Fish Research and the Institutional Animal Care and Use Committee

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Abstract

Fish represent the most diverse group of animals in the vertebrate phylum. The more than 25,000 species are characterized by an array of anatomical, biochemical, physiological, and behavioral repertoires. For this reason, it is difficult to develop a comprehensive guideline on the care and use of fishes. Institutional animal care and use committees (IACUCs) meet the challenge of ensuring adequate fish welfare using guidelines (Animal Welfare Act [AWA] and Public Health Service [PHS] Policy and their guides) derived mainly from the care and use of mammalian species, which may not be optimal for regulating fish research, teaching, or extension activities. Discussion focuses on various issues that often confront IACUCs in meeting regulatory requirements while assuring proper fish welfare. Issues include questions concerning animal tracking and inventory, utilization of fisheries bycatch, facility inspections in remote locations, and euthanasia. Common sense solutions appropriate for field and laboratory fish activities are suggested, which should help investigators, IACUCs, and regulatory agencies meet PHS and AWA objectives.

Key Words: anesthesia; animal welfare; aquaculture; bycatch; euthanasia; fisheries; husbandry; IACUC

Introduction

Since the early 1990s, studies on fish have increased significantly, due largely to the continued expansion of work in the aquaculture and fishery management arenas and the development of the pet aquaria industry. Clearly, aquaculture is now the fastest growing component of agriculture in the world whereas traditional fisheries are in decline (FAO 2002; USDA 2001). Emphasis has been placed on research to address the problems and opportunities associated with these emerging industries. Biomedical research on nonmammalian vertebrates, particularly fish, also has increased substantially because these vertebrates offer alternatives to research on warm-blooded animals and, in many cases, offer more simple systems to address diverse biomedical issues (reviewed by Fabacher and Little 2000). For example, fish offer advantages for studying carcinogenesis and its testing (Law 2001) as well as renal regeneration and development (Reimschuessel 2001), and they are good genetic models for neoplasia (Walter and Kazianis 2001). From the environmental perspective, fish and other aquatic organisms provide sentinel species for the study of environmental toxicology (Beaman et al. 1999; Kelly et al. 1998).

Zebra fish have been utilized as a primary model organism for the study of embryology and development (Lele and Krone 1996; Moorman 2001). The diverse reproductive strategies, varied levels of social organization, and more simple brain morphology of fish also make them ideal organisms for a myriad of behavioral studies (Fabacher and Little 2000). In addition, fish are represented by nearly 25,000 species worldwide, and each species is likely to offer unique characteristics for scientific studies ranging from the cellular to ecosystem level.

However, the sheer number of fish species used in research presents a problem for institutional animal care and use committees (IACUCs[1]) because each species has unique requirements that must be dealt with in research studies. Conversely, warm-blooded animals being used in laboratory research are represented by relatively few species, particularly in biomedical studies. Despite the varied number and types of fish species, regulations governing the use of animals in research are based largely on a few well-studied, predominantly mammalian species.

The focus of this article is to consider several concerns regarding fish welfare, husbandry, and research facing IACUCs, particularly because most of these committees have few if any fish biologists and the existing regulations governing fish welfare are based almost entirely on laboratory mammals. Specific topics of discussion are the inventory of fish numbers, animal inspections in remote locations, bycatch, and acceptable forms of euthanasia.

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[1]Abbreviations used in this article: *Ag Guide, Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching;* APHIS, Animal and Plant Health Inspection Service; AVMA, American Veterinary Medical Association; AWA, Animal Welfare Act; *Guide, Guide for Care and Use of Laboratory Animals;* IACUC, institutional care and use committee; OLAW, Office of Laboratory Animal Welfare; PHS, Public Health Service; USDA, US Department of Agriculture.

IACUC Requirements and Guidelines

Because of the diversity of species and research situations, regulatory agencies have charged the IACUC with complying with the Animal Welfare Act (AWA[1]) (AWA 1966) and Public Health Service (PHS[1]) Policy on Humane Care and Use of Animals (PHS 2000; PL 99-158). Although fish and other poikilotherms are excluded from the AWA, PHS-supported activities related to any live vertebrate require IACUC review and Institutional Assurance. Non-PHS-supported activities on fish, which do not require PHS Assurance from the Office of Laboratory Animal Welfare (OLAW[1], formerly the Office for Protection from Research Risks), may be exempt from IACUC approval. However, exemptions are rarely realized because institutions must clearly distinguish non-PHS- from PHS-supported activities both physically and programmatically. In addition, most academic institutions strive to avoid the appearance that they are conducting IACUC reviews only for those animals for which review is required or only for legal purposes (Williams 1999).

Although procedural matters of IACUCs may differ among universities and institutions, all must follow the principles of AWA and PHS Policy, which are central to the humane care of all animals. These principles are listed below, partially, as six general categories:

1. Animals should be used in teaching, research, and extension programs with due consideration of the relevance to human or animal health, for the advancement of knowledge, or for the good of society.
2. Procurement, transportation, care, and use of animals should be in accordance with the regulations and terms of the federal AWA and the Health Research Extension Act (PL 99-158) and subsequent revisions.
3. The animal species and quality selected for research/teaching should be appropriate. Their use should be limited to keep the number of animals in research to a minimum. Suggested alternatives for animal teaching, research, and extension programs are mathematical models, in vitro biological systems, nonanimal demonstrations, computer models, and audio/visual equipment that augments or replaces animal use.
4. Appropriately trained individuals must oversee the housing, care, feeding, observation, and procedures on all animals. Laboratory managers are expected to follow the guidelines set forth in the *Guide for Care and Use of Laboratory Animals* (*Guide*[1]) (NRC 1996) or the *Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching* (*Ag Guide*[1]) (FASS 1999). Other guides may be adopted by IACUCs.
5. Animal use shall be planned and conducted to avoid or minimize pain and distress to animals. Procedures with animals that may cause more than momentary or slight pain or distress should be performed with appropriate sedation, analgesia, or anesthesia. Procedures involving animals must be performed by or supervised by individuals skilled in the procedures to minimize or eliminate pain and distress.
6. Procedures for euthanasia must be performed in a manner consistent with the latest recommendations of the American Veterinary Medical Association (AVMA[1]) Panel on Euthanasia (AVMA 2000), and all proposed methods must be approved in advance by the IACUC.

The OLAW develops, implements, and oversees compliance with PHS Policy. It often assists institutions in implementing PHS Policy by responding to policy-related questions. Based on our experiences and those of our colleagues who have served on an IACUC, a common problem for investigators and IACUCs dealing with fish protocols is the clear lack of guidelines available for fish care and use. The OLAW addresses this problem by stating, "the PHS Policy is intentionally broad in scope and does not prescribe specifics about the care and use of any species, assigning the task to IACUCs and allowing for professional judgment" (Potkay et al. 1997, p. 2-3). The lack of guidelines for fish care and welfare leaves the decision to the discretion of individual IACUC members, who may do little or approve inappropriate or appropriate protocols, or disallow appropriate protocols, depending on the knowledge of ad hoc individuals or committee members. In few, if any, situations do committee members or other individuals possess the desired expertise on the numerous different species potentially under review.

Although the *Guide* and *Ag Guide* provide general guidelines and specific parameters for animal care and housing for traditional biomedical laboratory and farmed livestock animals, relatively little attention (only a few pages) has been devoted to fishes or "nontraditional species" in the *Guide*. This document is the "reference" for animal research adopted by the PHS and the one most often followed by IACUCs in their evaluation of protocols. Some literature is available on the care and use of fish in the laboratory and field, but the information is necessarily broad considering the number of fish species (ASIH 1987; DeTolla et al. 1995; Ostrander 2000; SCAW 1988; Williams 1999).

Indeed, it is impractical and undesirable to develop a specific guideline for the care and use of even a small fraction of the total number of fish species. Nevertheless, it would be of considerable benefit to IACUCs and principal investigators if general guidelines were developed that incorporate the basic requirements of fish husbandry, with some detail devoted to the most common fish species utilized in the laboratory teaching and research setting. Guidelines might include generally acceptable forms of animal tagging, anesthesia and euthanasia, the means for tracking fish numbers, and the conditions under which tracking numbers would or would not be required. Ideally, the guide would take into account practical differences in field versus laboratory research environments when adopting or developing fish care and use, tracking/inventory, anesthesia/euthanasia, and facility inspection guidelines. Most importantly, because most institutions or universities require IACUC-approved protocols for research on fish (emanating mainly from PHS policy), it is critical that this guide account for the realities of the biology of fishes rather than standards derived almost entirely from mammalian (biomedical and livestock animals, *Guide* and *Ag Guide*) species, for which there is ample detail on animal care and use.

Specific Issues or Problems Facing IACUCs and Investigators Related to Policies for Fish Care and Welfare

A controversy that often confronts IACUCs is the definition of when a fish becomes a regulated animal. Animals covered by the AWA become regulated at birth from the maternal animal. OLAW ruled for birds, which are not covered by the AWA, that they become regulated animals once they hatch from the egg. Based on these regulations, we define the same stage of development in fish to be when the embryo has absorbed the yolk-sac, or begins to forage on its own. This definition covers all reproductive strategies found in fish, including oviparous and viviparous species (Williams 1999). For the purposes of this article, the issues or problems discussed refer to regulated fish as defined above.

There are no clear-cut policies on the use of fish in research, teaching, and extension activities largely due to the lack of adequate guidelines. This situation leads to several problems for IACUCs. After consultation with various professionals, we provide several examples of problems encountered in approval of fish protocols by IACUCs. We specifically address problems of inventory and tracking of fish, remote inspections of fish facilities, utilization of bycatch, and forms of euthanasia. We offer suggestions for how IACUCs might better manage fish research protocols that involve these issues. These points also could be considered in the development of a guide for fish care and use in the field and laboratory.

Inventory and Tracking

IACUCs are charged with determining the appropriateness of animal numbers based on the AWA and the *Guide*. Although neither PHS Policy nor US Department of Agriculture (USDA[1]) regulations explicitly require institutional mechanisms to track animal use by investigators under IACUC-approved activities, both require that proposals to IACUCs specify and include a rationale for the approximate number of animals to be used. According to OLAW, these provisions implicitly require that institutions establish mechanisms to monitor and document the number of animals required and used in approved activities (Potkay et al. 1997). Some institutions interpret this implicit requirement to mean that no animals other than the number approved are to be used. Any activity exceeding this number is a violation. Other institutions are more flexible and may allow a small percentage of variance from the number originally documented in the approved animal protocol. For many field studies of fish, neither of these conditions can be met without potential detriment to the welfare of the animal and possibly the validity of research results.

In various situations, a determination of the exact number of fish in an experiment is unnecessary and impractical. Compared with mammals or other terrestrial vertebrates, when it is relatively easy to count a few animals in a cage or pasture by visual inspection, it is difficult to count fish without handling them and causing them undue stress. It is particularly challenging when conducting research on fish in the wild. Fish move and live in a three-dimensional environment. These environments (e.g., ponds) can be turbid, making it difficult to see and hence count the fish. Even in clear systems it is impossible to count large numbers of fish.

Stress is a primary factor that affects the health and welfare of fish. Excessive handling is associated with the activation of the hypothalamic-pituitary-interrenal (adrenocorticotropin-cortisol) "stress" axis (FSBI 2002; Kreiberg 2000; Wedemeyer et al. 1990). The response of this system to stress in fish is remarkably similar to that of warm-blooded animals. Other stress markers may include the following: increased blood glucose, red blood cell counts, and heartbeat and ventilation rates; and decreased digestive function (Wedemeyer et al. 1990). Even a small transient stress such as rapid netting and movement from one aquarium to another may affect these parameters, which are critical to the "fight-or-flight" response. Although a brief stressor is likely to produce only transient changes in certain biochemical and physiological variables, more prolonged stress (secondary and tertiary) from sustained or repeated handling (e.g., when counting hundreds of fish, which requires more than a few minutes) might include suppression of appetite, reproduction, and growth as well as an impairment of hydromineral balance (FSBI 2002; Schreck 2000). The latter is particularly noteworthy because osmoregulation is a major energy consumer in fish that is dramatically altered by both cortisol and adrenaline.

The burden of trying to restore physiological and metabolic parameters may leave fish less capable of fighting various opportunistic pathogens, including water molds, bacteria, and parasites (Powell 2000). Cortisol also suppresses the immune system (Wendelaar-Bonga 1997). If a disease does break out, it often occurs several days after the initial handling stress. If disease is present, then fish must be treated with medications that are virtually impossible to deliver to all affected animals, that are expensive, and that generate environmental concerns.

Although several factors, including the presence of certain salts, optimal water quality parameters, limited exposures to temperature fluctuations, and use of anesthetics, can limit the detrimental effects of handling stress, it is difficult to eliminate the stress response completely. The best method is to limit the number of times fish are handled, thereby reducing the potential for morbidity and mortality. With this approach, counting fish and tracking their numbers should be done only when it is critical to the experiment itself, rather than to fulfill regulatory requirements initiated for the purpose of reducing fish numbers (tracking animal numbers) and distress.

The diverse scenarios described below are often encountered in field and laboratory research or extension activities. Such cases make it difficult or unnecessary to count or track animal numbers accurately.

Scenario 1: Pond Production Experiments

In an aquaculture study, several ponds are stocked using thousands of fish. At the initiation of the study, the exact number of fish stocked could only be determined by handling each animal. The handling and anesthetization required to count each fish would cause some level of stress, which would result in mortalities. The current acceptable methodology for estimating total numbers of fish stocked in ponds is to count, weigh, and measure a subsample of the population and then estimate population weight and numbers. Based on this sample, weighing all fish to be stocked provides an estimate of the total number of fish stocked at the onset of the experiment.

Mechanical devices, if available, may also be used to help move and count fish. However, mechanical handling also disturbs fish and produces some level of stress and mortalities. As with manual counting, these mortalities often occur several days after initial handling. As the pond study progresses, the number of animals may decline to unknown, unanticipated levels due to natural mortalities, bird predation, cannibalism, or other factors. Hence, tracking the exact animal numbers being used in a protocol is often detrimental for humane reasons or for successful completion of a well-designed project.

Because the hypothesis of the project is related to factors that affect production, the final weight of the fish produced may be more important than the total number of fish at the end of the experiment. Although it is important for IACUC purposes to obtain a rough estimate of the number of fish needed for an experiment, the actual number at the end of the experiment is of minimal value to the experiment and to animal welfare.

Scenario 2: Larval Culture or Fry Production in the Field or Laboratory

Studies that involve small, fry (post-yolksac stage) are even more problematic than pond production experiments. Unlike most terrestrial vertebrates, many fish species produce millions of offspring with the expectation that only a few will live to adulthood and reproduce. How does one count millions of free-swimming fry? An estimate can be made as in Scenario 1, using counts by volume rather than counts by fish weight. However, tracking of fish numbers is usually even more unpredictable during production because fish mortalities are typically highest in the early life stages. This unpredictability is particularly noteworthy in carnivorous fish because slight differences in body size during the fry stage can lead to substantial cannablism of tankmates.

Because of these issues, we suggest that IACUCs not require animal tracking and final counts in Scenarios 1 and 2. Exact numbers should not be required for the initial application because one might over- or underestimate numbers based on standard weighing procedures. Because the number of animals changes during the experiment due to uncontrollable variables, there is no reason to track numbers.

Scenario 3: Fish Production for Experiments or Extension Activity

Often, fish cannot be purchased from commercial sources, or it is more cost-effective for research stations to produce their own animals. Thousands of fish may be produced for research activities with no specific experimental protocol that requires IACUC approval aside from production methods. In these cases, the rationale for number of animals may be vague and a rough estimate at best because animals are produced with no experiments planned.

Importantly, it may take one to several years to produce animals of a desired size for research and demonstration purposes. Hence, it is not always possible to project how many fish are required for "future" research. In addition, this production period will always be accompanied by some mortalities. Furthermore, the practice of counting—and hence handling—animals causes undue distress to the fish and is counterproductive to the goals of the project when animals are produced simply for distribution to subsequent research and extension projects that require standard IACUC-approved protocols. In such a case, IACUCs should provide some flexibility in the degree of justification of animal numbers and should exempt these protocols from tracking requirements.

Summary

We summarize our conclusions regarding the three scenarios described above as follows:

- In small-scale experiments, it is possible to obtain reliable numbers for tracking and inventory of fish by counting the input, mortalities, and output.
- In large-scale pond and tank production systems, where counting is impractical, an estimation of numbers should be acceptable with regard to maintaining inventories.
- When large numbers of animals are produced for use in subsequent research projects, it should not be necessary to track fish numbers.

Fisheries Catch Not Approved in the Original IACUC Protocol

Each scenario described below is an example of a fisheries catch that was not approved in an original IACUC protocol. We propose rational ways to deal with these cases.

Scenario 1. Catching Excess Targeted Species During Field Sampling

Some IACUCs require investigators to define the potential number and species of targeted and untargeted fish (bycatch) to be captured in the field. Investigators can clearly define the number of targeted species required to meet their research objectives, but they cannot predict how many will actually be caught. In some cases, more of the targeted species will be caught than are needed. Should these fish be used to increase the quality of data obtained, or should they be released because the number is beyond the limit indicated in the approved protocol?

If the captured fish is highly stressed and thus has a strong likelihood of dying upon release, it is irrational not to use it to advance the knowledge base to benefit society. An increase in animal numbers usually provides a more robust database for evaluating specific hypotheses.

In field experiments, we recommend allowing investigators to use excess fish numbers, particularly if they are moribund. We suggest that investigators submit an amendment with an appropriate justification post hoc for the excess number of fish.

Scenario 2. Catching Untargeted Species (Bycatch) During Field Sampling

An alternative scenario is the inadvertent capture of untargeted species (bycatch) and their utilization for data collection to increase the knowledge base of that species and its ecosystem. Some IACUCs require investigators to define the potential number and species of untargeted fish (bycatch) captured in the field. Although investigators can estimate the number of targeted species required to meet their research objectives, they cannot always predict the bycatch that is caught. As previously indicated, it seems wasteful that a fish with a high probability of dying will not be used for any purpose. We realize that this issue is complex and that predicting the survivorship of unanticipated catches is subjective when left to the discretion of the investigator. The problem here is that if these animals are to be used, there is no way to gain IACUC approval instantaneously for use of a specific species either in an application or a supplement.

In this case, we take a position that is similar to the issue of excess numbers of a targeted species. We believe in using only bycatch that have already died or that are morbid and unlikely to survive. The decision may ultimately depend on the nature of the funding received for the project. If the research is not under the auspices of PHS policy, then IACUCs should allow a degree of flexibility in utilizing unanticipated catches. The potential of using bycatch should be incorporated into the original protocol submission by the investigators. As with excess targeted species, investigators should also submit an amendment with appropriate justification for the species and numbers used post hoc. We also suggest that the PHS and its governing body (OLAW) consider these types of issues in future regulations. Currently, excess bycatch cannot be utilized by investigators funded by agencies that adopt the PHS policy unless an approved protocol is in place.

Animal Inspections in Remote Locations

Among their various responsibilities, IACUCs are also charged with inspecting animal facilities every 6 mo. By virtue of the AWA, the USDA and its regulatory unit, Animal and Plant Health Inspection Service (APHIS[1]), require inspection of all facilities that hold warm-blooded, AWA-covered animals for longer than 12 hr. The PHS Policy requires inspection every 6 mo of any buildings, rooms, areas, enclosures, or vehicles (including satellite facilities) used for animal confinement, transport, maintenance, breeding, or experiments (including surgical manipulations) by at least two IACUC members.

A satellite facility is defined as any containment outside a core facility or centrally designated or managed area in which animals are housed for more than 24 hr. Although the minimum period of animal holding in nonsatellite facilities that requires inspection is not explicitly stated by PHS Policy, we presume facilities that maintain PHS-regulated and AWA-covered animals (most warm-blooded vertebrates excluding birds, mice, and rodents) for longer than 12 hr must be inspected. All facilities where PHS-regulated vertebrates are surgically manipulated require inspection at least every 6 mo. Other PHS-regulated vertebrates not covered by the AWA (e.g., fish) that are held in permanent facilities or in field areas (satellite facilities) for at least 24 hr must also be inspected every 6 mo.

Because most academic institutions do not distinguish between PHS- and AWA-regulated animal activities, all fish research, teaching, or extension activity must receive IACUC approval, regardless of whether these activities are sponsored by the PHS. Therefore, permanent and satellite facilities holding animals that are neither covered by the AWA nor supported by the PHS may require inspection. Because a considerable amount of work on fish occurs at field sites, often in remote locations, problems arise for IACUCs inspecting these facilities when animals are held longer than 24 hr. Indeed, it is impractical and prohibitively costly for IACUC members to inspect facilities in other states, in other countries, or on remote bodies of water.

Below are listed several situations that are often problematic for inspection by IACUCs. With regard to the scenarios listed, we presume, unless indicated otherwise, that institutions require IACUCs to inspect fish facilities regardless of whether they are supported by the PHS. From this perspective, problems with inspection of distant fish facilities may also extend to those of other AWA-regulated or -nonregulated vertebrate facilities, as described in the five scenarios presented below.

Scenario 1: Inspection of an Institution-sponsored Facility in a Different State from the Investigator's Home Institution State, Involving Animals Held >24 Hr

Consider the case of a biologist from the University of Georgia, who conducts summer research at a marine laboratory in Hawaii that is operated by the University of Hawaii. In such a case, it would seem reasonable that the IACUC of the host institute (Hawaii) would approve the proposed research activity or would accept the home institution's (Georgia's) approved protocol. A memorandum of verification from representatives of the host institution's IACUC, that the facilities meet regulation, could also be required by the home institution. If this verification is not feasible (e.g., due to the short duration—perhaps only a few weeks—of the activity), then we suggest that the investigator seek sponsorship from individuals at the host institute, which either will possess adequate facilities or will attain appropriate approvals.

Scenario 2: A Facility Not Governed by Any Institution, Which Is in a Different State from the Investigator's Home Institution, Involving Extended Periods (6-24 Mo) of Activity

Consider the case of an individual from Michigan, who conducts ecological studies in Idaho and requires a net pen for holding fish in a lake located within a wilderness area. This area or "facility" is not managed by any institution but is located at the project field site. In this type of situation, it would be advantageous to use a fee-for-service program that would allow inspections by IACUC-approved individuals or committee members from an institution located within the state where the facility exists. Because all states possess a land grant university, this program might incorporate IACUCs from the respective institutions to inspect the sites ("facilities"). These banks of IACUCs, organized within the land grant university system, could serve the needs of projects emanating from all states or territories within the United States.

Scenario 3: A Facility Not Governed by Any Institution, Which Is Located in a Different State from the Investigator's Home Institution, Involving Activity of a Short Period of Time (Several Days to Months) Relative to the Required Frequency (Every 6 Mo) of Inspection

In this situation, the host institution's IACUC should consider an exemption from inspection. A signed self-evaluation checklist and photographs of the "facility" could be provided by the investigator in place of a physical evaluation by IACUC members. This possibility is supported by OLAW: Potkay et al. (1997, Question 8) state that laboratories where routine work is done can be monitered by others means, such as random site visits or evaluations. We believe self-evaluations should serve the purpose for such remote facilities. If the facility represents a PHS-supported activity, then an exemption from OLAW should be sought along with a self-evaluation and photographs of the facility.

Scenario 4: A Facility at a Foreign Site or on a Remote Body of Water

Often ecological and fisheries studies may require that fish be held for longer than 24 hr at distant foreign sites (e.g., sites outside the United States for US host institutions) or on research vessels in international waters, which are logistically difficult to inspect. In these situations, the suggestions for Scenario 3 may apply. If the fleet of boats or land-based site is associated with a US institution, inspection should follow standard procedures. If animals are held in a foreign institute that is under Animal Welfare governance, then the country's regulations or inspections should suffice. If the foreign institute is not under any Animal Welfare regulations, then a signed self-evaluation checklist and photographs could apply.

Scenario 5: Shipment of Animals

Transportation or importation of animals from international sites may take longer than 24 hr. In these situations, the IACUC has little control over the process, and the fish "facility" should not require inspection. Rather, a description of how animals will be held during transport should be provided in the IACUC protocol.

Summary

Overall, it must be kept in mind that the institution is accountable for all activities involving fish, despite technical differences in definitions and time limits. Therefore, institutional policies should be designed to obtain the desired outcome of both the PHS Policy and the AWA, which is the humane care of all fish used in research. We believe the solutions suggested above will accomplish the PHA Policy and AWA objectives while providing a common sense solution to remote site inspections.

Euthanasia

A critical aspect of the welfare of animals is to minimize pain and distress. Anesthetics are agents that sedate an animal and at higher concentrations cause them to lose equilibrium, consciousness, and reflex action (Summerfelt and Smith 1990). Anesthetics are used in fisheries, the aquarium trade, and aquaculture to immobilize and to mitigate, at least in part, the stress response(s) of fish during transport and handling and for more complex, surgical procedures. Various anesthetic agents (chemical and physical) have proven useful for fish research (Kreiberg 2000).

The most commonly used chemical anesthetics are listed in Table 1. Various other sources provide a more comprehensive list of fish anesthetics (AVMA 2000; DeTolla et al. 1995; Kreilberg 2000; Stoskopf 1993; Summerfelt and Smith 1990; Varner 2000).

Eugenol and Euthanasia

Certain anesthetics (e.g., tricaine, benzocaine, 2-phenoxyethanol) may also serve as effective agents for euthanasia. A new chemical that has received considerable attention only recently, and one we argue should be suitable for euthanasia, is eugenol or clove oil. Clove oil (95% eugenol) is used worldwide as a food flavoring as well as a local anesthetic in human dentistry. It exhibits antibacterial, antifungal, antioxidant, and anticonvulsive activity (Dallmeier and Carlini 1981; Feng and Lipton 1987; Sladky et al. 2001). Recently, clove oil has gained popularity as a fish anesthetic because it is considerably less expensive than other drugs, is widely available, and has a relatively short induction and recovery period. For these reasons, it has been used as an anesthetic for a variety of freshwater and marine fish (Anderson et al. 1997; Munday and Wilson 1997; Peake 1998; Prince and Powell 2000; Sladky et al. 2001; Soto and Burhanuddin 1995). Despite some concerns about the analgesic properties of clove oil and recent suggestions of the carcinogenic potential of closely related methyleugenol (Abdo et al. 2001; Waddell 2002), we believe that eugenol, when properly used, has potential as a means of inducing euthanasia in fish. For detailed perspectives on the legality of the use of clove oil as an anesthetic in fish being returned to the wild, see *Surgical Implantation of Transmitters into Fish,* also in this issue (Mulcahy 2003).

Animal activities that require euthanasia must use procedures consistent with the latest recommendations of the AVMA Panel on Euthenasia (2000), and any deviations must be justified and approved in advance by the IACUC. In the latest AVMA recommendation, clove oil is not acceptable because appropriate clinical trials have not been performed on fish to evaluate its effects. However, recent evidence, including that from clinical trials, suggests clove oil is equally or more effective than tricaine and other anesthetics (benzocaine, 2-phenoxyethanol) in immobilizing fish (Anderson et al. 1997; Munday and Wilson 1997; Sladky et al. 2001). It exerts a faster response and induces hypoxemia, hypercapnia, and respiratory acidosis similar to that seen with tricaine (Sladky et al. 2001). Interestingly, in comparing tricaine and eugenol, these authors suggest taking great care when using high concentrations of eugenol for induction of anesthesia because ventilatory failure may occur rapidly.

Because clove oil causes a reliable and rapid loss of consciousness and induces hypoxia (critical components to eliminating potential pain), it appears to meet the criteria (AVMA 2000) for euthanasia. Based on these recent results, we suggest that clove oil may be considered acceptable for euthanasia when used at high concentrations (>400 mg/L). As indicated by the AVMA for other chemical agents, fish should be left in solution for at least an additional 10 min after cessation of opercular movement. The use of eugenol should also be limited to applications in which fish will not be consumed.

Euthenasia in Fish by Decapitation Followed by Pithing

Because there is less information on euthanasia of aquatic species compared with companion, farm, and laboratory animals, the guidelines are limited. Indeed, in fish destined

Table 1 Common chemical anesthetics for fish

Compound	Dosage[a] (anesthetic)	Dosage[a] (euthanasia)	FDA[b]-approved	Other remarks
Tricaine methanesolfonate (MS-222)	50-200 mg/L	500 mg/L	Yes, 21-day clearance	Acidic—should be buffered with sodium bicarbonate in soft water; expensive
Benzocaine (Benzocaine hydrochloride)	25-200 mg/L	250 mg/L	No	Soluable in ethanol; Hydrochloride soluble in water; should be neutralized
Metomidate (etomidate)	2-10 m/L	N/A	No	Moderate as an anesthetic, better as a sedative
2-Phenoxyethanol	0.08-0.5 mg/L	0.5 mg/L	No	Narrow margin of safety; long induction time; health safety concern
Quinaldine (quinaldine sulfate)	5-30 mg/L	N/A[b]	No	Sulfate is soluble in water; relatively inexpensive
Clove oil	50-150 mg/L	N/A-see text	No	Inexpensive, physiological effects not well characterized
Carbon dioxide	300-400 mg/L	>800 mg/L	Yes	Induction stressful

[a]Effective dose depends on species, size, temperature, and other variables. Data adapted from various resources including those cited in the text (AVMA 2000; DeTolla et al. 1995; Kreilberg 2000; Summerfelt and Smith 1990; Varner 2000).
[b]FDA, US Food and Drug Administration; N/A, not applicable.

for human consumption in the United States, the only approved methods of euthanasia are CO_2, stunning, and decapitation. Unless justified otherwise, the AVMA Panel on Euthanasia recommends that decapitation of fish be followed by pithing. This ruling is based on the assumption that the central nervous system of fish, presumed to be like that of amphibians and reptiles, can tolerate hypoxia and hypotensive conditions. Although fish are grouped with reptiles and amphibians as "cold-blooded," no evidence is provided by the AVMA to support the notion that fish are tolerant to hypoxia or respond to stimuli once the head is severed, as has been demonstrated for other cold-blooded vertebrates (UFAW/WSPA 1989—cited by the AVMA in their rationale). Moreover, a premise for this argument is that the central nervous system of fish is similar to that of other vertebrates and that fish sense "pain."

We do not argue that fish lack pain perception, but instead suggest that such evidence in either the trunk or head region after decapitation is at best limited. Although the arguments for pain after decapitation require further debate, it would seem that decapitation alone would suffice in euthanizing fish, particularly because this method is most often used when anesthetics and damaged nerve tissue are incompatible with the research design. We recommend not requiring pithing after decapitation until additional evidence supports either that fish brains are tolerant to hypoxia and/or that they perceive pain or distress after decapitation. This revision would also eliminate the potential distress placed on the person who must perform pithings.

Conclusion

Due to the diversity of species and research situations, it has become increasingly more difficult for IACUCs to meet PHS policies for various fish research, teaching, and extension activities. This problem arises, in part, from the lack of general guidelines and policies that take into account the biology of fishes relative to those that have been established for several well-studied mammalian species. Clearly, certain procedures required to implement AWA or PHS policies (e.g., tracking animal numbers) may apply to activities surrounding terrestrial animals, but some procedures could prove detrimental to the welfare of fishes. We have attempted to address some of the concerns or problems that have arisen for IACUCs in evaluating and monitoring fish activities. Common sense solutions appropriate for field and laboratory fish activities are suggested, which should help investigators, IACUCs, and regulatory agencies meet PHS and AWA objectives. Considering the increased use of fish in biomedical and applied research, we propose the development of general guidelines on policies surrounding fish activities. The guide should incorporate the input of scientists, regulators, and industry personnel familiar with the biology of fish and the realities surrounding fish activities. We hope that the information provided in this article will aid in establishing such guidelines.

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