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Ecological Research Series

Acquisition and Culture of Research Fish: Rainbow Trout, Fathead Minnows, Channel Catfish, and Bluegills

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ACQUISITION AND CULTURE OF RESEARCH FISH:

RAINBOW TROUT, FATHEAD MINNOWS, CHANNEL CATFISH, AND BLUEGILLS

by

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ABSTRACT

Rainbow trout (Salmo gairdneri), channel catfish (Ictalurus punctatus), fathead minnows (Pimephales promelas), and bluegills (Lepomis macrochirus) are cultured widely for toxicological research. However, cultural conditions are sometimes suspected of compromising the test animals and, thus, results of research findings. Because optimum conditions for indoor maintenance and culture of the four species are not well defined, we have adopted standardized practices that are intended to reduce cultural conditions to a common variable status. Water quality, nutrition, genetic variation, diseases, fish handling, gross behavior, and required facilities are discussed. Well known propagation techniques provide the basis for the intensive care methods used. Special emphasis is given to diets, diet preparation, and residues of pesticides or other contaminants in diets and fish.

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**When reporting uses of, or research on drugs and pesticides, the authors do not imply that the drug or pesticide uses discussed have been cleared or registered. Clearance of drugs and registration of pesticides are necessary before recommendation. Appropriate state and federal regulatory agencies should be consulted to determine if the chemical in question can be used and under what conditions the uses are permitted.

SECTION I

RECOMMENDATIONS

Holding facilities for research fish should be adequate for the required size, number, and species of fish. A flow-through type of holding system is superior to closed, water recirculation systems. Round fiberglass tanks are generally superior to permanent concrete tanks for holding fish.

Water of uniform high quality should be supplied to holding facilities through chemically inert piping. Generally, a deep well is superior to surface water sources, shallow wells, or springs.

Fish to be used for toxicological research should be morphologically typical of the species and genetically consistent. Hatchery fish, rather than wild fish, are used because their history is relatively well known with regard to genetic strain, disease, diet, spawning times, and rearing water.

Contamination of fish diets and hatchery water supplies has resulted in the accumulation, to different degrees, of industrial compounds and pesticides by fish intended for research. Pre-shipment analyses of "candidate" test fish, and feeding a relatively uncontaminated diet assist in minimizing foreign residue accumulations in research specimens.

Stress producing factors should be minimized during transport of fish to the laboratory. After arrival at the laboratory, special considerations must be given to acclimating fish to laboratory water quality and temperature, depending on whether the fish were shipped by truck

or air. Incoming fish are quarantined for 2 weeks. During this time, we examine the fish for diseases and observe behavior. However, the best disease preventative measure is avoidance, or destruction of fish known to be diseased. Microscopic examination of gill lamellae and the skin is used during the quarantine period to identify possible disease agents, or to determine the severity of infestation. Some diseases that are extremely pathogenic to rainbow trout and channel catfish are discussed and control measures for these diseases are outlined.

To maintain the 4 species for extended intervals, acceptable loading capacities should not be exceeded. The capacity for rainbow trout can be used because their maintenance requirements are generally more restrictive than those of the other three species. Other recommendations for maintaining research fish include minimal handling or grading, observation of behavior, and routine feeding of the proper food. Bluegills and fathead minnows may require a brief period of temperature elevation to stimulate their conversion from natural to synthetic diets. This period, however, may not require conditioning if they have been fed artificial diets before. In general, conditioned behavioral patterns can reflect fishes' physical condition before more observable disease symptoms appear. Therefore, consistent feeding and other good cultural practices can be used to condition these behavioral patterns and provide an excellent diagnostic tool to the fish culturist.

SECTION II

INTRODUCTION

Use of fish for research in toxicology, physiology, and medicine has increased greatly in recent years. Lennon (1967) reviewed research needs for selected strains of fish and emphasized the importance of information on sources, care, handling, feeding, and testing various experimental fishes. Such information is essential to provide research animals that yield reproducible and comparable bioassay results (Henderson and Tarzwell, 1957; Lennon and Walker, 1964; American Public Health Association, 1971).

The need for high quality research fish is recognized, but methods for assessing the quality of prospective fish and procedures for their acquisition and maintenance are not well established. Most fish cultural techniques are directed at mass propagation of fishes and do not consider the intensive care necessary for obtaining and maintaining suitable research fish. Relative resistance of test fish to reference toxicants such as DDT or antimycin were used to estimate quality by Marking (1966) and Hunn et al. (1968). The latter authors also presented methods of handling and maintaining bioassay fish, but pointed out that the most appropriate methods are still in the developmental stage. They hoped to stimulate further discussion and exchange of information on this topic. In response, this paper presents culture methods currently in use at the Bureau of Sport Fisheries and Wildlife's Fish-Pesticide Research (FPRL) and the Fish Control (FCL) Laboratories for rainbow trout (Salmo gairdneri), channel catfish (Ictalurus punctatus), fathead minnows (Pimephales promelas), and bluegills (Lepomis macrochirus). We recognize that some of these methods are not yet supported by research data, but they do represent our best opinions based on empirical evidence.

SECTION III

FISH HOLDING FACILITIES

Inadequate, poorly designed facilities hamper research progress because they cannot sustain the required number, sizes, or species of fish for the duration of an experiment, or, more importantly, concurrent experiments. Potential water sources and supply systems in conjunction with special considerations, such as contaminants from construction materials, water additives (chlorine, fluoride), lighting, space limitations, and facility size and location should influence facility design and construction.

Many types of fish holding facilities are used to maintain research fish. Concrete tanks used at FPRL and FCL were designed to hold small numbers of various species for several months but they can be used to rear fingerlings to sexual maturity. Whereas concrete tanks are permanent and require little maintenance, tanks of redwood or fiberglass are moveable, durable, and versatile. Concrete tanks do not permit reallocation of space when fish holding requirements are reduced. Circular fiberglass tanks appear to meet space and versatility requirements and if inflows and outflows are properly designed, they are "self-cleaning" and require less water flow than rectangular tanks (Davis, 1967). However, Mairs (1961) noted fish death due to improperly "cured" epoxy resin cements and low water flows. The intensive culture concept of Buss and Waite (1961) is incorporated into some circular tank designs.

Concrete tanks are widely used in salmonid culture (Davis, 1967), but studies to determine the suitability of raceway troughs for culturing warmwater species were initiated only recently. Shell (1966) found that managed earthen ponds were more conducive to warmwater fish production, and thus they were preferred for culturing research fish over concrete ponds and plastic pools. We have found that the plasticizers (phthalic acid esters) in polyvinyl plastic pools contaminate fish and make this type of container undesirable for research use (Mayer and Sanders, 1973).

Outdoor culture provides natural light and at least some natural food (see DIETS FOR EXPERIMENTAL FISH), but fish may be exposed to broad temperature extremes and ponds may be covered with ice in winter. For this reason, our fish holding facilities are indoors. If a programmed photoperiod is desired, the apparatus designed by Drummond and Dawson (1970), or that by Wickham et al. (1971), may be incorporated into indoor lighting systems.

Many laboratories have only one water source, and if they must hold numerous species, optimal systems are often difficult to replicate. Aquarists have long recognized problems associated with maintaining optimal environments, and they have devised a number of water filtration and recycling systems (Lewis, 1962; Spotte, 1970; Clark and Clark, 1964). Partial recirculation or closed systems are desirable for holding research fish when optimal water quality is not possible. These systems may have a specific use, e.g., the channel catfish egg incubation system designed by Giudice (1966a). If only water requiring dechlorination or decontamination is available, recycling with small volume addition may be desirable. The probability of water contamination at FPRL is low because water is pumped from a deep (1,100 ft)

aquifer and used once in a flow-through system. We think this source is superior to most surface waters, shallow wells, or springs that could be intermittently contaminated. Water from deep wells may require aeration to increase dissolved oxygen, or to eliminate supersaturation of dissolved gases.

Water of excellent quality and quantity may be rendered useless for fish if pipes and valves that release heavy metals such as zinc (Pickering and Vigor, 1965: Eisler, 1967) or other contaminants are used. Vinyl plastic pipes, particularly the softer plastic, should not be used because these materials continually contribute plasticizers such as di-2-ethylhexyl and di-n-butyl phthalates to water (Mayer et al., 1972; Mayer and Sanders, 1973; Sanders et al., 1972; Stalling et al., 1973). Black iron, high density polyethylene and polypropylene piping do not contaminate water. Teflon (polytetrafluorethylene) and some types of nylon pipe are suitable but high cost limits their use.

SECTION IV

ACQUISITION

Fish used for research should be healthy, relatively free of pollutants, of known age, and "physiologically representative" of the species as a whole. Factors such as hybridization, disease, injury, nutritional state, and exposure to pollution may "compromise" the usefulness of wild fish in toxicologic and physiologic research.

SOURCES OF FISH

Fish are obtained primarily from national and state fish hatcheries (U.S. Bureau of Sport Fisheries and Wildlife, 1970a). Snow (personal communication)* selected "reference" strains of bluegills and channel catfish that are propagated at two national fish hatcheries. These strains are used for toxicologic research whenever possible. However, Eller (1970) noted an abnormally high incidence of ovotestis in third-generation bluegills of the reference strain. This finding may eventually preclude our using these bluegills for certain types of research. It also exemplifies the need for monitoring potential ill effects of inbreeding. Fish for experimental use are also available from commercial sources (U.S. Bureau of Sport Fisheries and Wildlife, 1970b).

The significance of research fishes' genetic background is not always clear, but many investigators believe that variation in results is reduced by using selected strains. Some types of research may require fish from polymorphic gene pools, but for reproducible laboratory results from a reasonably small sample size, a selected population

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* Mr. J. R. Snow, U.S. Bureau of Sport Fisheries and Wildlife, National Fish Hatchery, Marion, Ala. 1972.

having a restricted gene pool is desired. In most instances, minimal variation in animal responses due to homogeneity is essential for interpretation of experimental results. However, in other cases, heterogeneity may be desirable to estimate the range of responses in natural populations (Ferguson and Bingham, 1966). Lennon (1967) stressed the importance of establishing standardized reference strains of fish for research. Marking (1966) reported variability of bioassay results when fish from two sources were used. Also, bluegill response to standard toxicants was found to vary with the history of stress prior to bioassay use (Lennon and Walker, 1964).

Hatchery-reared fish are the alternative to wild fish, though some may argue that the former do not truly represent the species. However, for laboratory research, this factor is probably less important than the ease of acquisition and quality of hatchery fish reared under relatively controlled conditions.

Certain hatcheries promote inbreeding to maintain parent stock, whereas others replenish brood fish from wild stocks. In the past, wild fathead minnows, channel catfish, and bluegills were collected for brood stock. However, hybridization is common in wild populations of centrarchids (Childers and Bennet, 1961), and Giudice (1966b) reported different growth qualities of hybrid catfish. In contrast, Buss and Wright (1956) report that salmonid hybrids have poor survival rates and are less likely to be encountered than hybrids of other species. Therefore, to minimize experimental variation and insure known lineage, only hatcheries maintaining stocks that are several generations removed from wild populations should be considered as reliable sources of research fathead minnows, channel catfish, and bluegills. At least 12 established strains of rainbow trout are used

in commercial production (Dollar and Katz, 1964). These strains are geographically separate and spawn at various times throughout the year, and several research installations may use different strains of trout for similar research. The advantage of year-around availability of test fish from different strains may outweigh the potential disadvantage of response variability between strains.

In general, we discourage the use of wild fish because of the high potential for genetic variability, widespread pollution, and disease. Wild fish may be carriers of various parasites, bacteria, and viruses that, with crowding in the laboratory, may spread to healthy groups of research fish. The resulting changes in physiological stages of these fish may alter their susceptibility to toxicants as well as interfere with biochemistry and pathology observations (Lennon and Walker, 1964). Unavoidable epidermal and gill damage or sublethal stress induced in wild fish during netting, trapping, shocking, or transport will generally render these fish unfit for research use. Injuries and stress make the fish more susceptible to bacterial and fungal infections. Nutritional state may be unimportant except when stunted or starved wild fish are collected from overpopulated waters. The ubiquity of pesticides (Henderson et al., 1969) and other residues (Mayer, 1970) in wild fish may limit their utility in certain research because of variations in dose-mortality responses (Ferguson and Bingham, 1966), interference in studies of chemical kinetics (Mayer, 1970), and untoward embryological or other physiological effects (Grant and Mehrle, 1970a, 1970b, 1973; Grant and Schoettger, 1972).

RESIDUES

Hatchery fish may also contain undesirable residues if concentrations of contaminants in their diets and water supplies are sufficiently high. In 46 samples composed of eggs, fingerlings, and adults of the four species from 11 hatcheries, we found polychlorinated biphenyls (PCB) residues in 83 percent (0.01-2.7 µg/g), residues of the DDT complex (o,p'- and p,p'-DDT, DDE, and DDD) in 76 percent (0.01-0.29 µg/g), and phthalate acid ester plasticizers in 21 percent (0.16-1.0 µg/g). Other contaminants analyzed included chlordane in 11 percent (0.02-0.6 µg/g); dieldrin in 17 percent (0.003-0.37 µg/g); endrin in 6 percent (0.05-0.88 µg/g); toxaphene in 2 percent (9-20.0 µg/g in fat); and heptachlor, benzene hexachloride (BHC), and hexachlorobenzene (HCB) in 2 percent of the fish (0.002-0.03 µg/g). Accordingly, we now analyze a small sample of fish before accepting the main shipments. The analyses are made approximately 2 weeks before the requested fish reach the desired size at the supplying hatchery. The pre-shipment analyses for residues permit the researcher to decide whether or not contaminants are present in sufficient quantity to interfere with his research. The procedure minimizes the risk of invalidating research results and eliminates cost of transporting fish that cannot be used.

Hatchery diets can be the source of residues in research fish. Although commercial dry diets may provide adequate nutrition for rainbow trout, fathead minnows, channel catfish, and bluegills, special consideration should be required when one feeds fish destined for toxicologic research. Residues of pesticides and industrial contaminants in several fish diets have been analyzed by FPRL for several years. Analyses for 1972 and 1973 are shown in Table 1. Residues are also

Table 1. SELECTED CONTAMINANTS DETECTED IN DIETS ANALYZED INTERMITTENTLY AT FPRL FROM AUGUST, 1972 - AUGUST, 1973

Diets	Residues (µg/g)						
	DDT[a]	PCB[b]	Hexachloro-benzene (HCB)	Dieldrin	Endrin	Total organo-chlorine content	Phthalate esters[c]
Oregon Moist	0.06	0.30	-[d]	-	-	0.36	-
Glenco	0.11	0.30	-	-	-	0.41	-
Clark	0.11	0.20	-	-	-	0.31	-
Silver Cup	0.08-	0.20-	-	-	-	0.37	-
(2 samples)	0.17	0.32	0.06	0.01	-	0.555	-
EWOS	0.15-	0.20-	0.008-	0.01-	-	0.361-	-
(3 samples)	0.39	0.30	0.046	0.02	0.01	0.766	3.0
Colorado State Diets	0.11-	0.10-	-	0.01-	-	0.213-	-
(9 samples)	0.84	2.80	0.003	0.30	0.01	3.953	-
BSFW Hatchery Diets	0.12-	0.20-	-	-	-	0.32-	-
(2 samples)	0.19	0.30		0.01		0.50	-
Purina Catfish Chow	-	-	-	0.01	-	0.01	-
Reference research diet (4 samples)	-	<0.1	-	-	-	<0.1	-
Minimum detection limits	0.005	0.1	0.0001	0.01	0.01		0.1-0.5

[a] All DDT analogs
[b] All polychlorinated biphenyls, but usually Aroclor[R] 1254 and 1260
[c] di-n-butyl phthalate and di-2-ethylhexyl phthalate
[d] None detected

analyzed in various common components of fish diets (Table 2). In the past, we used the Cortland No. 7 diet (Phillips, personal communication)* that was formulated from selected ingredients shown in Table 2. The reference research diet used presently at FPRL is outlined in DIETS FOR EXPERIMENTAL FISH.

SCHEDULING

Even though hatchery fish are easily acquired, good planning is necessary for the best sequence of availability, shipment, receipt, quarantine, acclimation and final experimental use. Obtaining immature or adult fish may require more than a year of advance scheduling with the supplying hatchery. Therefore, maintaining a continuous flow of fish to the laboratory requires communication between the researcher, laboratory culturist (if present), and the supplying hatchery so that scheduling is periodically revised in light of current research needs.

Availability of any particular life stage of fish varies with latitude. For example, fingerling bluegills in southern states can be obtained approximately 60 days earlier than those at more northerly latitudes. Thus, with proper scheduling between southern and northern hatcheries, bluegills of a particular size would be available over 120 days.

Fish availability can also be extended by selecting strains with different spawning times, or by manipulating normal reproductive cycles. The scheduled acquisition of different strains insures adequate

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* Dr. A. M. Phillips, Sr., U.S. Bureau of Sport Fisheries and Wildlife, Tunison Laboratory of Fish Nutrition, Cortland, New York. 1967.

Table 2. SELECTED CONTAMINANTS DETECTED IN DIET COMPONENTS ANALYZED INTERMITTENTLY AT FPRL FROM 1970 - 1973

Sample	Residues (µg/g)								
	DDT[a]	PCB[b]	Hexachloro-benzene (HCB)	Diel-drin	Chlor-dane	Endrin	Lin-dane	Total organo-chlorine content	Phthalate esters[c]
Protein sources									
Fish protein concentrate	-[d]	-	0.0002	-	-	-	-	0.0002	-
Herring meal	0.005-0.05	0.10-0.20	- 0.0003	-	-	-	-	0.015-0.250	-
Menhaden meal	0.84	1.6	-	0.30	-	0.01	-	2.75	-
Peruvian fish meal	0.73	1.8	-	0.01	-	-	-	2.54	-
Rough fish meal	0.20	2.8	-	0.02	-	-	-	3.02	-
Casein	0.005	-	-	-	-	-	-	0.005	0.6
Gelatin	0.028	0.1	-	0.06	-	-	-	0.188	7.0
Skim milk	-	-	-	-	-	-	-	-	-
Soybean meal	-	0.1	-	0.03	-	-	-	0.130	0.6

Table 2 (continued). SELECTED CONTAMINANTS DETECTED IN DIET COMPONENTS ANALYZED INTERMITTENTLY AT FPRL FROM 1970 - 1973

Sample	Residues (µg/g)								
	DDT[a]	PCB[b]	Hexachlorobenzene (HCB)	Dieldrin	Chlordane	Endrin	Lindane	Total organochlorine content	Phthalate esters[c]
Oils									
Tuna oil (2 samples)	6.99-7.45	2.0-2.6	0.052-0.07	0.05-0.06	-	-	-	9.09-10.18	-
Menhaden oil	1.09	4.7	0.11	-	-	-	-	5.90	-
Redfish oil	2.14	1.6	0.07	0.06	-	-	-	3.87	-
Herring oil (3 samples)	0.69-3.40	1.0-2.3	- 0.07	- 0.46	-	-	-	1.69-6.23	-
Cod oil (3 samples)	0.87-0.96	3.0-3.5	0.06-0.38	0.07-1.10	-	-	-	4.00-5.94	-
Pollock oil	3.0	32.0	-	-	-	-	-	35.0	-
Salmon oil (3 samples)	0.23-0.90	0.20-1.60	- -	-	-	- 0.03	-	0.43-2.53	-
Linseed oil	-	-	-	-	-	-	-	-	-
Corn oil	-	0.1	-	-	-	-	-	0.1	1.1
Corn oil	-	-	-	-	-	-	-	-	-

Table 2 (continued). SELECTED CONTAMINANTS DETECTED IN DIET COMPONENTS ANALYZED INTERMITTENTLY AT FPRL FROM 1970 - 1973

Sample	Residues (µg/g)								
	DDT[a]	PCB[b]	Hexachlorobenzene (HCB)	Dieldrin	Chlordane	Endrin	Lindane	Total organochlorine content	Phthalate esters[c]
Carbohydrates									
Dextrin	-[d]	-	-	-	-	-	-	-	-
Wheat middlings	0.05	-	-	0.01	0.03	-	0.14	0.23	0.2
Cornstarch	0.005	0.10	-	-	-	-	-	0.105	-
Vitamins, minerals, and binders									
Bone meal	0.03	-	-	0.01	-	-	-	0.04	0.9
Distillers solubles	0.005	0.1-0.3	-	0.01	-	-	-	0.115 0.30	-
Brewers yeast	-	-	-	-	-	0.03	-	0.03	-
Carboxymethyl cellulose	-	-	-	-	0.72	-	-	0.72	-
Alphacel	-	-	-	-	-	-	-	-	-
Mineral mix	-	-	-	-	-	-	-	-	-
Minimum detection limits	0.005	0.01	0.0001	0.01	0.1	0.005	0.001		0.1-0.5

a/ All DDT analogs
b/ All polychlorinated biphenyls, but usually Aroclor® 1254 and 1260
c/ di-n-butyl phthalate and di-2-ethylhexyl phthalate
d/ None detected
NOTE: In addition to the above residues, 2.08 µg/g of methoxychlor and 0.5 µg/g of malathion were found in wheat middlings

numbers of rainbow trout fingerlings for testing throughout the year. However, strains spawning at different times are not developed for most species, and availability can be extended by altering normal reproductive cycles (Hazard and Eddy, 1950; Carlson and Hale, 1972). For example, channel catfish normally spawn in early summer, with fingerlings available for research in the fall. But, spawning can be controlled by holding adults at relatively cool temperatures and then gradually increasing temperature to stimulate ripening (Yamamoto et al., 1966; Brauhn, 1971). Similar techniques have been used to induce fall spawning in largemouth bass (Micropterus salmoides) (Brauhn et al., 1972; Carlson, 1973) and other teleosts (DeVlaming, 1972).

The importance of communication between the culturist and a proposed supplying hatchery cannot be overemphasized since hatchery rearing conditions may vary daily.

TRANSPORT

Truck transport is a reliable and economical method of transporting large numbers of adult or immature fish for short distances (Davis, 1967), but air transport is probably the most efficient and least stressful method of securing small numbers of fingerling fish (Nemoto, 1957; Hulsey, 1962; Gebhards, 1965). Air shipment is the only practical means of transporting fish eggs great distances.

The primary hazards in transport of research fish are O_2 deficiency, excess CO_2 and NH_3, pressure changes, and adverse temperatures (Leitritz, 1960; Moss and Scott, 1961; Doudoroff and Shumway, 1970). Of these, the first three are due to metabolism of fish and other

organisms such as bacteria that live on feces, mucous, and other organic material in transport water. Therefore, fish should be held without food for at least 48 hours prior to transport. Lane and Jackson (1969) determined the fecal voidance time for 23 species of fish including rainbow trout, fathead minnows, channel catfish and bluegills. They found that voidance was temperature dependent, but usually complete in 2 to 3 days. Haskell and Davies (1958) and Leitritz (1960) suggest the loading should not exceed 454 g (1 lb) per 3.8 l (1 gal).

Some hatcheries reduce bacteria in transport water by adding acriflavine or other bacteriostatic compounds. Since these compounds may be accumulated by the fish while in transport and later interfere with research, we discourage use of such chemicals. Healthy fish, minimal fecal accumulation, and proper loading rates make bacteriostats unnecessary.

All-season truck transport of fish requires special equipment including an oxygenation system, properly designed insulated tanks, a water recirculation system, and, in some instances, refrigeration equipment. It is generally impractical for research laboratories to invest in this expensive equipment. However, researchers should be aware that this kind of equipment is often necessary to transport rainbow trout during summer.

Because of the distances between supplying hatcheries and the laboratory (>500 miles), we ship many experimental fish by commercial air freight. Before air-shipment, fingerlings are not fed for 48 hours and then the water temperature is gradually lowered to 3°C during the second 24 hours. Approximately 450 g of fish are placed into a clear polyethylene

bag containing 7.5 liters of water at 3°C. Frequently, two bags are used, one inside the other, to minimize punctures or other leaks. Another bag containing 1.5 kg of ice is placed in the bottom of a 0.5-m^3 styrofoam box and the bag containing fish is set on the ice. The fish bag is partially inflated (to avoid bursting in unpressurized aircraft compartments) with pure O_2 and sealed tightly with rubber bands. Fingerling rainbow trout packed in this manner have survived 12 hours with less than 5 percent loss.

SECTION V

RECEIPT OF FISH AND ACCLIMATION

Constructing adequate facilities, finding a suitable water supply system and acceptable source of fish, and arranging transport of fish to the laboratory is wasted if attention is not given to the critical period immediately after receipt of fish at the laboratory. Water quality, stress, disease, or physical damage in this period may determine whether fish are fit for research. Therefore, we have established a fish receipt protocol that is followed whenever possible.

WATER QUALITY

Changes in water quality during shipment and differences between that of the supplying hatchery and receiving laboratory are common causes of stress and they should be given first consideration when fish arrive. Leitritz (1960) and Davis (1967) summarized water quality factors influencing trout culture. Because temperature, alkalinity, dissolved solids, and dissolved gases of a hatchery's water may differ appreciably from that of a laboratory, the researcher must allow incoming fish to adjust to the laboratory. Transfer of fish to water differing radically in temperature, hardness, pH, and O_2 may result in severe shock or death (Holmes and Donaldson, 1969). If possible, the researcher should consider acquiring fish from hatcheries with water qualities similar to those of the laboratory. However, when water quality differences are extreme, we modify a sufficient volume of receiving water in pH, alkalinity, hardness, and temperature to approximate that of the

hatchery. We then add laboratory water at a rate sufficient to give a complete exchange of water every 24 hours. If necessary, we adjust the rate of temperature change to approximately 3 to 4°C per day. Beamish and Mookherjii (1964) recommended a temperature change rate of 1°C per day when acclimating goldfish (Carassius auratus) to a research temperature, which we follow after quarantine.

Receiving air shipments requires a slightly different procedure. Except when water quality between the supplying hatchery and the laboratory differ greatly, it is less important than temperature difference. Dissolved CO_2 and NH_3 will accumulate during shipment and influence the pH. Also, O_2 concentration in the water will gradually decrease as the time of shipment increases. Therefore, the fish may be in water radically changed from that in which they were reared. Fish should be changed to a new water quality over 2 or more hours to prevent lethal stress. Temperature acclimation should receive primary consideration, because the shipments should be at a reduced temperature (see TRANSPORT). This is done by allowing the shipping bags to float on the surface of the tank in which they are to be placed. When temperatures are equalized, water may be added gradually at a rate of approximately 50 ml/min to the shipping bag. When a volume of water approximately equal to that in the shipping bag has been added, the fish may then be eased into their holding tank.

QUARANTINE

We assume that all incoming fish may be diseased. To prevent the spread of disease and to protect healthy resident fish, we quarantine incoming fish for 2 weeks while their behavior is observed and they are examined at least twice for ectoparasites (see DISEASE AND PROPHYLAXIS).

We designate a semi-isolated portion of the fish holding facilities as a permanent quarantine area. The quarantine procedure is initiated before fish arrive by sterilizing a tank with about 200 mg/1 HTH (calcium hypochlorite) for 24 hours (See DISEASE AND PROPHYLAXIS). During this time, the tank walls and plumbing are scrubbed with this solution. Then, the HTH is flushed from the tank and at least three exchanges of water are passed through the tank in the next 24 hours. All nets, screens, and buckets used in the tanks are treated similarly, then thoroughly rinsed with clean water.

Immediately after receipt, fresh preparations are made from the skin, gills, and gastro-intestinal (GI) tract of six fish and examined microscopically for the presence of ecto- and endoparasites, and physical damage to the epidermis or fins is noted. For ectoparasites, we initially apply a 1-hour flow-through treatment of one part formalin to 6,000 parts incoming water. Then, a mixture of formalin and malachite green is applied to give concentrations of 25 mg/1 and 0.1 mg/1, respectively, in three, 1-hour treaments on alternating days (Leteux and Meyer, 1972). Parasitism of the GI tract is treated with one dose of di-n-butyl tin oxide incorporated into the diet at a rate of 250 mg/kg of fish (Allison, 1957). We repeat the microscopic examination of skin, gills, and GI tract of fish on the 10th day of quarantine. Then, if there are no untoward side effects of treatment, and if no ecto- or endoparasites are present, the fish are moved from the quarantine section to a long-term holding tank or to experimental tanks.

Bacterial infections in newly-arrived fish warrant consultation with a qualified fish disease diagnostician as to the seriousness of infection, the causative organism, and possible therapeutic measures. (See DISEASE AND PROPHYLAXIS).

ACCLIMATION

Each of the four species under discussion must be acclimated to their new surroundings. Fish accustomed to unrestricted swimming in a rearing pond undergo intense competition for food and swimming space when placed in holding facilities. They may be frightened of nearby movements, and also respond to variations in background color and light intensity. The skin abrasions resulting from collisions with solid objects can lead to bacterial or fungal infections and, ultimately, the fish may have to be discarded. To prevent jumping, the facilities should be covered with screens, which may also reduce the light intensity. Channel catfish avoid light and appear to acclimate faster to new surroundings if the tank is covered with black polyethylene plastic. However, the cover should later be removed a section at a time over 2 days until normal lighting is reached. In general, fish should be maintained in facilities with color backgrounds and light intensities similar to those of research units to prevent a second acclimation period and lost research time. Rainbow trout seem less affected by restriction of swimming space and changes in background colors than are the other three species.

SECTION VI

MAINTENANCE

Fish often must be maintained for extended periods in laboratory facilities before they can be used, making capacity of facilities, diet, size, grading, disease, and behavior critical.

CAPACITY OF FACILITIES

Estimates of the proper number and weight of fish that can be held satisfactorily in the laboratory depend upon the size of fish, dissolved oxygen, feeding rate, temperature, and rate of water exchange in the holding unit. In general, the fish's oxygen requirement at constant temperature is associated with its caloric intake (Fry, 1957).

An acceptable loading capacity (weight of fish per liter of inflow) of holding facilities is estimated for salmonids by the method of Haskell (1955).

$$\text{Weight (g) of fish per liter of inflow} = \frac{\text{Weight (g) of feed per liter of inflow}}{\text{Fractional percent of body weight fed}} \quad (1)$$

Haskell's equation must be used in conjunction with the feeding tables of Deuel *et al*. (1952). This basic relationship was modified by Willoughby (1968), Piper (1971), and Liao (1971). Other investigators (Buterbaugh and Willoughby, 1967) have also developed correlations between trout length, weight, and the percent of body weight to feed for maximal growth. Examples and further discussion of these methods are presented by Phillips (1970).

Loading capacities have not, however, been adequately investigated for warm water species maintained in laboratory facilities. The minimum dissolved oxygen (D.O.) requirements of warm water species are generally lower than those for rainbow trout (Doudoroff and Shumway, 1970; Moss and Scott, 1961). So we utilize carrying capacities for rainbow trout. First, the permissible weight of fish is established empirically for a given water inflow rate (Piper, 1971). Permissible weight of fish is established by placing fish into a holding unit, varying inflow rates and monitoring behavior, O_2, and NH_3 at each rate. Safe limits of dissolved gasses for rainbow trout are: D.O., above 5 mg/l and NH_3, below 0.5 mg/l. Then loading is determined using the formula:

$$F = \frac{W}{L \times I} \qquad (2)$$

where F = loading factor
L = length of fish (cm or in)
I = 1 or gal per min water inflow
W = permissible weight of fish (kg or lb)

Subsequently, with additional growth, F can be substituted back into the equation to establish new holding requirements. Previously established feeding rates are used, when known, and as the fish grow, or as additional fish are placed into the unit, equation 2) is used to estimate the correct water inflow. This method is checked frequently according to the safe limits for rainbow trout. If a hazardous level of these gasses is noted, a new F is determined.

DIETS FOR EXPERIMENTAL FISH

The problem of residue accumulation in test fish from contaminated food is circumvented by preparing our diet from "non-contaminated" constituents. The diet is basically the H440 test diet reported by the

Subcommittee on Fish Nutrition, National Research Council (1973). We have modified the diet (Table 3) as suggested by the Bureau of Sport Fisheries and Wildlife's Committee on Fish Nutrition Research and Development. This Committee recommends that the diet be termed and used as a reference research diet. If fish meal is substituted for casein, we suggest that the meal and whole dry mixture contain less than 0.1 µg/g of organochlorine residues. Because casein is derived from milk, it is monitored closely by the U.S. Department of Agriculture for contaminating residues and discarded if residues exceed tolerance or action levels. Also, we require a pre-shipment analysis of all other components to permit selection of those relatively free of contamination. We prefer that fish oils contain no more than 2 µg/g of organochlorine residues. By purchasing a 6-month supply of components, the costly replication of residue analysis is eliminated and variations in background residues are minimized. Rainbow trout and channel catfish grow well on the diet with no apparent abnormalities. Its suitability for bluegill and fathead minnows remains to be demonstrated. We are using this diet for the four species but encourage future nutrition research to clearly define the requirements of bluegills and fathead minnows maintained solely on artificial rations.

The reference research diet can be made wet or dry, but we prefer the latter because it has longer shelf life and requires less refrigerated storage area. The feed is prepared at present as a rolled pellet in any selected size with a Dravo pelletizer.*

Since the nutrition of experimental fish may influence results, diets should be selected carefully by investigators. Unfortunately, many research fish are initially fed one diet, then offered another ration

*Dravo Corporation, Neville Island, Pittsburg, Pennsylvania.

Table 3. REFERENCE RESEARCH DIETS[a/] FOR COLD AND WARM WATER FISH

Component	Percent of dry diet for	
	Cold water fish	Warm water fish
Casein	35.0	28.0
Gelatin	15.0	12.0
Dextrin	28.0	28.0
α-cellulose mix[b/]		
α-cellulose	8.0	18.0
Vitamin mix	1.0	1.0
Mineral mix[b/]	4.0	4.0
Corn oil	6.0	6.0
Fish oil	3.0	3.0

a/ Recommended by the Committee on Fish Nutrition Research and Development, Bureau of Sport Fisheries and Wildlife.

b/ Subcommittee on Fish Nutrition, National Research Council (1973).

prior to, or during the experiment. Fish intended for toxicologic research should be maintained throughout their life-span on the ration to be used during research. For example, Mehrle et al. (in press) have shown that the susceptibility of rainbow trout to chlordane can be altered by the type of diet fed before testing.

Natural food organisms from lakes, streams, or ponds should not be considered for routine maintenance of experimental fish because of gross contamination by industrial pollutants and pesticides, variation in seasonal supply, diversity of weight and size, and species variation in protein, carbohydrate, and fat. However, rearing of small swim-up fry of species such as bluegills is difficult without live food. Eaton (personal communication)* recommends feeding very early instars of daphnia or copepods, or a small-size variety of brine shrimp from the West Coast.

GRADING

Hatchery fish may be delivered as a group of relatively mixed sizes, and certain research may require greater uniformity. The mechanical fish grader devised by Morton (1956) is useful for separating fish into groups of even size. The spaced-bar type of grader used by Pruginin and Shell (1962) is used at FPRL because it is efficient, accurate, and inflicts minimal injury to the fish.

Fish grading should be done only when necessary and then, very carefully. Only a few fish should be passed through the grader at one time and the transfer must be made with fine meshed nets of soft

* Mr. J. G. Eaton, U. S. Environmental Protection Agency, National Water Quality Laboratory, Duluth, Minn. 1974.

material. Overloading nets or graders may cause scale loss, skin puncture, eye damage, severe shock, or even mortality of some fish. Injuries promote infection by pathogenic bacteria which may later spread to healthy fish. We watch for delayed appearance of injuries for at least one week after grading before the fish are used in research.

DISEASES AND PROPHYLAXIS

Hygenic measures are required to prevent the initiation and spread of disease among experimental fish. Nets, buckets, fish graders, and hands are disinfected with 200 mg/l of Hyamine 1622 or a similar bactericide before they are placed in holding waters. Also, a 1-hour treatment with 2.0 mg/l Hyamine 1622 is applied to all fish at 14-day intervals. Routine maintenance schedules should include the recording of daily mortality and assessment of possible causes of death.

Most states now require that fish and eggs transferred, particularly of salmonids, across their boundaries be certified disease-free by a qualified fish pathologist. These regulations have helped to minimize disease problems among FPRL research fish.

When diseases occur, references of Davis (1967), Leitritz (1960), and the Fish Disease Leaflet series* published by the U.S. Bureau of Sport Fisheries and Wildlife can be used, among others, to identify common diseases encountered in fish culture and the methods for treatment. Disease diagnosis is difficult at times and success frequently depends on the biologist's experience. Many state conservation departments and

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* Available from the Superintendent of Documents, U.S . Government Printing Office, Washington, D. C.

the Bureau of Sport Fisheries and Wildlife maintain well-qualified disease diagnosticians who may be consulted. At the other extreme, our investigators have experienced problems among fish that were grossly over-treated by culturists with good intentions. Indeed, compatible host-parasite relationships may be more representative of fish found in natural ecosystems.

Unless seriously diseased fish are irreplaceable, they are immediately discarded or quarantined to prevent transfer of the disease to healthy fish. In exceptional situations, appropriate therapeutic measures are initiated when the disease is diagnosed and are continued until symptoms disappear. Post-treatment observation for 10 days is advisable before they are used for research.

Some therapeutants, particularly the antibiotics, may accumulate in fish tissues (Herman et al., 1969). The residues may interact with other compounds or cause side effects that will affect research results. For this reason, the use of therapeutants on research fish should be avoided whenever possible. If therapeutants are used, records should be maintained and the investigator should be informed of the disease, type of therapeutant, and its method of application.

FISH BEHAVIOR

Observations of fish behavior are a valuable tool to the culturist in assessing the day to day condition of fish. However, the natural behavior patterns of pond-reared fish are altered by placing them in restricted areas. Culturists and researchers should be aware of the behavior change. Experienced culturists know that abnormal behavior may be the first indication of stress-producing factors.

Hunn et al. (1968) observed behavior patterns of individual groups of fish for 10 days prior to their use as research animals. The culturist should observe the fish's response to food, their swimming posture, location in the tank, and unusual or erratic movements (Table 4). In addition, they are observed for visible injuries or changes in body conformation. Precision in the observations can be enhanced by inducing conditioned responses to scheduled, routine cultural practices. For example, Phillips (1970) suggested that small trout be fed small amounts of feed at hourly intervals in an 8-hour day and waste food and fecal material should be removed daily by siphoning or by partially draining the tanks. Deviations in the usual responses to these activities at appointed times may portend serious cultural problems.

Also, each species responds differently to laboratory space restrictions. It is normal for bluegills to disperse throughout the holding unit and maintain that posture, but it is abnormal for a schooling fish such as channel catfish fingerlings to do so, except when seeking food. Fathead minnows also exhibit a schooling tendency, and may exhibit a strong migration urge upon receipt. Thus, observations of fish behavior depend on the time of day that the observations are made, the species of fish, feeding schedule, the length of time in holding facilities, and the size of fish.

Table 4. OBSERVATIONS OF ABNORMAL FISH BEHAVIOR AND SOME COMMON CAUSES FOR THIS BEHAVIOR. SEVERAL OBSERVATIONS MAY BE MADE SIMULTANEOUSLY AND MAY BE CAUSED BY A COMBINATION OF TWO OR MORE PROBLEM AREAS.

Observation	Possible problem
1. Surfacing and swimming with mouth half emerged - a, b, c, d.	a. insufficient oxygen; toxic chemicals; high NH_3
2. Scraping against sides of tanks - [a] b, d, f	b. parasitism of skin or gills.
3. Erratic swimming - a, b, c	c. rapid temperature changes; parasitism of nervous system; virus disease
4. Crowding around water inflow - a, b	d. parasitism of intestine; bacterial disease
5. Distended abdomen and difficulty in swimming - d, e, f	e. diet consistency
6. Refusal to feed actively - a, c, d, e, f	f. nutrition deficiency
7. Listlessness, emaciation - b, c, d, e, f	

[a] Normal fathead minnow courtship behavior includes coloration change and display near tank sides.

SECTION VII

SPECIFIC CARE AND PROBLEMS

Whereas the preceeding subjects dealt with a broad spectrum of essentials for adequate fish maintenance, this section delineates specific cultural practices for each of the four species under discussion. The information presented here was determined empirically over 4 years by working with several hundreds of thousands of fish.

RAINBOW TROUT

Water that contains 5-10 mg/l of D.O.(but is not supersaturated), has a pH of 7-7.5, and is 9-13°C produces optimum vigor in rainbow trout (Leitritz, 1960). Dissolved oxygen and temperature are probably most critical since each limits the weight of fish held per unit of water volume (see CAPACITY OF FACILITIES). Water temperatures between 0° to 9°C cause sluggishness and reduction of appetite, whereas temperatures above 13°C stimulate appetite and growth. Temperatures near 25°C approximate the upper lethal temperature (Fry, 1957). In general, temperatures above 17°C to 20°C should be avoided.

Rainbow trout are susceptible to infectious viral diseases, such as pancreatic necrosis (Wolf, 1966a) and hemorrhagic septicemia (Wolf, 1966b). They are also susceptible to whirling disease (Hoffman, 1962), a highly contagious protozoan-caused disease. Since these diseases are not detected by routine microscopic analysis, a qualified fish pathologist should be consulted when appropriate symptoms appear. At present, since there are no therapeutic measures effective against these diseases, only complete sterilization of holding facilities will control them and permit introduction of new stocks.

Rainbow trout for use in static, acute toxicity tests (0.5-1.5 g) should be fed regularly for proper maintenance. In general, small fish consume more of their daily ration if portions are fed several times per day. Larger fish may be fed less often, down to once per day as the trout approach sexual maturity (Buterbaugh and Willoughby, 1967; Phillips, 1970). Bumgarner (1971) controlled trout growth by starvation. However, this practice must be used with caution because disease resistance may be lowered by starvation. Nevertheless, the technique may have some use in maintenance of rainbow trout, providing an optimum physiological state is reached before research use.

RAINBOW TROUT EGGS

On occasion, it is more convenient to obtain rainbow trout by shipping eggs, and specific studies may require eggs or alevins. Rainbow trout eggs may be shipped 24-36 hours after fertilization, but then they become more sensitive to disturbances during later development (Hayes, 1949). After eyes appear in the embryo, the eggs may be air-shipped great distances with minimal losses.

Shipment is accomplished by gradually reducing egg temperature to 1°C, packing the eggs in cheesecloth, placing an ample ice supply above the eggs and then putting both eggs and ice in a well-insulated box. When eggs are received, we reverse this procedure until the eggs reach 12°C, the appropriate incubation temperature. First, the eggs are gently removed from the shipping container and placed in an egg pan containing water at the same temperature as the eggs. Optimal water quality is similar to that for rainbow trout fingerlings. Then, clean water at 12°C is gradually added to limit the egg temperature increase to 2°C per hour. The egg pan is also floated in a tank of 12°C water to

prevent rapid warming from the air. When the incubation temperature is reached, the eggs are gently removed from the pan and placed in a Heath* incubator tray supplied with 5-10 liter per minute clean water. Davis (1967) described techniques for removing dead eggs and prophylactic treatment for fungal infections.

FATHEAD MINNOWS

Fathead minnows are commonly propagated in ponds (Dobie, et al., 1956) for use as forage or bait fish. They are fed supplementally to aid growth (Prather, 1957). Thus, before newly-acquired fathead minnows are used for research, they must be conditioned to accept a new diet, and they must be acclimated to their new surroundings. The length of "training" will vary with the feeding history of each group of minnows, but generally is not long.

We have found that water temperatures of 21-24°C apparently increase metabolism sufficiently to stimulate food intake and, therefore, make conditioning to artificial diets easier. Portions of the daily ration should be offered several times per day. Excess food and fecal material should be siphoned out of the tank because the common practice of partially draining the tank and brushing it may injure the fish or interfere with their conditioning to artificial feeds. Acceptance of food and a positive response to the culturist indicates that the fish are ready for long-term holding or research use. Temperature may then be varied gradually at 1°C per day to the desired experimental temperature and feeding rates are reduced to 1-2 percent of total body weight per day.

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* Heath-Techna Plastics Corp., Tacoma, Washington.

Altering the natural temperature regime of fathead minnows frequently alters their reproductive cycle. Water temperature elevation stimulates breeding behavior and spawning before and after the normal spawning period. Detailed recommendations for culturing this species are presented by the U.S. Environmental Protection Agency (1972).

CHANNEL CATFISH

Methods are available for economical pond propagation of channel catfish for sport fishing and for food (Snow, 1962; Tiemeier, et al., 1967; U.S. Bureau of Sport Fisheries and Wildlife, 1970c). The same methods are used to rear fingerlings for research use, hence, the fingerlings should be accustomed to competitive feeding on artificial rations. At FPRL they are fed a ration similar to that for trout, but, in general, their protein requirement is somewhat less (Phillips et al., 1957; Nail, 1962).

Channel catfish are more susceptible to several diseases than the other three species. One of these is the protozoan, Icthyophthirius multifilius, or ich, which is well known among culturists (Meyer, 1969). We assume that all newly-acquired channel catfish are carrying one or more life stages of ich. Probably the most effective control for ich in catfish is to place them in flowing water and elevate the temperature to 32°C for one week. This procedure hastens the life cycle of ich and is lethal to its infective stages (Meyer, 1969). The treatment is most successful with fingerling channel catfish, but it must be judiciously applied to adults. Adult channel catfish are sexually stimulated by this increase in water temperature (Brauhn, 1971) and they may initiate courtship behavior. This behavior includes competitive biting among males. The resulting lesions become infected rapidly with bacteria and/or fungus, and the fish cannot be used until a formalin treatment is applied.

Another serious disease of channel catfish is columnaris (Davis, 1967). Infected fish may be dipped into a 1:100,000 malachite green solution for 10 seconds to successfully control this disease. This treatment is repeated daily for 4 days. Also, daily applications of 25 mg/l Combiotic or Dystrillin for one hour under static conditions and repeated for 10 days is an effective control for this disease (see DISEASE AND PROPHYLAXIS).

During routine handling, the barbed pectoral spines of channel catfish frequently catch in nets, and soft fin tissue is easily damaged. Like other mechanical injuries, the surrounding tissues can then become infected with bacteria or fungus. This problem can be minimized by coating nets used for channel catfish with asphalt varnish.

BLUEGILLS

Since bluegills are reared in ponds on natural foods (Davis, 1967), their physiological state is tied closely with seasonal variations in light, temperature, and food. Thus, we have experienced difficulty in changing the reproductive cycle of bluegills with temperature alone. Previous experience shows that adult males establish a territory within indoor holding tanks when temperature is elevated but females appear unresponsive.

Bluegills also establish a "peck order," or sequence of dominant individuals (Breder and Rosen, 1966). Some fish, at the lower end of an established peck order, refuse to eat, become emaciated, and die. This dominance is usually established in the first 3 weeks after the fish are brought indoors, but is is disrupted each time they are handled or

graded. The dominance order, more frequent in adults than fingerlings, causes uneven growth and may be promoted by the change from natural to artificial diets. That is, some fish may adapt to the artificial diet sooner than others. Feeding the same quantity of food less frequently may ameliorate this problem.

Pond-reared bluegills are accustomed to natural food and the change to an artificial diet requires a conditioning period similar to that used for fathead minnows. This period is critical to bluegill survival and may extend over 2 weeks, with food offered at approximately the same time each day.

Bluegills are more susceptible to physical injury during handling than rainbow trout or channel catfish, therefore we use a 3-percent NaCl dip for 1-2 minutes (Hunn, et al., 1968) after their handling or grading. This reduces the possibility of external infection resulting from loss of mucous or injury to the skin.

SECTION VIII

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16. ABSTRACT

Rainbow trout (Salmo gairdneri), channel catfish (Ictalurus punctatus), fathead minnows (Pimephales promelas), and bluegills (Lepomis macrochirus) are cultured widely for toxicological research. However, vacillant or extreme cultural conditions are sometimes suspected of compromising the test animals and, thus, results of comparative or confirmatory research. Because exact optimum conditions for indoor maintenance and culture of the four species are not well defined, we have adopted standardized practices that are intended to reduce cultural conditions to a common variable status. Water quality, nutrition, genetic variation, diseases, fish handling, gross behavior, and required facilities are discussed. Well known propagation techniques provide the basis for the intensive care methods used. Special emphasis is given to diets, diet preparation, and residues of pesticides or other contaminants in diets and fish.

17. KEY WORDS AND DOCUMENT ANALYSIS

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