental factors will be maintained within the natural tolerance range for each species as follows:

(a) Temperature (°C): Tilapia mossambica.....24-26-28
Morone saxatilis.....16-18-20
Mugil cephalus.....20-22-24

(b) Oxygen (in % saturation): Tilapia mossambica.....40-80-120-ambient
Morone saxatilis.....60-100-140-ambient
Mugil cephalus.....60-100-140-ambient

Experimental series will be run at each temperature using one open container for ambient O_2 saturation and three closed containers for maintenance of experimental O_2 tensions. The total number of containers for each replicate will be 12 and all containers will use larval progeny from one female.

3. EXPERIMENTAL TECHNIQUE

Three to five-gallon plexiglass cylindrical containers will be used for all experiments. Sets (4) of these containers will be held in a constant temperature water tank. Each container will be connected to a separate reservoir or water supply tank (10-12 gal) where O_2 saturation will be monitored (Fry, 1951; Cech and Wahlschlag, 1973).

Each experimental container and its reservoir will represent a closed system. The flow or exchange rate within such a system will be 0.05 to 0.1 liters/min which is adequate to maintain larvae in the water column. Larvae will not be fed during experiments since larval starvation and "points-of-no-return" develop after swimbladder inflation and thusswell after the normal duration of these experiments.

4. PROCUREMENT AND HANDLING OF LARVAE

Eggs will be incubated at the optimal temperature for embryonic development of each species (25°C for Tilapia, 18°C for striped bass and 22°C for mullet). Only larvae from spawns exhibiting high-hatching success and normal embryonic development will be used.

On the second day post hatch, larvae will be transferred to experimental containers. Tilapia larvae will be stocked at the rate of 25 per container (due to the greatly lower fecundity but higher survival rate of these animals compared to striped bass and mullet) and striped bass and mullet at the rate of 100 per container.

Observations in our laboratory have shown that the primordial swimbladder is formed in Tilapia larvae 5-6 days post hatch and 4 days post hatch in striped bass. Swimbladder inflation occurs 11-12 and 6-8 days post hatch, respectively. Mullet larvae appear very similar to striped bass in terms of the timing of these events (Nash et al, 1977). Therefore, the expected duration of experimental times will be 12-14 days for Tilapia and 8-10 days for the other species.

5. SAMPLING

Larvae will be sampled periodically during experiments, narcotized with MS-222 and the following characteristics examined:

a. The formation of a gas bubble in the swimbladder.
b. The development of a functional jaw and intestine.
c. The development of the circulatory system.
d. Eye pigmentation.
e. Larval length.
f. The size of the yolk sac and oil globule.

Some of the above animals will be sacrificed for histological and fine structural examinations.

a. Histological studies: Larvae exhibiting both normal and abnormal swimbladder development and inflation will be fixed in Bouin's. Serial, 7 u parraffine sections will be prepared, including longitudinal, cross and frontal sections. These sections will be stained with iron haematoxylin and eosin and examined. The development of the swimbladder and adjacent organs (pneumatic duct, "gas gland" and circulatory system) will be described and correlated with the varying environmental parameters.

b. Fine structural studies: These studies will be run in conjunction with the histological work to determine if activity levels of the bladder secretory epithelium can be demonstrated and correlated with gas bubble formation.

Swimbladders and adjacent circulatory tissues will be exised from larvae exhibiting both normal and abnormal inflation. These tissues will be fixed in Karnovsky's solution (1965), post fixed in buffered 1% osmium tetroxide, dehydrated in a graded acetone series, and imbedded in a low viscosity plastic (Spurr, 1969).

Sections will be prepared on a Sorvall MT-2B ultramicrotome using glass and diamond knives, mounted on uncoated copper grids, stained with uranyl acetate (Venable and Coggeshall, 1965) and lead citrate (Watson, 1958) and viewed with an electron microscope. Thick plastic sections,

for light microscopy, will be stained with 1% methylene blue buffered in 1% sodium borate.

SUMMARY

The results and analysis of the data from these experiments will provide insight pertaining to the effect of environmental factors on swimbladder inflation as well as the relationship of inflation to larval size, yolk utilization and oil-globule absorption. This information is severely needed for the improvement of techniques related to hatchery systems for Physoclistous fish.

REFERENCES

Aronovich, T. M., S. I. Doroshov, L. V. Spectorova and V. M. Makhotin. 1975. Eggs incubation and larval rearing of navaga (Eleginus navaga Pall.), polar cod (Boreogadus saida Lepechin) and arctic flounder (Liopsetta glacialis Pall.) in the laboratory. Aquaculture 6:233-242.

Cech, J. J. and D. E. Wahlschlag. 1973. Respiratory response of the striped mullet, Mugil cephalus (L.) to hyposic conditions. J. Fish. Biol. 5:421-428.

Copeland, D. E. 1969. Fine structural study of gas secretion in the Physoclistous swimbladder of Fundulus heteroclitus and Gadus callarieas and in Euphysoclistous swimbladder of Opsanus tau. Z. Zellforsch, 93:305-331.

Doroshov, S. I. 1970. The biological peculiarities of the eggs, larvae and juveniles of Roccus saxatilis. J. of Ichthyology 10(2):235-248.

Fänge, R. 1953. The mechanism of gas transport in the euphysoclist swimbladder. Acta physiol. scand. 30(110):1-133.

Fänge, R. 1966. Physiology of the swimbladder. Physiol. Rev. 46:299-322.

Flüchter, J. 1965. Versuche zur Brutaufzucht der Seezunge Solea solea in kleinen Aquarien. Helgol. wiss. Meeresunters 12:395-403.

Fry, F. E. J. 1951. A fractioning column to provide water of various dissolved oxygen content. Can. F. Technol. 29:144-146.

Hoar, W. S. 1937. Development of the swimbladder of the atlantic salmon. J. Morph. 61:309-319.

Houde, E. D., S. A. Berkeley, J. J. Klinovsky and R. C. Schekter. 1976. Culture of larvae of the white mullet Mugil curema Valenciennes. Aquaculture 8(4):365-370.

Hunter, J. R. and C. Sunchez. 1976. Diel changes in swimbladder inflation of the larvae of the northern anchovy, Engraulus mordax. Fish Bull. 74(4):847-855.

Jones, F. R. H. and N. B. Marshall. 1953. The structure and function of the teleostean swimbladder. Biol. Rev. 28:16-83.

Karnovsky, J. J. 1965. A formaldehyde-glutaraldehyde fixative of high osmolarity for use in electron microscopy. F. Cell. Biol. 27:137A.

Kryzanovsky, S. G. 1946. Some basic principles of the early development in Perciformis fishes. Proc. Inst. An. Morph. 18:1-154 (in Russian).

Kuhn, W., A. Ramel, H. J. Kuhn and E. Marti. 1963. The filling mechanism of the swimbladder. Experientia, XIX, Fasc. 10:497-552.

Ledebur, J. F. von. 1928. Beitrage zur Physiologie der Schwimmblase der Fische. Zeit. Verg. Physiol. Bd. 8, S:445-460.

Ledebur, J. F. von and W. Wunder. 1937. Beitrage zur Physiologie der Schwimmblase der Fishe IY. Z. Vergleich Physiol. Bd. 25, S:149-155.

Marshall, N. B. 1960. Swimbladder structure of deep-sea fishes. Disc. Rep. XXXI:1-122.

McEwen, R. S. 1940. The early development of the swimbladder and certain adjacent parts in Hemichromis bimaculata. J. Morph. 67(1):1-57.

Nash, C. E., C. M. Kuo and S. C. McConnel. 1974. Operational procedures for rearing larvae of the grey mullet, Mugil cephalus L. Aquaculture 3(1):15-24.

Nash, C. E., C. M. Kuo, W. D. Madden and C. L. Paulsen. 1977. Swimbladder inflation and survival of Mugil cephalus to 50 days. Aquaculture 12(1):89-94.

Scholander, P. F. 1954. Secretion of gases against high pressure in the swimbladder of deep-sea fishes. Biol. Bull. 107:260-277.

Scholander, P. F. 1958. Counter current exchange. A principle in biology. Hvalradets Skriftert Norske Videnskaps-Akad. Oslo. 44:1-24.

Scholander, P. F. and L. van Dam. 1954. Secretion of gases against high pressure in the swimbladder of deep-sea fishes, I. Oxygen dissociation in blood. Biol. Bull. 107:247-259.

Shelbourne, J. E. 1964. The artificial propagation of marine fish. Adv. Mar. Biol. 2:1-83.

Spectorova, L. V., T. M. Aronovich, S. I. Doroshov and V. P. Popova. 1974. Artificial rearing of the Black Sea turbot larvae (Scophthalmus maeoticus). Aquaculture 4:329-340.

Spectorova, L. V. and S. I. Doroshov. 1976. Experiments on the artificial rearing of the Black Sea turbot (Scophthalmus maeoticus). Aquaculture 9:275-286.

Spurr, A. R. 1969. A low viscosity epoxy resin embedding meium for electron microscopy. J. Ultra-Struct. Res. 26:31.

Steen, J. B. 1963. The physiology of the swimbladder in the eel, Anguilla vulgaris. Acta physiol. scand. 59:221-241.

Venable, J. H. and R. Coggeshall. 1965. A simplified lead itrate stain for use in electron microscopy. J. Cell Biol. 25:407.

Watson, J. L. 1958. Staining of tissues for electron microscopy with heavy metals. J. Biophys. Biochem. Cytol 4:475.

Wittenberg, J. B. 1961. The secretion of oxygen into the swimbladder of fish. J. Gen. Phys. 44:521-527.

Wittenberg, J., M. J. Schwend and B. A. Wittenberg. 1964. The secretion of oxygen into swimbladder of fish. J. Gen. Phys. 48:337-355.

ATTACHMENT III

Scope of Work

The following experimentation will be conducted with three species of fish, Tilapia mossambica, Morone saxatilis, Mugil cephalus, and/or a common species of the family Sciaenidae native to the Southern California coast.

(1) Experimental animals will be obtained by inducing maturation and spawning of adults through hormonal injections, temperature manipulation and light manipulation.

(2) Larvae of each species will be reared under the following experimental conditions:

T. mossambica	24°, 26°, 28° C.
M. saxatilis	16°, 18°, 20° C.
M. cephalus	20°, 22°, 24° C.
Sciaenidae (if used)	(range dependent on species)

Trials will be conducted at four levels of oxygen saturation for each temperature as follows:

T. mossambica	40%, 80%, 120%, ambient
M. saxatilis	60%, 100%, 140%, ambient
M. cephalus	60%, 100%, 140%, ambient
Sciaenidae (if used)	(range dependent on species selected)

Each replicate of 12 experimental conditions will be run with offspring from a single female. Twenty-five Tilapia will be used per treatment and 100 individuals of the other species will be used per treatment.

Standard experimental conditions will be maintained during all experiments and no feeding will be done.

(3) Regular examination of live fry will be made to determine:

(a) extent of gas bubble formation in the swimbladder
(b) extent of development of the circulatory system
(c) extent of eye pigmentation
(d) extent of development of functional jaw and intestine
(e) length
(f) size of yolk sac and oil globule

(4) Regular samples from each treatment will be taken for histological and fine structural studies. For histological studies standard procedures outlined in the proposal will be followed. On the basis of these studies a description will be prepared of swimbladder and adjacent organ development at the cellular level as correlated with various environmental paramenters. Tissues for fine structural studies will be prepared as outlined in the proposal. These will be examined with an election microscope to determine if activity levels of the gas bladder secretory epithelium can be demonstrated, if this activity can be correlated with gas bubble formation, and if experimental conditions influence this activity.

ATTACHMENT IV

The following reports shall be prepared and submitted to A.I.D. as stated below:

(a) One hundred (100) copies of the Comprehensive Final Report on overall program and fiscal matters for the entire period for which the Grant was made:

(b) Ten (10) copies of such other reports as may be prepared or requested from time to time during the period for which the grant was made:

(c) Copies of the above stated reports in the quantities indicated shall be submitted to:

Director of the Fisheries Division
DS/AGR/F SA-18 (RP-C)
Agency for International Development
Washington, D.C. 20523

(d) One (1) copy of each report shall be submitted to the Grant Officer whose name appears on the Grant and three (3) copies of each report shall be submitted to:

The Documentation Coordinator
DS/DIU SA-18 (RP-C)
Agency for International Development
Washington, D.C. 20523

(e) The Final Report shall be due within sixty (60) days of completion of Project Activities and other reports shall be due within thirty (30) days following publication.

(f) Material for publication which may be prepared on the basis of project research shall be submitted to AID/W for approval prior to publication.