Abstract

The articles in this issue review pertinent and specific information about infectious and noninfectious disease, housing system design and function, husbandry practices and techniques, and Internet resources as they specifically relate to zebrafish (Danio rerio). This article explores aspects that members of institutional animal care and use committees, husbandry care staff, physical plant personnel, veterinarians, principal investigators, and research personnel should consider with regard to the appropriate and necessary care and use of this unique fish model in a teaching, laboratory, or research setting. This information is designed to enhance understanding and facilitate collegial discussions to inform decision making about zebrafish care and use at various institutions or facilities.

Key Words: Danio rerio; disease; housing systems; husbandry and care; institutional animal care and use committee (IACUC); resources; zebrafish

The Zebrafish Model and The Guide for the Care and Use of Laboratory Animals

As indicated by various authors in this issue, zebrafish (Danio rerio) have rapidly expanded from their humble origins of exclusive use as a model for vertebrate development and genetics to a become an increasingly popular biomedical research model with varying uses and applications. In contrast to the previous version of the Guide for the Care and Use of Laboratory Animals (hereafter, the Guide; NRC 2011), about which several articles have been written to assist the users of zebrafish models with the incorporation of this and other aquatic animal species into existing regulatory framework (Borski and Hodson 2003; DeTolla et al. 1995; Koerber and Kalishman 2009; Lawrence et al. 2009; Mason and Matthews 2012; Matthews et al. 2002; Smith and Noll 2009) with great explanation and detail. As a result and in the interest of brevity, I defer to those references.

Animal Care and Use Program

Although not exclusive to the use of zebrafish, this section of the Guide has been discussed in previous issues of the Journal and other publications (Borski and Hodson 2003; DeTolla et al. 1995; Koerber and Kalishman 2009; Lawrence et al. 2009; Mason and Matthews 2012; Matthews et al. 2002; Smith and Noll 2009) with great explanation and detail. As a result and in the interest of brevity, I defer to those references.

Environment, Housing, Management, and Physical Plant

In this issue, Lawrence and Mason (2012) explain that zebrafish housing systems should function to (1) provide a stable and favorable environment that produces and maintains healthy and productive fish and (2) support specific research goals of investigative staff. Although a state-of-the-art fish housing system is important, its functionality is maximized by the quality of the people who manage it. The rapid expansion in the use of the zebrafish model system to diverse applications in various fields may require the use of specialized housing equipment, especially when study designs call for the evaluation of fish over long periods of time. Zebrafish housing systems combining design principles from industrial aquaculture, laboratory rodent housing, and research genetics are now commercially available from a number of sources, and an increasing number of academic research institutions are constructing large, centralized facilities to support their growing zebrafish research programs.

Nasiadka and Clark (2012, in this issue) explain that zebrafish breeding is not a simple process and is still being elucidated. Mate choice and mating behavior depend on olfactory cues, visual stimuli, and social interactions, and spawning 2009), the 2011 version of the Guide incorporates aquatic animals much more expansively and specifically. This article is intended to help correlate the regulatory landscape described in the Guide (chapters 2–5) with the articles in this issue as they relate to the use of the zebrafish model.

Information about the zebrafish model has become widely available and is increasing at a tremendous rate. A good source of this information is the Internet. In this issue, Smith (2012) provides a resource for locating information about methodology, techniques, and organizations associated with zebrafish.
is affected by fish age and size, intervals of egg production, light cycle, diet, and health status. Regarding line propagation and vigor, current breeding strategies avoid inbreeding and focus on maintaining genetic diversity. However, this often results in a high degree of polymorphic variations, which can affect the effectiveness of the research and its reproducibility. Because there are no zebrafish inbred lines in which all individuals are identical and homozygous (as has been described for mouse isogenic lines), attempts should be made to develop breeding schemes that yield relatively robust inbred lines characterized by a high degree of genetic homogeneity.

In an article on larval rearing and husbandry, Wilson (2012, in this issue) considers both published literature and unpublished methods to describe current techniques. She notes that there is, in fact, minimal knowledge about the requirements of larval zebrafish; instead, their care tends to be a function of the constraints and pressures of the facilities and laboratories in which the zebrafish are reared. Even though many aspects of zebrafish early larval physiology, anatomy, and genetics are well studied, this knowledge has seldom been used to improve larval rearing and husbandry techniques. Successful larval rearing is dependent on matching the biological and morphological needs of the larval fish to the rearing protocol. Studies in this area need to be completed and published so that standardization in this field can be based on sound scientific evidence.

**Veterinary Care**

In terms of their effects on veterinary care, biosecurity, and research model integrity, yet-identified viral agents pose a significant threat to zebrafish models. According to Crim and Riley (2012, in this issue), no naturally occurring viral infections have been reported in the zebrafish. However, because many aquatic viruses can infect multiple species, zebrafish are likely to be susceptible to viral pathogens that have been identified in other fish species. It is imperative that naturally occurring viruses of zebrafish be identified and characterized so sensitive diagnostic tests can be designed and adequate health monitoring can be implemented. This process is crucial to the improvement of zebrafish health, reduction of unwanted variability, and continued development of the zebrafish as a model organism. Furthermore, continued research to elucidate the specific pathogenesis and transmission of each virus will be necessary to determine which pathogens are of concern for various areas of research and will aid in the design of biosecurity protocols.

Similarly, the impacts of subclinical disease in zebrafish are of considerable concern. In this issue, Kent and colleagues (2012), Whippes and colleagues (2012), and Sanders and colleagues (2012) report that the two most common infectious diseases of zebrafish in research laboratories are mycobacteriosis and microsporidiosis, which present with both clinical and subclinical signs.

For mycobacteria, a combined diagnostic approach is the most informative, with histology and special staining as a first-level screening tool, polymerase chain reaction for rapid detection and species identification, and the slower culture method reserved for strain differentiation. However, complete elimination of mycobacteria from a large facility supplied with recirculating water is probably not feasible given the ease by which mycobacteria can colonize or re-colonize such systems.

For microsporidia, a combined spore identification diagnostic approach includes wet mount preparation from infected tissues, histology with special staining, and polymerase chain reaction for more rapid confirmation. Because of the horizontal and potential vertical transmission of this parasite by spores that are resistant to the standard method of embryo and egg surface disinfection used for zebrafish, the most effective method for prevention is avoidance. Routine morbidity, mortality, and disease and pathogen surveillance is a very important tool not only for the control of mycobacteriosis and microsporidiosis but also for the detection of other pathogens and the monitoring of the overall health of zebrafish colonies.

Intestinal parasitism by the nematode Pseudocapillaria tomentosa is less frequently observed (Kent et al. 2012, in this issue). This disease usually exhibits clinical signs that are associated with the level of parasitism. A myxozoan parasite is occasionally found in the common mesospheric ducts but does not appear to be associated with any clinical disease. A common condition that may or may not have an infectious etiology is egg-associated inflammation in reproductively mature females.

Noninfectious diseases that are often clinically unapparent in zebrafish include hepatic megalocytosis, bile and pancreatic ductal proliferation, and neoplasms of the ultimobranchial gland, gastrointestinal tract, and testis. Most of these lesions are only evident histopathologically and normally do not display clinical signs unless the lesions are severe.

Facilities should monitor stocks of zebrafish for the presence of these concurrent diseases, and be aware of their existence when interpreting study results. Understanding the cause, modes of transmission, and distribution of these pathogens can provide useful information for the development of control, prevention, and possibly treatment strategies.

With the increasing maintenance of adult zebrafish for longer periods of time, neoplasia is becoming a more common finding in these populations. Spitsbergen and colleagues (2012, in this issue) explain the importance of considering spontaneous tumors in diagnostic cases and retired broodstock as well as carcinogenesis bioassays and mutant tumor models because neoplasia has been observed in a wide variety of histologic types and has affected nearly every organ and most cell types in zebrafish.

Data regarding dietary influences on neoplasia in zebrafish are not yet published. Both diet and husbandry system type have shown a strong influence on tumor incidences in zebrafish. Thus one of the most urgent issues in the rapidly growing field of cancer research using the zebrafish model is the need to optimize husbandry systems and diets to eliminate or minimize the natural carcinogens and possible tumor promoters.
that confound research in many recirculating systems and commercial diets. Some studies have shown a clear link between these, whereas others have not. The role of infectious disease agents in the development and types of neoplasia observed in zebrafish is an ongoing topic of concern.

Finally, Matthews and Varga (2012, in this issue) describe standard anesthesia and euthanasia agents and doses for zebrafish. They also discuss why anesthesia and euthanasia of the different stages of zebrafish can be challenging. As per the Guide, the specific agents and methods chosen for zebrafish anesthesia and euthanasia depend on several host factors, professional veterinary judgment, and the needs or scientific objectives of research protocols. Because zebrafish, like most other animals, cannot communicate states of (dis)comfort, distress, or pain directly and subjectively, it is necessary to rely on human interpretation and knowledge of zebrafish’s species-specific behavior to assess their condition. Typically the difference between sedation, anesthesia, and euthanasia is closely related to drug/agent, dose, and/or contact time. However, current techniques are limited at best and depend on an interpretation of behavior or a measuring of physiological responses, both of which add a layer of complexity.

Conclusions

The articles in this issue elucidate standards set forth in the updated Guide for the Care and Use of Laboratory Animals. In addition, it is critically important that all individuals associated with the care and use of zebrafish at a given institution or facility work collaboratively toward the goal of promoting excellent research with this aquatic animal model. It is essential that everyone understand their particular role(s) in the process and strive to do the best they can to contribute in a collegial and constructive manner to meet the established goal(s). Mutual education and communication among all groups are imperative for success.

References