Journal of the American Association for Laboratory Animal Science
Copyright 2025
by the American Association for Laboratory Animal Science

Vol 64, No 3
May 2025
Pages 520–527

Zebrafish Health, Environmental, and Water Quality Monitoring in Research Facilities: Longitudinal Trends of South Korea from 2018 to 2024

Sang Wha Kim, DVM, MS, PhD,[1] Bo-Kyung Kim,[2] Eun-Suk Lim,[2] Young Hann Kim,[3] Jeong Hwan Che, DVM, PhD,[4] Byeong-Cheol Kang, DVM, PhD,[3,4,5] Yi Rang Na, DVM, PhD, DKCLAM,[6,7,8,*] and Seung Hyeok Seok, DVM, PhD, DKCLAM[8,9,10,*]

Regular monitoring of laboratory zebrafish health status is crucial for ensuring both animal welfare and scientific validity in aquatic research. While zebrafish usage in research has increased substantially due to their biological advantages and experimental benefits, including high fecundity and vertebrate similarity, systematic health monitoring remains uncommon in South Korean facilities. This study presents a comprehensive assessment of zebrafish colony health monitoring practices in South Korea, combining comparative survey data from 2018 and 2024 with microbiologic and environmental analyses of 11 facilities. Our survey revealed a trend: despite facility scale expansion (proportion of the large-scale facilities with >200 tanks increasing from 41.7% to 54.5%) and universal adoption of recirculation systems, monitoring efforts have declined. The percentage of facilities without active monitoring increased from 50.0% in 2018% to 81.8% in 2024, while awareness of monitoring necessity decreased from 91.7% to 72.7%. To investigate these issues, we conducted analyses across 11 facilities (6 research institutes and 5 local suppliers). The analysis encompassed multiple parameters: 1) detection of key infectious agents (*Mycobacterium* spp., *Aeromonas hydrophila*, *Flavobacterium columnare*, *Pseudocapillaria tomentosa*, *Pseudoloma neurophilia*, *Pseudomonas aeruginosa*) in sump tank biofilm, zebrafish specimens, and feed samples; and 2) evaluation of water chemistry parameters (pH, nitrate concentration, conductivity) in tank water. Our findings revealed that *Mycobacterium* spp. were present in biofilm samples from all facilities and in >80% of fish samples from research facilities. *Aeromonas hydrophila* was detected across all sample types. Both *Mycobacterium* spp. and *A. hydrophila* are opportunistic pathogens that necessitate careful consideration in long-term zebrafish experiments. Furthermore, evaluation of water quality analyses indicated widespread deviations from acceptable parameters, particularly in nitrate levels and pH values. Our results underscore the need for implementing standardized monitoring protocols and enhanced water system management to safeguard research integrity, animal health, and occupational safety in zebrafish facilities.

DOI: 10.30802/AALAS-JAALAS-24-158

Introduction

Zebrafish (*Danio rerio*) have emerged as a powerful model organism in diverse research fields, including biomedicine, developmental biology, genetics, and aquaculture.[1] Beyond their traditional use in toxicology studies, which capitalize on their transparent bodies and high fecundity, zebrafish have recently gained prominence as a model organism for studies in cancer microenvironment[2–4] and biomedical research for immunologic evaluation.[5,6] Recognizing the growing importance of zebrafish in research, South Korean facilities began incorporating fish into their animal care and use programs even before the 2011 revision of the *Guide for the Care and Use of Laboratory Animals* expanded its coverage of aquatic animals.[7,8] By 2021, fish had become the second most commonly used experimental animals in South Korea (18.93% of all research animals), surpassed only by rodents.[9]

The proliferation of zebrafish models and their diverse usage have heightened the need for comprehensive health and environmental monitoring in research facilities, encompassing both the animals and their environment. The aquatic habitat of zebrafish presents unique challenges, as infectious agents typically replicate more readily in water versus dry environments. In addition, although zebrafish can tolerate and survive variation in a wide range of water parameters, precise water quality control is essential as variations can stress the fish and alter the experimental results.[10] Consequently, maintaining optimal fish health requires vigilant monitoring of both microbial content and water quality parameters. Consensus opinions with respect to the needs for regular and precise monitoring have, thus, been formed within the zebrafish research community, with the goals of enhancing research reproducibility, animal health, and the safety of personnel. In this regard, several publications have emphasized the necessity of regular

Submitted: 15 Dec 2024. Revision requested: 20 Jan 2025. Accepted: 26 Feb 2025.
[1]College of Veterinary Medicine and Institute of Veterinary Science, Kangwon National University, Chuncheon, Gangwon, Republic of Korea; [2]WooJung BSC, Suwon, Gyeonggi-do, Republic of Korea; [3]Institute for Experimental Animals, College of Medicine, Seoul National University, Seoul, Republic of Korea; [4]Biomedical Center for Animal Resource Development, Seoul National University College of Medicine, Seoul, Republic of Korea; [5]Department of Experimental Animal Research, Biomedical Research Institute, Seoul National University Hospital, Seoul, Republic of Korea; [6]Translational Immunology Lab, Department of Transdisciplinary Medicine, Seoul National University Hospital, Seoul, Republic of Korea; [7]Immunology Core Facility, Department of Translational Research Center, Biomedical Research Institute, Seoul National University Hospital, Seoul, Republic of Korea; [8]Department of Biomedical Sciences, Seoul National University College of Medicine, Seoul, Republic of Korea; [9]Macrophage Lab, Department of Microbiology and Immunology, and Institute of Endemic Disease, Seoul National University College of Medicine, Seoul, Republic of Korea; and [10]Cancer Research Institute, Seoul National University College of Medicine, Seoul, Republic of Korea
**Corresponding author. Email: yirangna@snu.ac.kr or lamseok@snu.ac.kr*

and systematic microbial monitoring of both zebrafish and their aquatic environments.[11–13]

Despite the global consensus on the importance of periodic monitoring that emerged a decade ago, health monitoring reports and facility guidelines for zebrafish have primarily originated from North America and Europe,[1,14] with no comprehensive reports from South Korea. This gap is concerning, as ethical and fiscal responsibilities mandate that animal research studies clearly document the health status of their subjects, including any clinical or subclinical infections.[15] Researchers not only have an ethical responsibility to ensure optimal animal health and welfare, but by protecting these elements, they can simultaneously enhance reproducibility and consistency of experimental outcomes, thereby promoting scientific validity. Although there have been repeated calls for standardized health monitoring programs, information about the health status of laboratory zebrafish in South Korean institutions remains largely unavailable.

To address this knowledge gap, we conducted the first comprehensive investigation of zebrafish facility monitoring practices in South Korea from 2018 to 2024, spanning the period from when global monitoring standards were established to the present. Our study encompasses 2 main components: 1) survey-based assessments of zebrafish health and environmental monitoring practices in South Korean zebrafish research facilities; and 2) actual health, environmental, and water quality monitoring statuses of zebrafish colonies across research facilities and local vendors in South Korea. This study represents the first systematic investigation of zebrafish monitoring in South Korea and aims to provide a foundation for developing standardized monitoring protocols tailored to regional research needs.

Materials and Methods

Bibliometric analysis of zebrafish research trends in South Korea. To assess the trends of zebrafish research in South Korea, a comprehensive bibliometric analysis was conducted. Google Scholar and Web of Science were chosen as scientific literature search databases, and the annual numbers of publications from either database were collected from 1980 to 2023 with the following keywords: 'zebrafish' OR 'danio rerio' and 'Korea'. The keywords were searched in title, abstract, and keywords fields with publication types of original research articles and reviews.

Survey design and administration. A formal survey questionnaire was developed and reviewed by the subject matter experts for clarity and relevance before survey conduction. Zebrafish research facilities in South Korea were identified through Web-based searching, and among the facilities registered in the Korean Zebrafish Society,[16] a large zebrafish researcher society. A total of 12 facilities in 2018 and 11 facilities in 2024 were randomly selected for this study. Managers in charge of each zebrafish facility were requested to complete the survey. The survey questionnaire was distributed by email after the principal investigator of each facility had consented to allow the results to be shared. Survey responses were collected and analyzed to assess current status of zebrafish monitoring practices and results in South Korea.

Sampling procedures. Zebrafish facilities of 11 different locations in South Korea (6 research facilities and 5 local suppliers) participated in the microbial surveillance and water chemistry analysis. Water (5 mL) was sampled from the end of the outlet pipe, where water circulates back to the sump tank, using disposable pipettes, and kept at 4 °C during transportation and until analysis. Five wildtype zebrafish at 12 to 18 mo old in each facility were randomly selected and later euthanized by hypothermia, and the whole carcass was collected for microbial detection. Biofilm was swab-sampled from the sump tank of the water circulation system and kept in a transport medium swab tube at 4 °C until analysis. Feed (brine shrimp and/or commercial feeds) was also sampled in a conical tube and kept at 4 °C until analysis. Samples collected from each facility were sent to the laboratory of Seoul National University for proper sample preparation, and then submitted to QM Diagnostics (Nijmegen, the Netherlands) for microbiologic monitoring and water chemistry analysis. Six zebrafish infectious agents were selected and used for PCR detection based on the company's Circulum sampling kit PCR panel (*Mycobacterium* spp., *Aeromonas hydrophila*, *Flavobacterium columnare*, *Pseudocapillaria tomentosa*, *Pseudoloma neurophilia*, *Pseudomonas aeruginosa*), and 3 criteria were selected for water chemistry assessment (pH, conductivity, nitrate concentration) (Table 1). All animal work was approved by the Institutional Animal Care and Use Committee of Seoul National University.

Results

Bibliometric analysis of zebrafish research in South Korea. Publication trends from 1980, when the zebrafish first became widely used as a laboratory animal, to 2023 show a steady increase in publications worldwide and in South Korea (Figure 1A). The proportion of the number of zebrafish-related publications from South Korea to that from the entire world also shows a constant increase (Figure 1B).

Survey results on zebrafish facility management. A survey questionnaire was developed focusing on 3 key aspects: 1) facility, 2) zebrafish husbandry, and 3) health and environmental monitoring system (Table 2). Two independent surveys were conducted in 2018 and 2024 using the same questions.

Twelve randomly selected zebrafish research facilities in South Korea were surveyed to describe the current status of

Table 1. Sample characterization and tested criteria for heath/environmental/water quality surveillance in zebrafish facilities

Purpose	Test	Samples	Tested criteria
Health and environmental monitoring	PCR	Fish (n = 5 per a facility), biofilm, feed	*Aeromonas hydrophila* *Flavobacterium columnare* *Mycobacterium* spp. *Pseudocapillaria tomentosa* *Pseudoloma neurophilia* *Pseudomonas aeruginosa*
Water quality monitoring	Chemical analysis	Tank water	Conductivity Nitrate pH

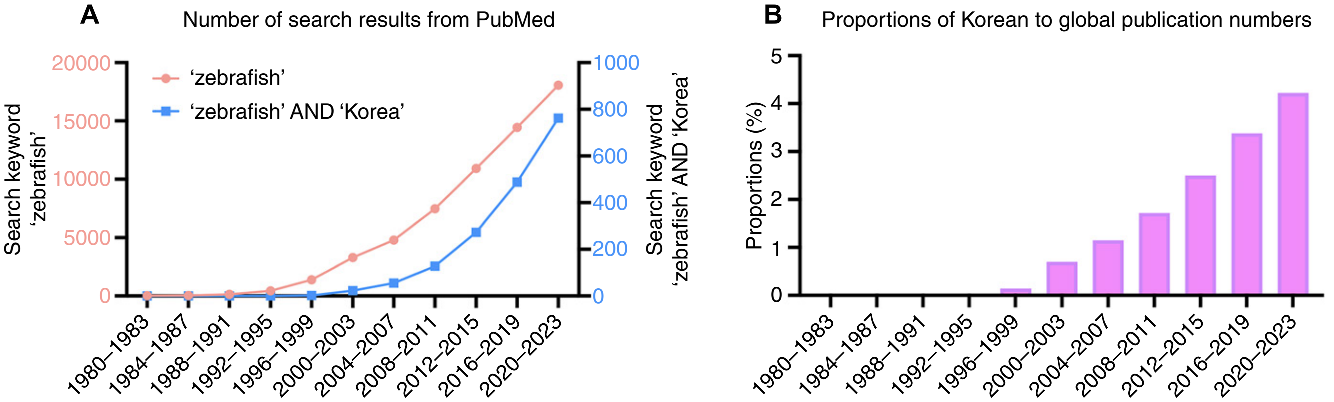

Figure 1. Publication trends of zebrafish studies from PubMed (1980 to 2023). (A) The numbers of search results using keywords 'zebrafish' and 'zebrafish' AND 'Korea' are shown. Both global and Korean publication trends have steadily increased since 1980. (B) The proportions of the search results of 'zebrafish' AND 'Korea' to 'zebrafish' are shown. The proportion of the Korean to global publications is increasing.

zebrafish monitoring in 2018. The survey questionnaire was distributed by email and the response was accepted within a 2-wk period, with a 100% (12/12) response rate (Figure 2A–G). Analysis of survey responses revealed that 66.7% (8/12) of respondents were affiliated with universities, while 33.3% (4/12) were from research institutes. A notable bimodal distribution in facility size was observed, with large-scale facilities (>200 tanks) accounting for 41.7% (5/12) of respondents, followed by small-scale facilities (<50 tanks) at 33.3% (4/12). The survey also revealed that most facilities (83.3%, 10/12) were managed by individual researchers rather than institutional management systems (16.6%, 2/12), indicating a predominance of investigator-driven facility operations. Regarding awareness of zebrafish microbial monitoring concepts, 83.3% (10/12) of institutions reported familiarity with regular monitoring protocols, while 16.7% (2/12) indicated no such awareness. However, when questioned about actual implementation, 50.0% (6/12) of

Table 2. Survey questionnaire for zebrafish facilities in South Korea

Survey questions
1. Facility
Location of the facility
Duration of the facility operation
Entity of facility management (individual laboratory; institution)
Scale of the facility (number of tanks)
System type (recirculation system; single tank system)
2. Zebrafish husbandry
Acquisition route of zebrafish (local commercial suppliers; domestic researcher; international researcher)
Feed (brine shrimp; commercial feed)
Age of the zebrafish (adult; juvenile; larva)
3. Health and environmental monitoring system
Are you aware of the concept of 'zebrafish health monitoring'?
Does your facility have its own zebrafish health monitoring program?
Does your facility regularly conduct a zebrafish health monitoring program?
Does your facility regularly conduct environmental monitoring?
How satisfied are you with the microbial infection control in your facility?
To what extent do you believe there is a need to monitor microorganisms in your facility?

facilities reported no active health monitoring programs. The gap was even more pronounced for environmental monitoring, with 66.7% (8/12) of institutions reporting no regular monitoring practices in place.

Six years later, in 2024, 11 zebrafish research facilities in South Korea were requested to complete the second and successive survey to describe current status of the zebrafish health and environmental monitoring (Figure 2H–N). Selected facilities were not necessarily the same ones selected in 2018. The survey questionnaire was distributed by email and the response was accepted within a 2 wk-period with a 100% (11/11) of response rate. Analysis of survey responses revealed that 72.7% (8/11) of respondents were affiliated with universities, while 27.3% (3/11) were from research institutes. Large-scale facilities (>200 tanks) accounted for 54.5% (6/11) of respondents, showing an increase compared with 2018, while small-scale facilities (<50 tanks) decreased to 18.2% (2/11), indicating a clear trend toward larger facility operations. All institutions (100%, 11/11) reported using recirculation systems, with none relying solely on a single-tank system. While individual researcher management (72.7%, 8/11) still predominated over institutional management systems (27.3%, 3/11), this ratio showed a slight decrease compared with 2018. Regarding awareness of regular zebrafish microbial monitoring concepts, 72.7% (8/11) of institutions reported familiarity with monitoring protocols, while 27.3% (3/11) indicated no such awareness, revealing a decline in monitoring awareness compared with 2018. The implementation rates of monitoring programs showed further deterioration from 2018 levels: 81.8% (9/11) of facilities reported no active health monitoring programs, and 72.7% (8/11) conducted no environmental monitoring practices.

Zebrafish health surveillance sampling from facilities in South Korea. Six of the research institutes that participated in the survey agreed to sampling for zebrafish health surveillance, as illustrated in Figure 3. As the survey results indicated that most institutions procure wild-type zebrafish from local suppliers (that is, local aquariums), health surveillance was extended to include 5 local suppliers.

Microbial surveillance findings. In research facilities, 3 out of the tested 6 infectious agents were detected (Figure 4). Infectious agents found in samples of diet were also identifiedin fish and biofilm samples. *Aeromonas hydrophila* and *P. aeruginosa* were identified in all 3 types of samples. However, though *Mycobacterium* spp. were not detected in diet samples, it was identified in samples of fish (83.3%; 5/6 samples and biofilm (100%; 6/6 samples).

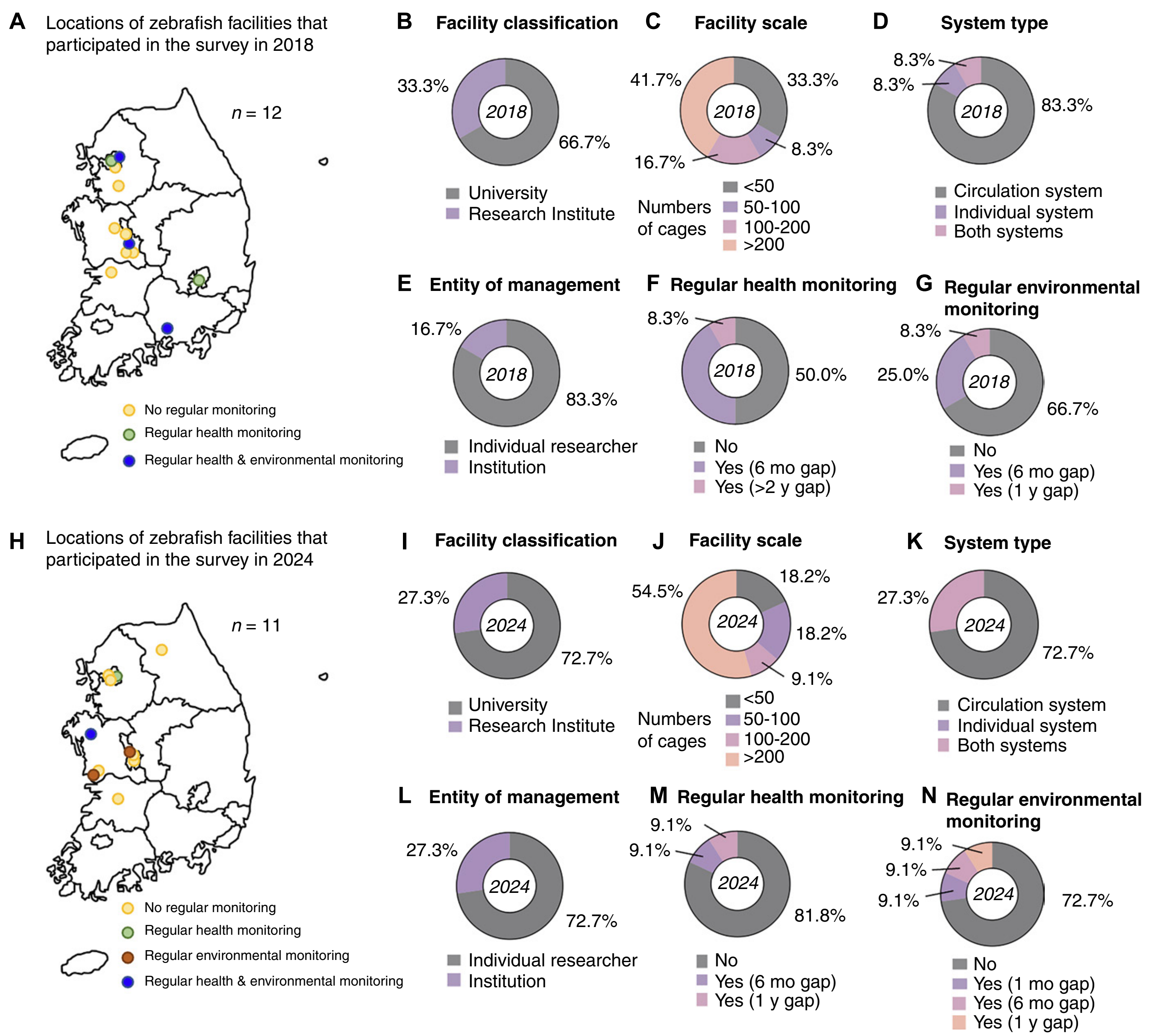

Figure 2. Survey results of zebrafish facilities regarding zebrafish health monitoring and environmental monitoring in the Republic of Korea in 2018 and 2024. (A–G) Survey results of 12 zebrafish facilities in 2018 are summarized. (H–N) Survey results of 11 zebrafish facilities in 2024 are summarized.

Of note, the prevalence of detected infectious agents differed among local suppliers (Figure 4). All 6 tested infectious agents were found in samples from the local suppliers, showing plentitude of infectious agents in uncontrolled environments. *Aeromonas hydrophila* was found in the highest frequency from all 3 samples indicating the difficulty of environmental hygiene control. Diet from the local suppliers showed 3 infectious agents including *A. hydrophila*, *P. neurophilia*, and *P. aeruginosa*, which were reflected in the fish and biofilm samples. In addition, *F. columnare* and *P. tomentosa* were detected from biofilm and fish, respectively and solely.

Water chemistry analysis. All 3 tested criteria of water chemistry analysis did not show any statistically significant difference between research facilities and local suppliers. Most of the facilities (90.9%, 10/11) showed conductivity values in the acceptable range (550 to 650 μS/cm) (Figure 5A).[17] However, the nitrate concentration was over the acceptable range in most of the facilities (<50 ppm), indicating that removal of the final products of the nitrogen cycle was not effective (Figure 5B).[18] Samples from only one facility had pH levels within the acceptable range (6.7 to 7.3), and all others had values below the lower limit (Figure 5C).[17]

Discussion

Publications from South Korea that describe use of zebrafish as experimetal models have shown continuous growth since the early 2000s, with Korea's contribution to global zebrafish research now >5%.[9] This growth can be attributed to various factors, notably: 1) collaborative efforts between academic and regulatory sectors to implement alternative testing methods, and 2) the establishment of an essential infrastructure for zebrafish research. The synergistic combination of ethical considerations driving zebrafish usage, recognition of the platform's unique advantages, and the establishment of robust national support systems (e.g. the Korea Zebrafish Resource Center) and academic societies (e.g. the Korean Zebrafish

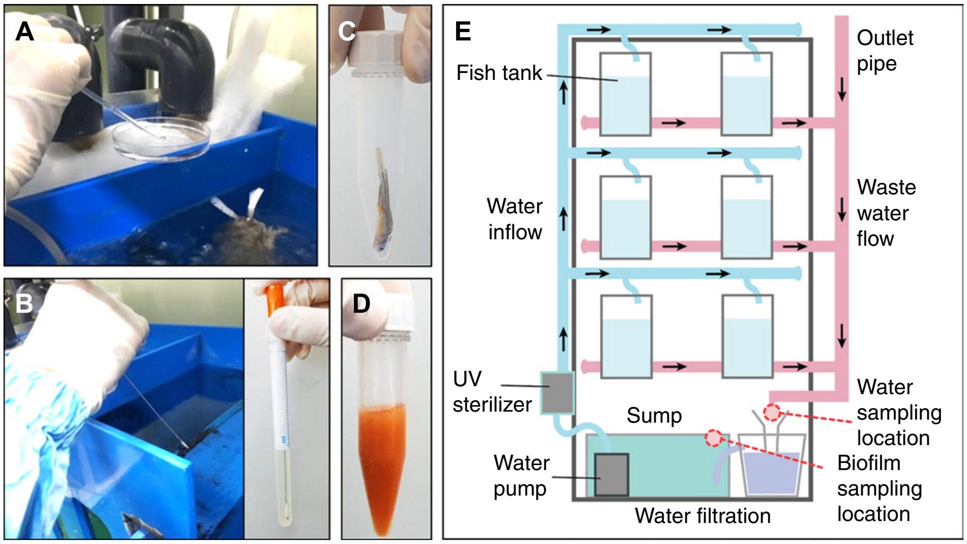

Figure 3. Sample preparation for heath/environmental surveillance in zebrafish facilities. All samples were kept at 4 °C until analysis. (A) Circulating water was sampled using a culture dish and disposable pipette for chemistry analysis. (B) Biofilm on the sump tank was swab-sampled and kept in the transport medium tube for further transportation. (C) Zebrafish were euthanized by hypothermia and sampled in a conical tube for health surveillance. (D) Feed (brine shrimp and/or commercial feed) was sampled in a conical tube. (E) Biofilm and water sampling locations of the zebrafish husbandry system are indicated with a red-dotted circle.

	Research facility			Local supplier		
	Fish	Biofilm	Feed	Fish	Biofilm	Feed
Aermonas hydrophila	33.3%	66.7%	16.7%	80%	80%	60%
Flavobacterium columnare	0%	0%	0%	0%	20%	0%
Mycobacterium spp.	83.3%	100%	0%	40%	100%	0%
Pseudocapillaria tomentosa	0%	0%	0%	20%	0%	0%
Pseudoloma neurophilia	0%	0%	0%	60%	0%	20%
Pseudomonas aeruginosa	33.3%	50%	50%	0%	40%	40%

(Color scale: 0% to 100%)

Figure 4. Detection prevalence of representative zebrafish infectious agent detection throughout the South Korean zebrafish facilities by PCR.

Society) has led to this dramatic acceleration of zebrafish research in Korea.[16,19]

Our survey results reveal that, despite this increasing research trend, most Korean facilities do not conduct health monitoring of their zebrafish. The importance of zebrafish health monitoring was globally emphasized in the mid-2010s, with numerous guidelines and publications addressing this issue.[11–13] In our 2018 survey, while 83.3% (10/12) of institutions reported awareness of zebrafish microbial monitoring concepts, only half (5/10) implemented regular monitoring, highlighting a disconnect between concept awareness and practical implementation. In addition, 16.7% (2/12) of institutions reported no awareness of zebrafish microbial monitoring concepts, indicating a need for institutional education and awareness expansion. Notably, 91.7% of institutions (11/12) acknowledged the necessity of monitoring, suggesting the opportunity for specific protocols and information related to service providers to for expand implementation.

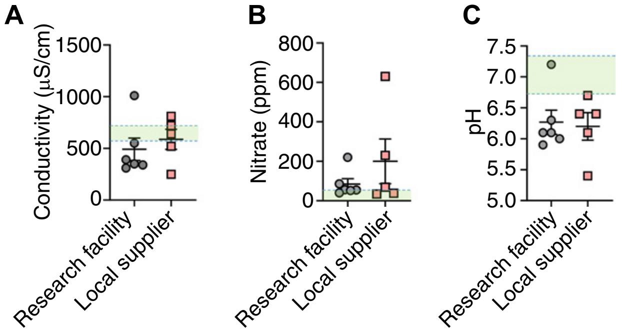

Figure 5. Water chemistry analysis of samples from research facilities and local zebrafish suppliers in South Korea. Acceptable ranges of each criterion are highlighted with green-colored boxes, and upper and lower limits are marked with blue dotted lines. (A) Conductivity of the environmental water (acceptable range, 550 to 650 µS/cm). (B) Nitrate concentration of the environmental water (acceptable range, <50 ppm). (C) pH of the environmental water (acceptable range, pH 6.7 to 7.3).

Since the 2010s, Korea has lacked guidelines, regulations, and legislation mandating zebrafish health monitoring. Our 2024 survey reveals a significant decline in monitoring practices compared with 2018. The proportion of institutions reporting no knowledge of zebrafish microbial monitoring increased to 26.3% (3/11) institutions, while only 2 out of 8 institutions that claimed awareness actually conducted monitoring. The percentage of institutions not performing regular monitoring increased dramatically from 50.0% (6/12) in 2018% to 81.8% (9/11) in 2024, while acknowledgment of monitoring necessity decreased from 91.7% (11/12) to 72.7% (8/11). Due to the variation in institutional sampling between the 2 time points (2018 and 2024), discrepancies in 1 or 2 institutions could be attributed to sampling error. However, the aggregate data analysis revealed that the observed patterns exhibited variations across a substantial number of facilities, extending beyond the margin of sampling error.

Contrasting with this declining monitoring trend, zebrafish research facilities in Korea have grown in scale. Large facilities (>200 tanks) increased from 41.7% (5/12) of all facilities in 2018, to 54.5% (6/11) in 2024; and all facilities (11/11) used recirculation systems in 2024, compared with 8.3% (1/12) using such systems in 2018. This expansion in facility size and universal adoption of recirculation systems, which can amplify the impact of microbial infections, underscores the increasing importance of monitoring practices.

To directly assess the implications of the current zebrafish monitoring status in South Korea, we conducted microbial, environmental, and water quality monitoring at 11 research facilities and local vendors that previously had no monitoring programs in place. The findings from this preliminary assessment strongly indicated the necessity for implementing regular monitoring protocols in these facilities:

1. Within specific facilities, infectious agents detected in feed were consistently found in samples from either fish and biofilms. Although a causal relationship between feed-derived infectious agents and those detected in fish and biofilms has not been proven, there is the possibility that controlling feed-derived infectious agents could contribute to controlling infectious agents detected in zebrafish and their husbandry environment. This observation emphasizes the importance of maintaining feed freshness and implementing microbial surveillance. Even meticulous maintenance of fish and research environments becomes ineffective without control of the microbial quality of feed, which represents an ongoing external input into the system.
2. Local suppliers exhibited greater diversity of infectious agents compared to research facilities, potentially due to factors such as inadequate management practices or concurrent cultivation of various fish species within the same facility. A notable example is the detection of *P. neurophilia* in feed samples from local suppliers. As this organism is an obligate parasite requiring a fish host for reproduction, its presence in feed samples suggests the possibility of reverse contamination from infected fish. This contamination pathway presents a risk for transmission of infectious agents to other fish within the facility through feed distribution. Consequently, specimens sourced from local suppliers may require more stringent quarantine measures and comprehensive health monitoring protocols.
3. Two pathogens, *A. hydrophila* and *Mycobacterium* spp., were frequently detected in samples of both fish and biofilms, despite their absence in feed samples. As common residents of aquatic milieus and potential zoonotic agents capable of causing disease in both humans and fish, these organisms are priority targets for surveillance programs.[20,21] Notably, *Mycobacterium* spp. were detected in 100% of biofilm samples from both research institutes and local suppliers, making them the most prevalent pathogens identified. As significant zoonotic agents,[22] these findings underscore the necessity for regular monitoring not only for zebrafish health and experimental accuracy but also for researcher safety. This situation requires vigilant observation of pathogen detection patterns and fish infection dynamics not only of clinical cases but also of subclinical cases, along with strict adherence to personal protective equipment protocols and careful consideration exposures that might occur during the conduct of experimental procedures.

The monitoring conducted in this study has several limitations that warrant discussion. The analyses were limited to a small number of samples from each facility (5 randomly selected zebrafish, a single biofilm sample, and a single feed sample), which likely resulted in the detection of only the most prevalent infectious agents. This sampling limitation supports the possibility that the results may not fully represent the complete microbiologic status of each facility. Furthermore, while our initial assessment focused on a limited panel of representative zebrafish infectious agents to evaluate the overall monitoring status, comprehensive zebrafish health and environmental monitoring require evaluation for a broader spectrum of potential pathogens (e.g. covert mortality nodavirus, *Edwardsiella ictaluri*, *Piscinoodinium pillulare*, *Pleistophora hyphessobryconis*, zebrafish picornavirus). Finally, although this study conducted detection at the broad *Mycobacterium* spp. level, further studies would benefit from more specific species-level detection to differentiate between zoonotic pathogens and zebrafish-specific pathogens.

Given that zebrafish must be maintained in aquatic environments, it is difficult to achieve complete control over environmental infectious agents. While it would be ideal if all zebrafish were free from infectious organisms, maintaining such conditions in an aquatic system is likely unfeasible.[12] If necessary, establishing and maintaining specific pathogen-free status for zebrafish is certainly possible.[23] However, in situations where specific pathogen-free status is not required, it is recommended to carefully monitor whether environmental infectious agents, when detected, are also present in samples from fish or if they are associated with clinical manifestations of disease.[11]

In our study, while no fish exhibited significant clinical symptoms associated with infectious agents, certain infectious agents (*A. hydrophila, Mycobacterium* spp., *P. aeruginosa*) were detected not only in the environment but also in samples from the fish body, indicating that it may be advisable for some facilities to conduct monitoring with consideration for potential subclinical infections. It is well known that even well-managed facilities can experience background infections in zebrafish by *Mycobacterium* spp., such as *M. chelonae*, as these organisms are difficult to completely eradicate from the environment.[24] Among infectious agents that do not produce clinical llness, those considered capable of significantly impacting the other fish housed in the same recirculating system (e.g. *P. neurophilia*, *Mycobacterium* spp.) require ongoing efforts directed at environmental elimination effort and clinical infection surveillance.[25] This requires the implementation of effective disinfection methods, stringent quarantine procedures for newly introduced fish, and comprehensive health and environmental monitoring protocols. In particular, periodic and accurate identification of resident microorganisms in the environment is crucial. Previous studies showed that for detecting facultative pathogens, microbial detection through sampling of sludge or biofilm from sumps or biofilters is more sensitive and reliable than direct sampling of the fish.[26–29] Therefore, environmental sampling holds significant importance and should be routinely performed together with the examination of fish specimens themselves.

Because infections by commensal organisms typically occur when the host's immune system is compromised, environmental conditions must be carefully monitored to maintain fish immunity (for example, in cases involving *A. hydrophila* and *Mycobacterium* spp.). The most critical factors that can compromise the fish immune system include 1) temperature, 2) pH, and 3) nitrogen waste concentrations, underscoring the importance of water quality management.[30] However, our investigation revealed that nitrate concentrations, the end product of nitrogen waste, exceeded acceptable ranges in most facilities, while pH values were below normal levels. These findings suggest the possibility of chronic stress and resulting inconsistent experimental results from inadequate water quality management.[10] Suboptimal water quality parameters, even when not directly causing infections, may introduce experimental inconsistencies by disrupting fish ionic and osmotic homeostasis, thereby serving as a significant source of non-protocol–based variation of an experiment.[18] Therefore, systematic approaches to managing nitrogen cycle end-products (e.g. water changes, use of refugium) and pH regulation methods based on water source characteristics must be established.

Subclinical infections can also impact the consistency and accuracy of experimental outcomes, resulting in the nonprotocol variations.[11,12,31] To ensure robust and reproducible experimental results, fish health must be maintained even at the level of subclinical infections. Therefore, the potential impact of microbial factors must be considered even in the absence of severe clinical symptoms such as mortality from infectious diseases. In this regard, regular microbial and environmental monitoring becomes particularly crucial in situations in which the fish lack any evidence of clinical disease.

This study describe here demonstrates, using South Korea as an example, that regular health, environmental, and water quality monitoring may be inadequate even in regions and countries having robust communication between researchers who work with zebrafish. Our results revealed the presence of significant human and fish infectious agents across facilities, with some institutions even failing to maintain appropriate water quality conditions. This provided participating institutions with both a wake-up call and practical evidence of the need for increased rigor of monitoring. We aimed to use these findings to highlight current conditions and emphasize the necessity of zebrafish monitoring across institutions. Implementing standardized monitoring protocols is not merely a procedural consideration but a fundamental requirement for ensuring experimental reproducibility and reliability, protecting researcher health through proper zoonotic disease surveillance, maintaining animal welfare through systematic health, environmental, and water quality monitoring, and supporting the sustainable growth of zebrafish research globally. As zebrafish continue to gain prominence in biomedical research, the scientific community must transition from acknowledging the importance of monitoring to actively implementing comprehensive surveillance programs.

As the growth trend in zebrafish research is global rather than limited to the South Korea, broad efforts should be made with respect to best practices related to monitoring water quality and microbial status. The Zebrafish International Resource Center provides standard operating procedures through their health monitoring guidelines as well as FELASA-AALAS–published recommendations for monitoring and reporting disease and health status in laboratory fish, particularly zebrafish.[14,32] Given the availability of well-established monitoring guidelines, institutions should implement systematic approaches based on these resources. The establishment of periodic and systematic monitoring protocols at a global scale, concurrent with the expansion of zebrafish research, is paramount for ensuring experimental consistency, reproducibility, and biosafety. Through the implementation of robust monitoring protocols based on established guidelines, research institutions can simultaneously safeguard scientific rigor, occupational safety, and laboratory animal welfare. This comprehensive approach to health monitoring serves as a cornerstone for the sustainable advancement of zebrafish-based research, facilitating its continued growth while maintaining the highest standards of both scientific excellence and ethical conduct.

Conflict of Interest

The authors have no conflicts of interest to declare.

Funding

This work was supported by the National Research Foundation of Korea grant funded by the Korea government (Ministry of Science and ICT) (Grants RS-2024 to 00356146, 2021R1A2C2010219, and 2022R1C1C2010114). This study was also supported by the Seoul National University Hospital research fund (Grants 0320223010, 0320210190, and 0320240050).

References

1. Aleström P, D'Angelo L, Midtlyng PJ, et al. Zebrafish: housing and husbandry recommendations. *Lab Anim.* 2020;54(3):213–224.
2. Mealiea D, Boudreau E, De Silva N, et al. Modeling oncolytic virus dynamics in the tumor microenvironment using zebrafish. *Cancer Gene Ther.* 2021;28(7–8):769–784.
3. White R, Rose K, Zon L. Zebrafish cancer: the state of the art and the path forward. *Nat Rev Cancer.* 2013;13(9):624–636.
4. Yan C, Do D, Yang Q, Brunson DC, Rawls JF, Langenau DM. Single-cell imaging of human cancer xenografts using adult immunodeficient zebrafish. *Nat Protoc.* 2020;15(9):3105–3128.
5. Povoa V, Almeida CR, Maia-Gil M, et al. Innate immune evasion revealed in a colorectal zebrafish xenograft model. *Nat Commun.* 2021;12(1):1156.
6. Torraca V, Gomes MC, Sarris M, Mostowy S. Meeting report: zebrafish infection and immunity 2019. *Lab Anim (NY).* 2019;48(10):284–287.
7. Institute for Laboratory Animal Research. *Guide for the Care and Use of Laboratory Animals.* 8th ed. National Academies Press; 2011.
8. Sanders GE. Zebrafish housing, husbandry, health, and care: IACUC considerations. *ILAR J.* 2012;53(2):205–207.
9. Kim SW, Jung WJ, Han SH, et al. Current status of the zebrafish euthanasia and humane endpoint in the Republic of Korea and guideline suggestion from nationwide expert elicitation: a model for other countries. *Zebrafish.* 2024;21(1):53–66.
10. Barton B. Stress in fishes: a diversity of responses with particular reference to changes in circulating corticosteroids. *Integr Comp Biol.* 2002;42(3):517–525.
11. Borges AC, Pereira N, Franco M, et al. Implementation of a zebrafish health program in a research facility: a 4-year retrospective study. *Zebrafish.* 2016;13(Suppl 1):S115–S126.
12. Collymore C, Crim MJ, Lieggi C. Recommendations for health monitoring and reporting for zebrafish research facilities. *Zebrafish.* 2016;13(Suppl 1):S138–S148.
13. Murray KN, Varga ZM, Kent ML. Biosecurity and health monitoring at the zebrafish international resource center. *Zebrafish.* 2016;13(Suppl 1):S30–S38.
14. Mocho J, Collymore C, Farmer S, Leguay E, Murray K, Pereira N. FELASA-AALAS recommendations for monitoring and reporting of laboratory fish diseases and health status, with an emphasis on zebrafish (Danio rerio). *Comp Med.* 2022;72(3):127–148.
15. Kilkenny C, Browne WJ, Cuthill IC, Emerson M, Altman DG. Improving bioscience research reporting: the ARRIVE guidelines for reporting animal research. *Osteoarthritis Cartilage.* 2012;20(4):256–260.
16. Korean Zebrafish Society. Group introduction. Accessed November 10, 2024. https://kzfs.co.kr/.
17. Goodwin N, Westall L, Karp NA, et al. Evaluating and optimizing fish health and welfare during experimental procedures. *Zebrafish.* 2016;13(Suppl 1):S127–S131.
18. Hammer, HS. Water quality for zebrafish culture. In: Cartner SC, Eisen JS, Farmer SC, Guillemin KJ, Kent ML, Sanders GE, eds. *The Zebrafish in Biomedical Research.* Academic Press; 2020:321–335.
19. Korea Zebrafish Resource Center. Group introduction. Accessed November 10, 2024. https://kzrc.org/.
20. Boylan S. Zoonoses associated with fish. *Vet Clin North Am Exot Anim Pract.* 2011;14(3):427–438, v.
21. Igbinosa IH, Igumbor EU, Aghdasi F, Tom M, Okoh AI. Emerging *Aeromonas* species infections and their significance in public health. *Sci World.* 2012;2012:625023.

22. Lowry T, Smith SA. Aquatic zoonoses associated with food, bait, ornamental, and tropical fish. *J Am Vet Med Assoc.* 2007; 231(6):876–880.
23. Kent ML, Buchner C, Watral VG, et al. Development and maintenance of a specific pathogen free (SPF) zebrafish research facility for *Pseudoloma neurophilia*. *Dis Aquat Organ.* 2011;95(1): 73–79.
24. Whipps CM, Matthews JL, Kent ML. Distribution and genetic characterization of *Mycobacterium chelonae* in laboratory zebrafish Danio rerio. *Dis Aquat Organ.* 2008;82(1):45–54.
25. Max-Planck-Gesellschaft. Zebrafish husbandry and breeding. Accessed November 10, 2024. https://www.mpg.de/10973805/husbandry-breeding.
26. Crim MJ, Lawrence C, Livingston RS, Rakitin A, Hurley SJ, Riley LK. Comparison of antemortem and environmental samples for zebrafish health monitoring and quarantine. *J Am Assoc Lab Anim Sci.* 2017;56(4):412–424.
27. Miller M, Sabrautzki S, Beyerlein A, Brielmeier M. Combining fish and environmental PCR for diagnostics of diseased laboratory zebrafish in recirculating systems. *PLoS One.* 2019;14(9):e0222360.
28. Mocho JP. Three-dimensional screen: a comprehensive approach to the health monitoring of zebrafish. *Zebrafish.* 2016;13(Suppl 1):S132–S137.
29. Naumann U, Brazzell JL, Crim MJ, Hoppe B. Comprehensive colony health management and emerging pathogens of the annual killifish species *Nothobranchius furzeri*. *J Am Assoc Lab Anim Sci.* 2024;63(1):20–33.
30. Smith SA. *Fish Diseases and Medicine.* 1st ed. CRC Press; 2019.
31. Kent ML, Harper C, Wolf JC. Documented and potential research impacts of subclinical diseases in zebrafish. *ILAR J.* 2012;53(2):126–134.
32. Zebrafish International Resource Center. Health monitoring protocols: sentinel fish program. Accessed November 10, 2024. https://zebrafish.org/wiki/protocols/health/.