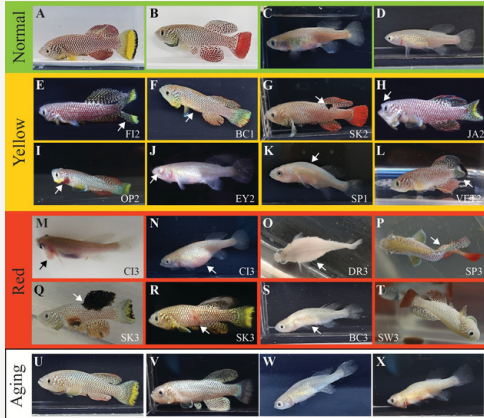


## Introduction

As *N. furzeri* attracts the attention of a growing number of research groups, the number of institutional fish facilities that keep and breed this aging model organism is also increasing. However, maintaining a stable and healthy colony for this species can be challenging. At the Leibniz Institute on Aging, *N. furzeri* have been maintained for more than 15 years with continuous improvement of husbandry practices, resulting in a modern fish facility and a large healthy fish colony. One of the challenges in keeping *N. furzeri* is that aged fish are needed for research. Aged *N. furzeri* can pose a hygiene risk if kept in larger numbers. Hygiene measures such as daily scoring and quarterly health monitoring are appropriate tools to monitor and ensure colony health, especially of aged fish. For more than 6 years, daily health checks for all fish with the help of the self-developed score sheet, complemented by quarterly colony health monitoring for a specified list of infectious agents is an inherent part of standardized processes in the fish facility. The resulting data set provide new evidence for appropriate colony health surveillance and health problems specific to *N. furzeri*.

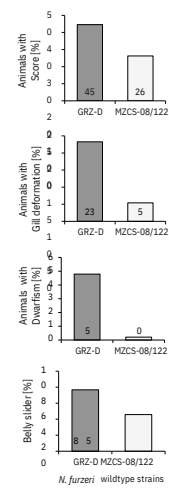
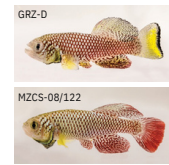
## Daily Health Monitoring and Health Score Sheet

By developing a fish health score sheet for daily scoring, special requirements of an aged fish colony is addressed and humane endpoints are defined. Consistent use of the fish health score sheet results in an overall improvement in fish health and welfare as well as a reduction in pain and distress experienced by individual fish. Analysis of the scores allows prognosis about emerging pathogens or other disease outbreaks and even provides insights into age- and strain-specific symptoms.

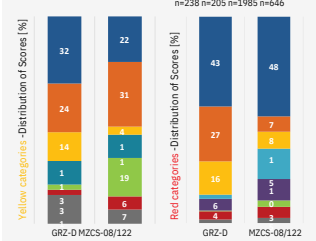


Category	Severity	Clinical signs	Abbr. for documentation	Score
Swimming	Physiological	Upright position, balanced, without tilting	SW0	0
	Altered	Sloping position, cannot hold stable	SW2	2
	Strongly altered	Negative or positive buoyancy, tilting	SW3	3
Feeding	Physiological	Active feeding and hunting	FB0	0
	Reduced	Decreased response to food	young FB2, old FB1, 2-11	
	Strongly reduced	Starvation, completely unreactive towards food	FB3	3
Social	Physiological	Responsive and purposeful swimming	SB0	0
	Reduced	Very delayed reaction or very aggressive	young SB2, old SB1, 2-11	
	Strongly reduced	Apathy, no swimming movements	SB3	3
Body condition	Physiological	Body bigger than head, well-conditioned	BC0	0
	Underconditioned	Head, belly and base of anal fin in a line	young BC1, old BC0, 1-10	
	Severe emaciation	Body narrow than head, concave shape along the belly, juveniles: much smaller than mates	BC3	3
Eyes	Physiological	Clear eyes, scales attached to body, no lesions, intact fins, normal coloration, even skin, enophthalmos	young EY2, old EY1, 2-11	
	Moderate damage	One protruding or deformed eye, loss of one eye, enophthalmos	EY3	3
	Severe damage	Bleeding, neovascularization, both eyes missing	EY3	3
Jaw	Moderate deformation	Slight changes, no influence on feeding behavior	JAW2	2
	Severe deformation	Deformed jaw, strongly influenced feeding behavior	JAW3	3
Operculum	Malformations	Missing or deformed gill cover	OP2	2
	Changed appearance	Small lesion, dark white, discolored foot, pale coloring	SK2	2
Skin	Severe changes	Large discolored patch, hemorrhage, abscess wound with organ exposure, fungal infection	SK3	3
	Circumferential increase	Possible tumor growth, possible spawning problem	C3	3
	Droopy	Severe changes, massively enlarged abdomen in appearance	DR3	3
Fins	Mild damage	Slightly injured or partially missing fins	FI2	2
	Severe damage	Missing, severely injured or rotten fins	FI3	3
Spine	Mild deformation	Moderate skeletal curvature or deformity of the spine	young SP1, old SP0, 1-10	
	Severe deformation	Strong deformation of spine	SP3	3
Other symptoms	Other reasons	Rarely occurring clinical signs, unclear conditions	VET	1-3

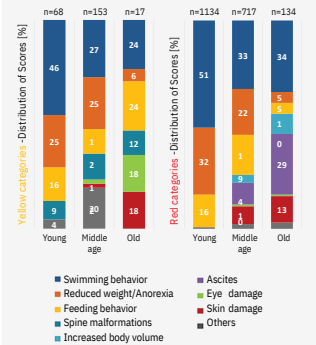
Developing a fish health score sheet that was simultaneously applicable to young and aged fish was a complex task, as several factors must be taken into consideration. The fish health score sheet should include most clinical signs and yet be universal and simple to use. Therefore, a traffic light system was established which reflects the animal's experience of pain or distress. Green state comprises physiological conditions with no apparent pain or distress, Yellow state initiates a closer observation of conspicuous fish exhibiting signs of mild pain or distress and Red state defines humane endpoints requiring immediate euthanasia to eliminate severe pain or distress.



## Strain-specific Symptoms

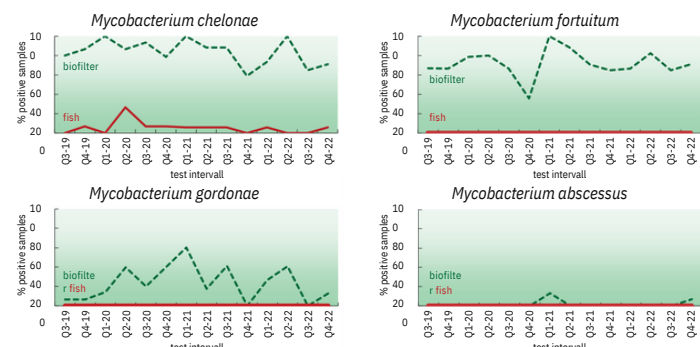
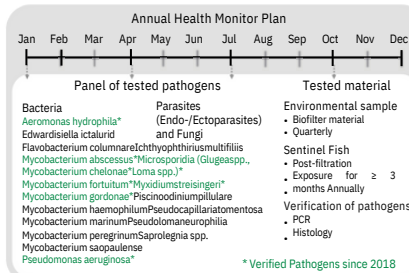


## Age-specific Symptoms



## Quarterly Colony Health Monitoring Program

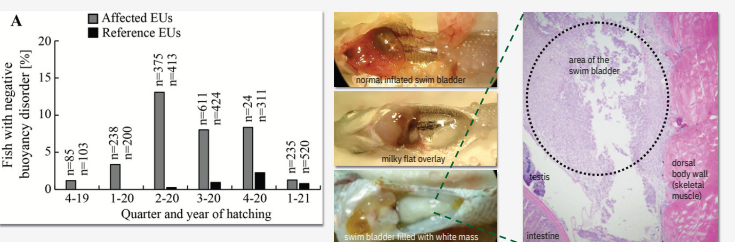
A fish specific health monitoring was developed and is provided to the users on a quarterly base. Our routine health monitoring program supports the colony health and helps to identify disease outbreaks. Verification of pathogens is carried out by PCR and histology from collected material of environmental samples and sentinel fish. Results are available to users as health monitoring certificate. By using healthy fish with consistent physiological conditions, researchers increase the reproducibility of their results.



*M. chelonae* was identified regularly, but at a low prevalence in sentinel fish, indicating that this species infects *N. furzeri* and is enzootic in the colony. Increased mortality was not apparent in the sentinel fish or positive hygiene units, suggesting that most *M. chelonae* infections are subclinical as has been reported for other species.

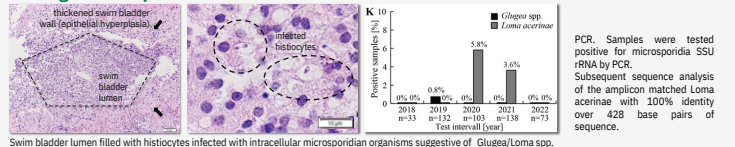
## Case Study: Swim Bladder Disease in Adult *N. furzeri*

Sudden increase of young adult fish with swim bladder lesions presented with a negative buoyancy disorder. Clinical signs appeared acutely during the daily visual inspection (Scoring SW3, "Belly Sliders") and were confirmed by the histological and molecular biological analysis at the quarterly health monitoring.

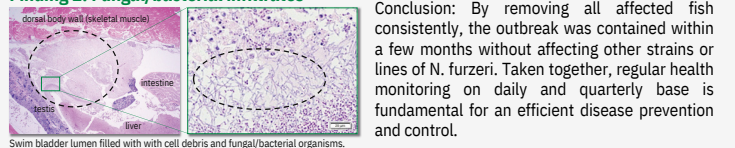


Graph shows percentage of affected animals with swim bladder defects compared to a reference hygiene unit. 13% of GRZ-D animals hatched in Q2-20 were affected.

### Finding 1: Microsporidian infiltrates



### Finding 2: Fungal/bacterial infiltrates



Conclusion: By removing all affected fish consistently, the outbreak was contained within a few months without affecting other strains or lines of *N. furzeri*. Taken together, regular health monitoring on daily and quarterly base is fundamental for an efficient disease prevention and control.

