Implementation of a Biosecurity Program at the UCL Zebrafish Facility

Jenna Hakkesteeg, Visila Moiche, Carole Wilson
j.hakkesteeg@ucl.ac.uk, ana.vega@ucl.ac.uk, carole.wilson@ucl.ac.uk

UCL Zebrafish Facility, Division of Biosciences

Aim: To prevent the introduction of new pathogens into the UCL Zebrafish Facility and reduce the spread of those that might be already present.

Introduction

Biosecurity is a set of measures taken to preserve the health of living organisms from any type of infectious diseases. Recommendations for an aquatic biosecurity program should include the identification, control, prevention, and elimination of infections that cause disease or lead to abnormal responses in animals that affect their welfare. Furthermore, it has been demonstrated that research studies can be affected by subclinical diseases, sometimes leading to unreliable results[1], increasing the number of animals used. Hence, the implementation of a biosecurity program at the UCL Zebrafish Facility promotes the refinement and reduction of animals in research.

Prevention and Control

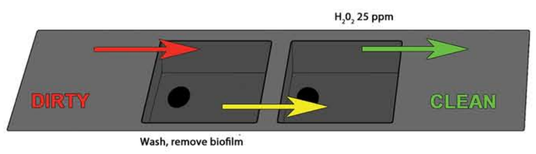

Fig. 1: All equipment is disinfected before entering the room. Equipment in contact with fish are autoclaved to render free from bacterial spores. Thorough cleaning and disinfecting of equipment within the room to remove biofilm is done prior to autoclaving.

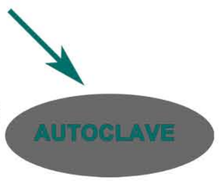

Fig.7: New colonies are from parental stocks that are less than 1 year old. Fish that are moved out of the SPF Room cannot be returned and our wild type stocks used for outcrosses are transferred to separate tanks.

MAIN PREVENTION AND CONTROL MEASURES IMPLEMENTED

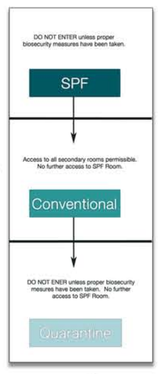

Fig. 2: Controlled movement of staff between rooms.

Fig. 3: Staff training is obligatory.

PVPI

Fig. 6: Embryos are disinfected with buffered povidine-iodine (PVPI) where PVPI is shown to reduce the spread of *Mycobacterium spp.* and also safe for zebrafish embryos[2].

Fig. 5 Food is stored at controlled temperatures reducing the risk of contamination. As Artemia, a live food, is a vector for pathogens, frozen irradiated copepods are used as a replacement. Salt water rotifer (*Brachionus plicatilis*) is used to reduce potential pathogen risk.

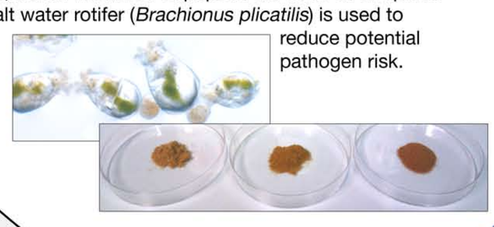

Fig. 4: PPE. The use of gloves, scrubs and change of shoes is mandatory. Showering if in contact with fish 24 hours prior to entering.

Diagnostics

Molecular diagnostic techniques, such as PCR and histology, allow us to accurately detect the specific pathogens affecting our zebrafish colonies. The biosecurity program implemented in the SPF Room has helped to avoid the spread of *Mycobacterium haemophilum* found in the conventional rooms.

PCR Evaluation

Pathogen	SPF Embryos[A]	SPF Fish[A]	Conventional 2 Fish[A]	Conventional 1 Fish[A]	SPF Biofilm Racks[A]	SPF Biofilm Sentinels[A]	Quarantine Biofilm[A]	Conventional 2 Biofilm[A]	Conventional 1 Biofilm[A]	SPF Biofilm Racks[A]	SPF Biofilm Sentinels[A]
Mycobacterium spp.	-	-	-	-	+	+	+	+	+	+	+
Mycobacterium abscessus	-	-	-	-	-	-	-	+	+	-	-
Mycobacterium chelonae	-	-	-	-	+	+	-	-	+	-	-
Mycobacterium fortuitum	-	-	-	-	-	-	+	-	+	+	-
Mycobacterium haemophilum	-	-	-	-	-	-	-	+	-	-	-
Mycobacterium marinum	-	-	-	-	-	-	-	-	-	-	-
Mycobacterium peregrinum	-	-	-	-	-	-	-	-	-	-	-
Pseudoloma neurophilia	-	-	-	-							
Pseudocapillaria tomentosa	-	-	-	-							
Edwardsiella ictaluri	-										
Flavobacterium columnare	-										
Ichthyophthirius multifiliis	-										
Infectious spleen & kidney necrosis virus (ISKNV)	-										

Table 1 (left): PCR results for fish tissues, biofilms and embryos submitted to reference laboratories from different rooms. [A] =May 2017; [B] =August 2017

Histopathological Evaluation

Tissue	Conventional Room 1	Conventional Room 2	SPF Room
Brain	-	-	-
Epidermis	-	-	-
Gills	-	-	-
Heart	-	-	-
Intestine	-	-	-
Kidney	-	-	-
Liver	-	-	-
Reproductive organs	-	-	-
Skeletal muscle	-	-	-
Spinal cord	-	-	-
Swim bladder	-	-	-
Vertebral column	-	-	-

Table 2 (right): Histology results for fish tissues samples submitted to reference laboratories from different rooms in 2017.

Health Monitoring

The health monitoring program is implemented equally in both the SPF and conventional rooms; it includes the flagging of unhealthy fish and immediate removal of dead fish. All findings are recorded in a database. Fish found deceased in the SPF room compared to the conventional rooms were significantly lower (P=0.0343). This difference may be associated with the lower biosecurity measures in the conventional rooms versus the higher measures taken in the SPF room (see *Prevention and Control Measures*).

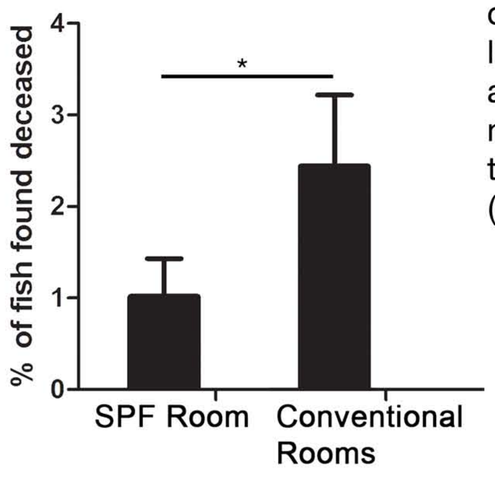

Fig. 8: Fish deceased in the SPF room vs. conventional rooms over a 10 month period. We ran a non-parametric statistical analysis. The values, presented as Means ± SEM, were compared using the Mann Whitnney test and considered to be significant at $^*P < 0.05$.

Considerations and Challenges

Biosecurity Rules: Assuring that procedures already implemented are clearly understood and followed[3].

Maintenance of specific pathogen free-stocks: The different techniques to identify the presence of infectious diseases does not guarantee a total lack of pathogens. However, they can help us to identify specific pathogens absent in the fish population.

Sampling: Determine the best sampling types (sentinel fish, water, feaces, tissues, biofilms, etc.) to increase the probability of pathogen detection.

Understanding system functionality

Further Work

In order to reduce the potential impact of pathogens on zebrafish welfare and research studies, our further work will include in-house use of quantitative polymerase chain reaction (PCR) assays[4], allowing us to identify some of the most virulent pathogens and increase the frequency of testing. We will conduct regular health screenings of a representative number of our fish colonies, allowing us to evaluate the prevalence of pathogens over a one year period.

References:
1 Kent ML, Harper C, Wolf JC. Documented and potential research impacts of subclinical diseases in zebrafish. ILAR J. 2012;53:126-134.
2 Chang CT, Amack JD, Whipps CM. Zebrafish embryo disinfection with povidone-iodine: Evaluating an alternative to chorine bleach. Zebrafish 2016;13:S96-101.
3 Murray KN, Varga ZM, Kent ML. Biosecurity and health monitoring at the Zebrafish International Resource Center. Zebrafish 2016;13:S30-38.
4 Whipps CM, Lieggi C, Wagner R. Mycobacteriosis in zebrafish colonies. ILAR J 2012;53:95-105.

Acknowledgements:
Many thanks to the UCL Zebrafish Facility:
H. Callaway, K. Dunford, M. Wicks, and P. Barwood.