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Care of Fish in Biological Research¹

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ABSTRACT: Fish live in a very complex, dynamic environment. Their use as biological research subjects during the past three decades has increased almost exponentially because of the demand for an increased knowledge base in response to the need for better aquaculture technology. To use fish as biological research subjects requires the investigator to take into account approximately 40 interactive environmental variables, if the research data are to be free of unwanted biases. These environmental factors are classified into five major groups, all important to the well-being of fish. These five include intrinsic factors

(fish associated) and extrinsic factors (water, container, nutrition, and management associated). The stress response is the primary intrinsic factor of concern, and associated pathological changes should be used to monitor animal well-being and prevent secondary infectious disease problems. The water-associated factors are the primary extrinsic factors affecting the well-being of fish. Thus, the investigator must design research protocols that maintain fish within documented environmental limits for the species.

Key Words: Fish, Aquaculture, Well-Being, Research

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Introduction

As biological research subjects, fish are unique among the vertebrates. First, they constitute some 42% of the living vertebrate species. Second, they afford a researcher the opportunity to study living examples of the evolutionary innovations that have occurred in these animals over the past 300 million years. Third, because they are irrevocably oriented to their environment (i.e., a quantitative change of an environmental factor can induce a predictable quantitative change in the animal), the researcher has the opportunity to measure induced physiological responses to a wide variety of environmental conditions (Klontz and Smith, 1968).

Research on the comparative aspects of fish physiology, immunology, hematology, and anatomy has been conducted over the past 100+ yr. In the past 20 to 30 yr the major focus of research has been on those species involved in aquaculture, with the primary emphasis on developing the technology for improved fish health management. This increased activity has

involved researchers having backgrounds in using mammalian species as research subjects, for which there are specific guidelines for their use. For fish, however, there is but one publication describing in very general terms the recommended conditions under which fish could be maintained in the laboratory (NRC, 1974).

The primary objective of this presentation is to identify and define the major environmental variables together with their potential impacts on the well-being of fish.

Factors Affecting the Well-Being of Fish

Fish are poikilothermal vertebrates living all or nearly all their lives in a complex and dynamic environment. Their environment includes water depths up to 4.4 km beneath the surface, water temperatures from <0°C to >30°C, and salinities of .005 to 14‰ (Hoar, 1966; Klontz and Smith, 1968).

Conceptually, the aquatic environment consists of five major groups of factors: the fish, the water, the container, nutrition, and management. Within each of these major groups are several individual factors, each acting in a mutually interdependent fashion with other factors (Table 1). That is, if one factor changes quantitatively, this initiates a series of quantitative changes in several other factors.

The environmental dynamics can be visualized in the following example involving an increase in water

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Table 1. Environmental factors affecting the well-being of fish^a

Fish-associated	
Ammonia	Disease history
Behavior	Condition factor
Nutritional requirements	Oxygen demand
Environmental requirements	Oxygen uptake
Physical factors	Fecal solids
Chemical factors	Cannibalism
Growth rate potential	Stress response
Water-associated	
Dissolved oxygen	Contaminants
Nitrite	Municipal
Alkalinity	Industrial
pH	Agricultural
Inflow rate	Natural
Solids	Use
Suspended	Single pass
Settleable	Multiple pass
Salinity	Hardness
Container-associated	
Water volume	Water replacement time
Water velocity	Outfall design
Composition	Shape
Water inflow pattern	
Nutrition-associated	
Nutritional quality	Feeding rate
Proximate analysis	Feed efficiency
Metabolizable energy	Feed style
Management-associated	
Sampling techniques	Size grading
Feeding frequency	Feeding technique
Record keeping	Pond cleaning

^aKlontz (1991a,b).

temperature from 9°C to 15°C in the environment of a 100-g rainbow trout (*Oncorhynchus mykiss*). This increase generates the following fish-associated changes: 1) 67.5% increase in metabolic rate (oxygen demand), 2) 97.8% increase in daily length gain potential, 3) 66.7% increase in daily weight gain potential, 4) 89.6% increase in ammonia generation potential, and 5) 33.1% decrease in oxygen-carrying capacity. In addition, water-associated changes occur: 1) 12.8% decrease in oxygen concentration, 2) 58.8% increase in environmental un-ionized ammonia, and 3) 67.5% decrease in dissolved oxygen in the outfall water.

Thus, the life support (oxygen-based) carrying capacity of the pond is greatly reduced, possibly to the point of adversely affecting the health of the fish, depending upon the biomass involved. A method frequently employed, where possible, to restore the oxygen-based carrying capacity is to increase the water inflow, which in turn generates the following series of changes within the system: 1) the oxygen-based carrying capacity of the pond is increased, 2) the water velocity is increased, 3) the swimming energy expenditure of the fish is increased, 4) the oxygen demand of the fish is increased, and 5) the

oxygen-based carrying capacity of the pond is decreased.

The final effect of this act further compromises the well-being of the fish. The overall net effects could seriously compromise the validity of the data collected during the process of the study. A simpler method to correct the original change would be to reduce the population, but this could interfere with the original experimental design and subsequent data analyses. Thus, it is quite important to understand the potential interactive impact(s) of these factors and to anticipate them in the planning stages of research.

Fish-Associated Factors

The fish-associated factors are termed intrinsic, in that they pertain to the very nature of the fish. Their function is governed to a major degree by the genetic make-up of the animal. The water-associated, container-associated, nutrition-associated, and management-associated factors are termed extrinsic. These factors influence changes within the physiological milieu of the fish.

The major intrinsic factor affecting the well-being of the fish is the stress response, which is a complex physiological response to environmental conditions. Stress affects fish both directly and indirectly (Wedemeyer et al., 1976).

Causal factors in the acute (short-term) stress response are day-to-day husbandry activities such as population inventorying, pond cleaning, blood and tissue sampling, transportation, and the administration of a therapeutic regimen. Common causal factors in the chronic (long-term) stress response are population density and water quality (i.e., ammonia, low level toxic contaminants, and hypoxia).

The main clinical feature of the acute stress response is respiratory hyperactivity, which involves many physiological alterations. The important alterations are the rapid depletion of interrenal ascorbic acid, an increase in circulating cortisol, cessation of renal and intestinal activity, hemoconcentration, leukocytosis, and an increase in blood ammonia (Wedemeyer and Yasutake, 1977). This response, according to Selye (1950), is the alarm reaction. With the removal of the stressor from the system, the physiological activities return to their original state.

If the stressor persists in the system, the stage of adaptation ensues. In this stage of the stress response the fish "adjusts" its physiological activities to cope with the situation. For the most part, all physiological variables are within normal ranges. However, the longer the fish must accommodate the stressor the more pronounced are the deleterious effects. Growth rate begins to decline measurably, and a generalized melanosis becomes apparent. Concomitant with this is the loss of tissue integrity between the fin rays, especially in the caudal, anal, and pectoral fins. The physiological explanation for this is

that these tissues have become ischemic, resulting in necrosis ("frayed fin" syndrome). In this stage of the stress response, if an acute stressor is applied, the fish may die rather unexplainedly. This is the stage of exhaustion in that the fish is no longer capable of mounting another alarm reaction. This aspect of the stress syndrome is seen quite often as post-transportation mortality. One of the clinical features of this occurrence is that the "fish is the healthiest dead fish seen" (i.e., there are no significant postmortem lesions).

Another significant effect of the stress response, particularly to a severe acute stressor, is the activation of a latent bacterial or viral infection (Snieszko, 1974). In many cases, physical handling has been followed within 2 to 3 d by a severe episode of a systemic bacterial or viral disease. Some researchers ascribe this to the "risks of doing business." This is unfortunate because there are means to prevent such occurrences were the researcher aware of the processes "triggering" the event.

The environmental gill disease (**EGD**) syndrome is considered to be the major stress-mediated and environmentally mediated noninfectious disease (Klontz et al., 1985). By itself, i.e., uncomplicated by pathogens or other noninfectious factors, it is more a debilitating process than it is lethal. This aspect is, perhaps, what makes it such an economically significant disease process. There is no specific recommended treatment regimen, largely because the causal factors are often quite obscure, if evident at all.

The gross and microscopic pathological changes in gill tissues, especially the lamellae, are dose-response dependent. The inflammatory process ensuing from the initial exposure begins with lamellar epithelial hypertrophy in which there is focal-to-generalized involvement of the squamous epithelial cells. If the irritant exposure is short-lived, the hypertrophic condition subsides within a few days, usually without notice.

If the irritant exposure continues, the hypertrophic condition is joined by lamellar epithelial-capillary endothelial separation (**ECS**). In this lesion the squamous epithelium becomes separated from the underlying capillary endothelium, and the resultant space is filled with a serous exudate. At this point in the process, especially if there is generalized involvement, the fish could be exhibiting clinical respiratory distress, particularly during or immediately following handling.

In the subacute inflammatory process, the lamellar hypertrophy is replaced with epithelial hyperplasia, first of the lamellar epithelium and then of the filamental epithelium. The hyperplastic response can be terminal (i.e., involving only the distal portions of the lamellae), or it can involve the entire lamellae. This condition, over a period of 1 to 2 wk, gradually worsens to become interlamellar hyperplastic occlu-

sion, in which the interlamellar spaces are completely obliterated. At this point the fish is often visibly distressed. Gill tissues frequently protrude from the opercular cavity, and there is incomplete closure of the opercula. Grossly, the excised gill is quite characteristic with filamental separation due to the increased mucus production and the entrapped aquatic particulates. In some cases in the EGD process secondary involvement by bacterial and protozoan pathogens occurs. In contrast, many reports of myxobacterial gill disease include the conclusion that the myxobacterium was the exciting agent in this condition (Bullock, 1972).

With proper chemotherapy and management practices the foregoing responses can be healed. The repair process in the more severe cases requires 2 to 3 wk, provided there are no further insults to the physiological respiratory process.

There are several approaches to preventing and controlling EGD episodes. The first is avoidance of the conditions that are conducive to the occurrence of subclinical and clinical episodes. This is best accomplished by maintaining the fish within the generalized environmental "no-effect" limits with respect to settleable and suspended solids, ammonia nitrogen, dissolved oxygen, and population density (Table 2) (Klontz, 1991b; Wedemeyer and Wood, 1974). However, because these limits are not specific for a species, the unique limits for the species of fish being considered should be established. To accomplish this, measuring the environmental factors and their effects on growth and gill tissues begins with sac fry and continues throughout the production cycle. One caveat is that the process is time-consuming and often frustrating, but always rewarding in the long term.

Table 2. The physical and chemical limits of water supporting most finfishes^a

Factor	Limit
Dissolved oxygen	>90 mm of Hg pO ₂ ca. 60% of saturation
pH	6.7 to 8.5
Alkalinity	30 to 200 mg/L as CaCO ₃
Carbon dioxide	<2.0 mg/L
Calcium	>50 mg/L
Zinc	<.04 mg/L at pH 7.5
Copper	<.006 mg/L in soft water <.3 mg/L in hard water
Iron	<1.0 mg/L
Ammonia (as NH ₃)	<.03 mg/L constant <.05 mg/L intermittent
Nitrite (as NO ₂)	<.55 mg/L
Nitrogen (as N ₂)	<100% of saturation
Suspended solids	<80 mg/L
Dissolved solids	50 to 200 mg/L
Temperature	Standard environmental temperature for the species

^aWedemeyer and Wood (1974).

During the process of the research project, wet mounts of gill tissues should be examined on a regular basis. Fish should be taken from the "healthy" and the "unhealthy" or "sickly" portions of the populations. The fish are prepared for the examination by killing them with a sharp blow to the dorsum of the head and exsanguination by severing either the causal peduncle or the spinal cord immediately posterior to the base of the skull. An entire gill arch is removed and placed into 10% neutral buffered formalin for no longer than 20 to 30 s. It is either examined in toto (small fish only) or a few of the filaments are removed with scissors, mounted in pH 7.2 phosphate-buffered normal saline and examined using the 10× and 100× objectives. Paraffin-embedded sections of the lamellae and filaments may be prepared, but these are very time-consuming and expensive and not any more illustrative than are wet mount preparations, which can be done on site very rapidly.

When a clinical episode of gill disease occurs, an accurate diagnosis must be made before any therapeutic regimen is initiated. The sequence of changes occurring in the gill tissues is the best indicator of the nature of the causal factors involved. Lesions such as hypertrophy, ECS, and occlusive hyperplasia all suggest basic physiological upsets that may be reflected by alterations in other systems. The presence of bacteria and the so-called gill parasites often is a reflection of an underlying environmental problem, most common of which is poor housekeeping.

After the problem is defined, i.e., the major causal factors identified, the next step is to "re-balance" the system. This is best accomplished by, first, withholding feed for 3 to 4 d, if the fish are of sufficient size to permit this. This will 1) reduce the oxygen demand of the fish, 2) reduce the ammonia nitrogen generation by the fish, and 3) reduce the fecal and uneaten solids in the system. Second, administer sufficient salt (as granulated NaCl) to the system to obtain a 1 to 2% solution. This will 1) reduce the blood ammonia nitrogen levels, 2) stimulate mucus secretion, and 3) have an astringent effect on the gill tissues. Third, reduce the population density to approximately one-half the oxygen-related carrying capacity of the system. This should be accomplished without unduly stressing the fish.

Other fish-associated factors, without regard to degree of significance, are as follows:

Ammonia. Most bony fish produce ionized ammonia (NH_4^+) as the end product of protein metabolism. There are two major pathways of generation: endogenous and exogenous. The endogenous pathway is a catabolic function of the body in which the vital processes of the body are accommodated. The exogenous pathway is an anabolic function in which the dietary protein is metabolized for growth and other physiological functions. The ammonium ion is excreted across the gill membranes under the influence of the

sodium ion. In the aquatic environment the measured ammonia occurs in two forms: dissociated or ionized (NH_4^+), which is nontoxic for fish, and undissociated or un-ionized (NH_3), which is toxic for most finfish at continuous levels exceeding .03 mg/L. The ratio of $\text{NH}_4^+:\text{NH}_3$ is both temperature and pH dependent (Trussel, 1972).

Behavior. Behavior patterns of finfish are classified as territorial, schooling, or sedentary. Not to accommodate these innate patterns could, in all likelihood, provide sufficient stimulus for the chronic stress response to occur.

Rainbow trout, for example, are territorial animals that prefer moving (stream-like) water conditions. When in free-living or confined conditions they establish their required amount of space based on water conditions and food availability. They will defend these areas quite actively. In confined conditions, the defensive acts are mostly in the form of nipping the dorsal fin and(or) pectoral fins of the transgressor. This gives rise to the injured areas appearing as a "target" to other aggressive fish in the population. The fin-nipping sometimes becomes so severe that the condition called soreback occurs. Thus, it is desirable to maintain these fish in ponds that permit the establishment and maintenance of territories. Mullet (*Mugil cephalus*), however, are a schooling fish and require rather still water conditions and adequate space in which to roam.

Nutritional Requirements. Fish are carnivorous, herbivorous, or omnivorous, and the dietary formulation must take this into account. Nutritionally adequate diet formulations are now available for most species of finfish.

Environmental Requirements. The environmental requirements can be classified as physical and chemical. The major physical requirements are water temperature and space. The major chemical requirements are dissolved oxygen, organic and inorganic ions (primarily calcium, carbonate, and bicarbonate), and pH.

Fish have been categorized by their water Standard Environmental Temperature (**SET**) preferences (e.g., coldwater, 12 to 15°C; coolwater, 21 to 22°C; warmwater, 26 to 28°C). The SET is considered the water temperature the fish would select if given the opportunity to do so. For each degree below the SET there is a decrease in metabolic rate. For rainbow trout there is an 8.25% decrease in metabolic rate for each degree below 15°C. For each degree above the SET there is an increase in metabolic rate but a decrease in growth rate. The upper limit of thermal tolerance is termed the thermal death point.

All species of finfish have limits for the degree of crowding they will tolerate. Above these limits, the fish will exhibit the stress response, thereby perhaps compromising the purpose of the research process. For rainbow trout, the accepted limit of population density

is 3.0 kg per m³ per cm of body length. The limits for species other than salmonids have not been determined, largely because they are not commonly raised under intensively managed conditions.

The main chemical preferences are dissolved oxygen levels >60% of saturation and continuous levels of ammonia (as NH₃) <.03 mg/L. These and other environmental preferences are fairly well established (Table 2).

Growth Rate Potential and Allowable Growth Rate. Growth of fish can be measured as an increase in length, weight, or both over time. The growth rate potential (**GRP**) is largely under genetic control and influenced primarily by water temperature.

The allowable growth rate (**AGR**), on the other hand, is the growth rate that the system will permit. The major factors that positively or negatively affect the AGR are water temperature, oxygen availability, water osmolarity, feed quality, and feed quantity.

Under ideal conditions, the AGR and the GRP are equal; however, in the majority of cases this is not so. Thus, a researcher should be capable of taking all the factors influencing growth into consideration to establish the AGR of the system.

Disease History. The impacts of clinical and subclinical episodes of infectious and noninfectious diseases of free-living and confined finfish have been documented (Stoskopf, 1993). However, the documentation relates more to the impact of dead fish rather than those that are clinically ill but do not die. When fish are exposed to environmental conditions exceeding the accepted limits for an extended period, there comes a point at which the fish can no longer cope with the situation (Wedemeyer et al., 1976). Two of the first signs of this are the loss of tissue between the fin rays (the frayed fin syndrome) and a generalized melanosis (body darkening).

Condition Factor. This factor, the length:weight relationship, is calculated by dividing the weight (grams) by the cube of the body length (millimeters). In many cases the number of fish per kilogram is determined by weighing a sample of fish from the population and using this number to determine the mean body length from a prepared weight:length table. Unfortunately, most tables assume a constant condition factor, which is not true; thus, an incorrect length is obtained, which then leads to further complications because many feeding rate models use body length as their basis. To avoid this, fish should be individually weighted ($\pm .1$ g) and measured (± 1.0 mm) to calculate the correct condition factor and to measure growth accurately.

Cannibalism. The major impact of this factor is having a progressive loss of small fish in the population, which can lead to errors in estimating biomass. The problem can be circumvented by grading and(or) feeding regimen. With respect to the latter, if populations are fed in such a fashion (i.e., hand

feeding) that the size variance is minimal, then cannibalism is minimized. This, plus judicious grading, will nearly always preclude cannibalism from being a serious problem.

Oxygen Uptake. The rate of oxygen uptake across the gill lamellar membranes is a function of the differences in partial pressures of dissolved oxygen between the water and the lamellar capillaries. Under ideal conditions the partial pressure difference should be 15 to 20 mm of Hg. However, if the gill lamellar epithelia become hypertrophic or hyperplastic the pO₂ difference must be greater. If the pO₂ difference is insufficient, the oxygen demand of the fish cannot be met and growth suffers accordingly.

Oxygen Demand. The oxygen demand of fish is regulated by the metabolic rate. The Standard Metabolic Rate (SMR) is under the influence of water temperature primarily and fish size secondarily. If the oxygen demand cannot be met because of insufficient pO₂ differential between the water and the lamellar capillaries, the metabolic rate is negatively influenced, which, in turn, negatively influences the growth rate and the general well-being of the fish.

Fecal Solids. If fecal solids are left to accumulate, one or more of the following health-compromising changes in the system can occur: 1) increased biological oxygen demand (**BOD**); 2) sestonosis, an accumulation of solids and other detritus on the buccal aspect of the gill rakers, which will interfere with water flow, oxygen uptake, ammonia excretion, and osmoregulation via the gills; 3) lamellar thickening (hypertrophy or hyperplasia) resulting from the physical irritation due to solids passing over the lamellar tissues; and 4) the accumulation of toxic by-products of fecal solid decomposition.

Water-Associated Factors

The well-being of fish used in research studies is largely dependent upon the quality and quantity of water available (Table 2). The container, nutrition, and management factors are subordinate to the water quality and quantity. The factors to be identified and defined must all be considered as equally important to maintaining the well-being of the fish.

All water supplies used for maintaining fish under controlled laboratory conditions should be analyzed annually for the following: dissolved oxygen (milligrams per liter and saturation percentage), temperature, dissolved nitrogen (milligrams per liter and saturation percentage), dissolved carbon dioxide (milligrams per liter), ammonia nitrogen (milligrams per liter of NH₄⁺ and NH₃), pH, nitrite nitrogen (milligrams per liter), nitrate nitrogen (milligrams per liter), carbonate-bicarbonate alkalinity (milligrams per liter), calcium (milligrams per liter), calcium-magnesium hardness (milligrams per liter), total dissolved solids (milligrams per liter), copper ions (milligrams per liter), zinc ions (milligrams per liter), and iron ions (milligrams per liter).

If municipal water supplies are used, facilities for dechlorination must be installed. Also, most municipal water supplies are quite deficient in dissolved oxygen and have an excess of dissolved nitrogen and carbon dioxide. Thus, these waters must be passed through gas stabilization chambers before being used for the fish (Hackney, 1981).

If recycled water systems (i.e., biofiltration) without a constant make-up inflow are used, the water added to replace the evaporative loss should be a quantitative solution of make-up ions (calcium, carbonate-bicarbonate, sodium, chloride) in distilled or deionized water, to prevent osmoregulatory changes that could interfere with the experimental design (Lai and Klontz, 1980).

Dissolved Oxygen. Ideally, the dissolved oxygen in the water should be >95% of saturation (Davis, 1975). The dissolved oxygen in the water exiting a container should have a pO_2 of >90 mm of Hg. This is a departure from the traditionally accepted dissolved oxygen limit of 5 mg/L, which under certain temperature circumstances is less than 90 mm of Hg pO_2 .

Nitrite. Nitrite is the oxidation product of ammonia. It is under the influence of *Nitrosomonas* sp. The accepted tolerance level of nitrite is .55 mg/L. Levels exceeding this create methemoglobinemia in which the iron in the heme molecule becomes reduced and cannot transport oxygen, thus inhibiting the satisfaction of the oxygen demand of the fish (Bowser, 1983).

Alkalinity and Hardness. In freshwater systems, fish are hypertonic to their environment, i.e., water is attempting to equilibrate the differences in osmolarity. Thus fish must excrete large quantities of urine to maintain their internal physiological balance. Fish in marine environments are hypotonic to the environment and must drink water to maintain their physiological balance. Thus, in freshwater situations, fish in hard water (>250 mg/L alkalinity) will spend less metabolic energy on osmoregulation than fish in soft water (<100 mg/L alkalinity), thus providing more metabolic energy for growth.

Contaminants. Waters used in laboratory systems must be virtually free of municipal, industrial, and agricultural contaminants. In addition, natural contaminants, such as heavy metals (Cd, Cu, Zn, and Hg) and iron must all be in the <.1 mg/L range to preclude an unwanted toxicity. One of the major natural contaminants is nitrogen supersaturation of the water. Excesses higher than 100% of saturation will create gas-bubble disease, a syndrome in which nitrogen comes out of solution in the plasma and creates gas emboli that interfere with blood flow to organs and tissues (Bouck, 1980).

Solids. Waters containing high levels of certain types of suspended or settleable solids can create impairment of oxygen uptake by causing an inflammatory response in the gill lamellar tissues. In addition, certain plant pollens (especially pine pollen) can

cause similar problems in gill tissues. The net results are often a reduction of growth rate and an increase in feed conversion ratio.

Container-Associated Factors

This group of factors that can affect fish health are largely hydraulic in nature. The water replacement time and the water velocity function to provide adequate available dissolved oxygen for the fish and to remove the potentially deleterious metabolic waste products (fecal and uneaten feed solids and NH_3).

The water velocity for riverine species (salmonids) should not exceed 1.0 to 1.5 body lengths per second. For lacustrine species, the water velocity should be minimal.

The composition of the container (circular or rectangular tank) should be of inert, nontoxic fiberglass or glass. Unpainted and water-based painted wood containers are to be avoided, if possible, because of the potential for toxic materials leaching into the system. If wooden containers are to be used, then several coats of high quality clear epoxy should be applied followed by several days of curing time before being put into use.

A new system should be flushed without fish for 1 to 2 d. Then it is advised to put two or three fish into the system and monitor their activities. If these sentinel fish appear not to be negatively affected after 3 to 5 d, then the system can be considered usable.

Nutrition-Associated Factors

Adequate nutrition is a key factor to optimizing growth and health. The diets must be formulated and presented with the requirements of the fish in mind. If these requirements are not met, the research goals could be compromised.

Most salmonids require 3,525 to 3,650 metabolizable kcal of energy/kg of weight gain. Channel catfish require 3,650 to 3,800 metabolizable kcal of energy/kg of weight gain. Most species can derive 4.0 kcal/g of digestible crude protein, 9.0 kcal/g of digestible lipid, and 1.8 kcal/g of digestible carbohydrate. The majority of commercial fish feeds are designed energy-wise to provide feed conversions of 1.2:1 to 1.4:1.

Fish under controlled laboratory conditions should be fed a suitable diet formulation daily. The amount to be fed can vary from maintenance, which is approximately 20 to 25% of the AGR level, to that which achieves the AGR (Klontz et al., 1991). However, there are at least three feeding conditions to avoid: 1) overfeeding will create overly fat fish and the uneaten feed and feces will increase the oxygen demand on the system, 2) underfeeding will cause weight loss and an increased susceptibility to environmentally induced physiological changes, and 3) commercial feed formulations >6 mo old often are vitamin C deficient and high in oxidative rancidity.

Management-Associated Factors

This group of factors consists of the discretionary activities that a researcher can exercise during the research process. The major activities are growth assessment, blood and tissue sampling, killing of the fish, size grading, and container cleaning. Of these, the blood and tissue sampling and killing procedures probably have the greatest potential for creating unwanted physiological changes. The main cause of these changes, in addition to the handling stress, is the type of anesthetic used. Each has its unique physiological "target", which, when altered, could induce chemical changes (Herwig, 1979).

Regularly scheduled growth assessments of the populations, especially the untreated control populations, should include the following performance criteria: 1) Gill condition: the gills should be uniform in color and lie completely within the opercular cavity. 2) Gross appearance: the body color should be that of a healthy fish and not melanotic. Generalized melanosis and fin fraying are the two major clinical signs of the chronic stress response. 3) Observed vs expected length and weight gains: deviations from the expected growth are often the first signs that all is not well within the system. 4) Changes in size variation within the population: increases in size variation are largely a reflection that the entire population is not receiving its daily food ration. 5) Feed conversion ration (**FCR**): the FCR is an indication of proper feeding level, impending clinical episode of either an infectious or noninfectious process, and proper feeding technique. Most commercial fish feeds are formulated to give an FCR of 1.1:1 to 1.2:1. An FCR higher than that should be considered as an indication of a possible breakdown in fish health maintenance.

The most common anesthetic used for fish is tricaine methanesulfonate (MS-222). It is used at 20 to 25 mg/L for tranquilization and at 75 mg/L for complete anesthesia. Other anesthetics are carbon dioxide, Propoxate, and Quinaldine (Herwig, 1979). When using a water-administered anesthetic it is advisable to conduct a bioassay to establish the dosage producing the desired depth and duration of anesthesia. The container should be either glass or plastic to prevent a possible toxic interaction of the anesthetic with the container.

It is not the intent of this presentation to describe the various methods of blood and tissue sampling. The publications of Ames et al. (1966), Blaxhall (1972), Hunn (1967), Klontz and Smith (1968), and Stoskopf (1993) should be consulted for these techniques.

Killing can be accomplished by terminal anesthesia, electrical shocking, anoxia, exsanguination, or a sharp blow to the base of the skull. All involve varying degrees of stress response induction. Perhaps the least stressful is terminal anesthesia, whereas the most stressful is anoxia.

Implications

For fish to be used to their best advantage in basic and applied research studies, the investigator must take into consideration the potential physiological alterations induced by the aquatic environment. Also, the potential effects of physical handling and type of chemical restraint used must be considered. Not to do so could relegate the final data analyses to the "wastebasket of history."

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