DOI: 10.2478/cjf-2022-0015

CODEN RIBAEG ISSN 1330-061X (print) 1848-0586 (online)

## **\$** sciendo

# HEALTH MANAGEMENT OF ENDEMIC AND NON-ENDEMIC FISH IN THE AQUATIKA – FRESHWATER AQUARIUM KARLOVAC

Krešimir Kuri<sup>1\*</sup>, Marijana Sokolović<sup>3</sup>, Krešimir Drašner<sup>2</sup>, Juraj Petravić<sup>1</sup>, Margarita Maruškić Kulaš<sup>1</sup>, Goran Jakšić<sup>1</sup>

<sup>1</sup> Public Institution Aquatika - Freshwater Aquarium Karlovac, Karlovac, Croatia

<sup>2</sup> IHOR park fish farm, Jastrebarsko, Croatia

<sup>3</sup> Croatian Veterinary Institute, Zagreb, Croatia

\*Corresponding Author: kkuri@aquariumkarlovac.com

ARTICLE INFO	ABSTRACT		
Received: 23 February 2022 Accepted: 6 June 2022	This study presents data on regular health monitoring of fish in the Aquatika – Freshwater Aquarium Karlovac between October 2016 and December 2019. The Aquatika Aquarium houses 85 different freshwater fish species, 31 of which are endemic in Croatia. The study included an evaluation of the results of the aquarium health monitoring programme. It determined the most common fish diseases in the aquarium (at the species and individual levels) and determined whether endemic or non-endemic freshwater fish are more susceptible to diseases and disorders. The regular health monitoring programme revealed different diseases and disorders in endemic and non-endemic fish. During the monitoring, 3104 fish specimens were analysed. The most frequent disease was ichthyophthiriasis which occurred at a similar frequency in endemic and non-endemic fish species. The results proved to be valuable for the evaluation of risks and measures to minimise the risk of the introduction and spread of pathogens in the		
<i>Keywords:</i> Aquatika Health monitoring Endemic fish Ichthyophthiriasis	aquarium. Preventive fish medicine is extremely important for effective aquarium management. A comprehensive health monitoring programme, including quarantine systems, control of feed and environmental parameters, along with regular fish observation, are critical for the early detection of fish diseases.		
How to Cite	Kuri, K., Sokolović, M., Drašner; K., Petravić, J., Maruškić Kulaš, M., Jakšić, G. (2022): Health management of endemic and non-endemic fish in the Aquatika – Freshwater Aquarium Karlovac. Croatian Journal of Fisheries, 80, 141-150. DOI: 10.2478/cjf-2022-0015.		

#### INTRODUCTION

Aquatika – Freshwater Aquarium Karlovac is the largest freshwater aquarium in Croatia, established in 2016. It exhibits some of the rarest protected freshwater fish species in Croatia. The aquarium houses 85 freshwater fish species, of which 31 are endemic in Croatia. All species are kept in 25 separate aquarium tanks of various sizes. The order of aquarium tanks follows the river zones, based on the fish species naturally occurring in each zone (Jakšić, 2019). Each tank is equipped with its own mechanical, biological and sand filters, chiller, two pumps and a UV steriliser, and as such can be considered a single epidemiological unit (Grozdanić Begović, 2013). Consequently, each tank contains a group of fish that share the same risk of exposure to a pathogenic agent (OIE, 2021).

Comprehensive fish health management is necessary to ensure the health and welfare of fish contained within a closed system such as an aquarium. Isolating fish from their natural habitat and housing them in tanks (at a high stocking density) is not only stressful but also increases the risk for the onset and development of diseases (Gjurčević et al., 2006). Generally, fish health management is focused on four main factors: fish, water, tank and nutrition (Floyd and Beleau, 1986; Noga, 2010). Therefore, selecting the appropriate tank for each fish species is important for the well-being and longevity of fish in the aquarium (Fairfield, 2000).

The adequate water supply of suitable quality, water circulation and/or filtration within tanks should ensure maintenance of water quality parameters within acceptable levels (Plumb, 1999; Johansen et al., 2006). Water quality depends upon physical, chemical and biological factors and affects fish to a much greater extent than other animals. Optimal ranges for water parameters are also specific to the fish species, life stage and housing system, making monitoring of water quality one of the most important keys for the prevention of fish diseases (Floyd and Beleau, 1986; Noga, 2010; Smith, 2019). A nutritionally balanced feed of high quality and appropriate feeding regime is another critical factor for the well-being and normal growth of fish (Fijan, 2006). Unutilised feed creates an environment of increased organic load and can contribute to the onset and spread of diseases in fish (Webster and Lim, 2001). Another aspect of feed is the risk of transmission of pathogenic agents. The biosecurity plan should include appropriate control measures so that only feed that has been adequately processed, manufactured, stored, transported and delivered is used for feeding aquatic animals in such a way that prevents the presence of infectious and parasitic agents (OIE, 2021).

Quarantine and sanitation procedures are the main biosecurity measures that aim to prevent the introduction, spread and release of pathogenic agents in an aquaculture establishment (OIE, 2021). The placement of animals in an unfamiliar environment can cause stress (physical, chemical, biological or social). This can result in the susceptibility of fish to different infectious agents, and disease outbreaks can occur (Webster and Lim, 2001). In addition, even routine activities (including translocations, handling, crowding, switching to managed diets, trauma, lesions, etc.) are stressful for fish in the aquarium tank. Therefore, to prevent disease, eliminating stress is important. An effective health management plan should include a quarantine and sanitation programme with appropriate measures and operations in which the benefits and potentially harmful effects are evaluated specifically for each case (Stoskopf, 2010; WHO, 2020; OIE, 2021).

Surveillance for diseases of fish is an important element of health management in an aquarium. It aims to avoid the introduction of diseases, detect the emergence of diseases and provide control and contingency strategies before the agent becomes widespread (Hadfield, 2021). A disease is any deviation from normal or good health and may be a condition resulting from infectious agents, nutritional deficiencies, toxicants, environmental factors and genetics (Plumb, 1999). In this study, the term disease will represent all of these deviations. Disease in an aquarium can occur if some of the procedures of the health management plan have not been applied properly. In the scope of the health management plan, special attention is given to monitoring physical (trauma) and biological causes (infections by viruses, bacteria and fungi), and signs of potential intoxication. Under normal living conditions, most parasites cause little pathology, and wild fish can have a variety of parasites without developing clinical symptoms. However, in aquaculture, parasites can cause serious disease and mass epidemics due to the high stocking density and stressful conditions (Stoskopf, 2010).

The aim of this study was to investigate the health status of the fish housed in the Aquatika aquarium between October 2016 and December 2019. Three main goals were defined: (1) evaluation of the results of the aquarium health monitoring programme; (2) determination of the most common fish diseases in the aquarium (at the species and individual levels); and (3) determination of whether endemic or non-endemic freshwater fish are more susceptible to diseases and disorders in the aquarium. Endemic fish species are particularly vulnerable and commonly have little ability to adapt to changes in external factors (Mrakovčić et al., 2006).

#### MATERIALS AND METHODS

#### Acquisition of animals

The fish for stocking the aquarium originated from commercial fish farms or were obtained by electrofishing or angling from their natural habitats by various fishing techniques on streams, rivers and lakes. To obtain fish of high quality, they were acquired only from farms that

regularly monitor animal health. Wild fish were captured using two main fishing techniques: electrofishing and angling. Electrofishing was conducted only during daylight hours with a spatially continuous single-pass electrofishing screening from the shore and along the riverbanks at depths from 0.2 to 1.5 m (Jakovlić et al., 2014). On canals and streams, at depths from 0.3 m to 1.6 m, electrofishing was performed in the water using fish catcher IG600 (Hans Grassl Apparatebau, Germany). The device consists of a battery electrofisher with alternating current (AC) and direct current (DC) (AC maximum power output 1.2 kW, and DC power 0.65 kW) and a control box. The anode pole was 1.8 m long with a 0.3 m diameter ring at the end with an extra flat net of 6 mm mesh. The cathode was a 5-meters long copper strip type. Angling was conducted during daylight hours using bait float fishing and spinning techniques. After collection, fish were transported in a vehicle equipped with a fish tank and oxygen. All procedures (capture, transport and handling) were performed in a manner that ensures the least impact on the animals and the ecosystem. The capture, acquisition, handling and transportation of fish followed the relevant regulations of the ethical and legal frameworks of the European Union. All activities were performed with the permission of the Croatian Ministry of Agriculture and the Ministry of Economy and Sustainable Development.

#### Introduction of new fish into aquarium tanks

After the acquisition, all fish were handled according to standard procedure for new arrivals (Chadfield and Clayton, 2011; Mihinjač et al., 2016). Animals were soaked in antibacterial, antifungal and antiparasitic baths of formalin (saturated solution with 34-38% aqueous solution of formaldehyde gas) and placed into quarantine tanks for a period of time. Cold-water and warm-water fish were quarantined and monitored for a period of six and four weeks, respectively. Afterwards, only the fish not showing disease symptoms were transferred to the public display tanks.

#### Fish feeding

Fish were fed with commercial sinking and floating pellet feed (carp, trout, sturgeon; Aller Aqua, Denmark), floating feed for pond fish (Versele Laga, Belgium) and sinking feed for smaller aquarium fish (Tropical, Poland). Once a month, pellets were supplemented with NutriSel Plus vitamin and mineral supplement (Lek, Slovenia), salmon oil (Fish for Dogs, England) and garlic oil (Aqua forest, Poland). Every three weeks, zooplankton and phytoplankton that were grown in our aquarium, were added into the large aquarium for those fish that feed on plankton (big-head carp *Hypophthalmichtys nobilis*). Earthworms, from aquarium domestic cultivation, and other homemade natural feed supplements were introduced into aquariums as a natural supplement for omnivorous fish and for fish that do not eat artificial food. Whole and chopped sprats *Sprattus sprattus* were used as the main food or a natural food supplement for carnivorous and omnivorous fish such as pike-perch *Sander lucioperca*, perch *Perca fluviatilis*, chub *Squalius cephalus*, catfish *Silurus glanis*, largemouth bass *Micropterus salmoides*, Siberian sturgeon *Acipenser baerii*, Russian sturgeon *Acipenser gueldenstaedtii*, Adriatic sturgeon *Acipenser nacarii*, Ship sturgeon *Acipenser nudiventris*, sterlet *Acipenser ruthenus*, Stellate stugeon *Acipenser stellatus* and *Beluga Huso huso*.

### Implementation of procedures for the prevention of infectious diseases

The infectious disease prevention programme in the aquarium includes regular disinfection of fish tanks, nets and mats used for the transport and handling of fish, and equipment used for tank cleaning (Fijan, 2006). Mechanical filtration was performed by filtering aquarium water through the filtration material (100% polyester). The filtration material was changed two to three times a week, depending on the amount of accumulated impurities. Some of the tanks are equipped with a granular filter (0.5-1 mm) used for the removal of tiny particles. This filter was cleaned once or twice a week, depending on the amount of impurities. Each replacement of the filter material and cleaning of the granular filter was routinely recorded.

#### Monitoring programmes

Regular monitoring of fish health included analysis of basic water parameters, implementation of procedures for feeding, prevention of infectious diseases and surveillance of diseases.

### Monitoring of water quality, water changes and other factors of the health monitoring programme

Regular monitoring (weekly) of water quality included the measurement of basic parameters: temperature, dissolved oxygen, pH, ammonia, nitrate, nitrite and water clarity. The ideal temperatures for breeding salmonid fish species are between 9° and 12°C, with maximum deviations up to 5°C and below 18°C, as are the sources of mountain streams (Aganović, 1979). Water temperatures in aquarium tanks for fish from the Salmonidae family were never above 17°C. The optimum range of temperature tolerance for carp is between 23° and 30°C (Plumb, 1999). Water temperatures in aquarium tanks for warm-water fish were always from 16° to 24°C. Cold-water fish require a minimum of 8 mg/L of dissolved oxygen, while warmwater fish require a minimum of 5 mg/L. Water quality criteria, regarding pH for optimum health management for warm-water fish, are from 6.5 to 9, and for cold-water fish from 6.5 to 8 (Plumb, 1999). All freshwater fish in aguarium tanks tolerated a water pH range from 7 to 8.5. A concentration of 0.01 mg/L ammonia or more was taken as an optimal water quality level for cold-water fish, while 0.02 mg/L ammonia for warm-water fish (Plumb, 1999). The maximum concentration for nitrate was 50 mg/L and

for nitrite it was 1 mg/L. Monitoring was conducted in all 25 tanks, containing in total 3,000,000 litres of water, on a daily (temperature, dissolved oxygen) or weekly basis (all others), depending on the parameter. Temperature and amount of dissolved oxygen were measured by a portable dissolved oxygen meter for fish farming (Hanna 9147), pH with a pH meter (Hanna 98107), and ammonia, nitrate and nitrite with a photometer (Aquaculture Bench Photometer HI 83203). Finally, monitoring included documenting all other data that might aid in the evaluation of fish health, including feeding practices, data on water changes and other routine procedures.

Partial weekly water changes of 10 to 15% were carried out on regular basis and as needed. The frequency of regular water change (daily, weekly and/or as needed) depends on the species and number of fish in the aquarium. Therefore, in tanks with cold-water fish, such as grayling *Thymallus thymallus* and huchen *Hucho hucho*, daily water changes of 5-10% were applied. In all other tanks, recommended regular water changes of 20-30% were carried out every two to four weeks (Trivedi, 2020). All parameters and procedures were regularly recorded.

#### Surveillance of diseases and defining the most common fish diseases in the aquarium (at the species and individual levels)

The survey of the health status of fish was performed between October 2016 and December 2019 in all 25 aquarium tanks of various sizes, housing 85 different freshwater fish species. In all, 3104 individuals from all 85 species were analyzed during the study. Each examination was documented and included data on the number and species of disease-affected individuals. When necessary and possible, animals were moved to hospital tanks in the guarantine room equipped with separate aeration and filtration. In some biotope aguariums, easily diseased fish could not be removed from the aquarium so they were treated in it. Health monitoring included a clinical examination and evaluation of other available background data (history, environment, etc.). Fish that showed clinical signs characteristic of the observed behavioral, physical and pathological changes of the disease (Plumb, 1999) were treated with medications. In case it was not possible to diagnose a specific disease, the most severely affected individuals were taken alive, if possible, to a professional fish diagnostic veterinary laboratory for fish diseases (for further examination for external parasites, possible attempts to isolate bacteria or viruses with the aim of pathogen identification). The most obvious external clinical signs of fish diseases were inflammation and hemorrhage of fins, skin (Plumb, 1999) and on the fish head, but also various lessions and change in appetite and behavior of fish. When examining external parasites, a glass slide was taken from the gills, skin and fins, a drop of water was added and a cover slip placed (Plumb, 1999). The slide was examined at low (40-100x) and bigger (440-440x) magnification. Clinical signs of some parasitic diseases like ichthyophthiriasis (white spots), lernaeasis (tail of the parasite from the fish body) and argusilasis (whole parasites all over the fish body) were relatively easy to notice. For a person working with fish in an aquarium, it is very important to be able to recognise clinical signs of fish diseases as soon as possible, as well as lack of oxygen and chemical toxicants (Plumb, 1999). When it comes to bacterial diseases, it is hard to diagnose a specific etiological agent only from the clinical signs. It is always very useful to take the fish with clinical symptoms to the professional fish diagnostic veterinary laboratory for fish diseases for pathogen identification. At the same time, it is also very important to start antibacterial therapy if a bacterial infection is suspected.

# Determining whether endemic or non-endemic freshwater fish are more susceptible to aquarium diseases and disorders

The survey data were analysed to answer the questions on the susceptibility of endemic fish species in comparison to non-endemic species to various diseases in this controlled environment. Especially, if it was assumed that they are more sensitive and less able to adapt to external factors.

#### **RESULTS AND DISCUSSION**

In most cases, infected fish from public display tanks were moved to hospital tanks in the quarantine room. On several occasions, two or more diseases appeared simultaneously. Sacrificing fish was reduced to a minimum to safeguard animal welfare and protect valuable and rare fish for public display.

Sources of water pollution in the aquarium were fish excrements, decay of potentially hidden dead fish, decay of plants or excess fish feed. High values of ammonia in the aquarium were often a good reason to search for a dead fish hidden in the aquarium. If the ammonia level reached 0.1 mg/L or more, an additional water change of 50% was carried out for this tank. During the threeyear surveillance programme, the majority of water quality parameters were within the appropriate range for all fish species. Also, during the feeding of scavengers (which eat dead fish parts) and carnivorous fish species, the aquarium water was more polluted than during the feeding of herbivores (plant-based diet) and omnivorous fish. In winter, when the controlled tanks had only a few or no plants that might aid in the reduction of ammonia, tank bacteria were removing it. In the first days of aquarium operation, the pump and filtration stopped during the night, so in the morning two dead koi carp and a very high ammonia concentration of 0.3 mg/L and a very low oxygen level of 0.2 mg/L were detected. After that incident, security guards who guard the aquarium at night were trained to be able to regularly control the aquarium technology during their night shifts.

Optimal nutrition is essential for the growth and

well-being of fish species. Feeding fish is particularly challenging due to the diversity of their anatomical and physiological characteristics. Generally, the dietary nutrient requirements for major economically important fish species are known (NRC, 2011). Therefore, the composition of the feed must be adapted to the fish species, environment and age (Fijan, 1975). When the exact nutritional requirements of certain species of fish in the aquarium are unknown, it is generally acceptable to use a combination of commercial feed and different supplements (Floyd and Beleau, 1986). The best results were achieved with a combination of commercial pelleted feed and the addition of different supplements. Considering the differences and habits of various fish species, it is important to prepare fish feed individually for every fish species and sometimes for every fish individually, if necessary. All pelleted feed was used fresh and before the expiration date.

Prophylactic treatments of fish with formalin or salt are generally recommended before introducing new fish specimens. Their application is helpful for the removal of external parasites and bacteria on the surface of the fish epithelium (Fijan, 2006; Noga 2010; Smith, 2019). However, excessive exposure and/or inadequate concentration of prophylactic solutions might cause negative effects on the fish and the environment (Leal et al., 2018). In this survey, the application of formalin baths before placing the fish into aquarium tanks has proven effective, especially when wild-caught fish are introduced. Furthermore, such practices are applied in similar facilities worldwide. In a review of fish guarantine procedures in zoos and aquariums in the United States, 64% successfully use formalin as a common immersion treatment (Hadfield and Clayton, 2011).

Between October 2016 and December 2019, 92 diseases were documented. A total of 3104 fish individuals housed in aquarium tanks were analysed for the presence of clinical signs of diseases. The diseases were detected in 44 species of freshwater fish from ten fish families and 381 fish individuals (12.3% of all individuals examined) (Table 1). Such a number of fish diseases at the beginning of the aquarium operation can be explained by the stress caused by the movement of fish from various fish farms and nature into the tanks. Previous studies have shown that when environmental conditions worsen, the number of fish diseases and mortality increases (Fijan, 2006). The occurrence of diseases is individual for each fish and fish species and is closely related to the fish environment (water quality).

The most common diseases in Aquatika aquarium fish were parasitic, including ichthyophthiriasis, lerneosis, argusilosis and ichtyobodosis. Ichthyophthiriasis is one of the most important parasitic diseases of the skin and gills of most freshwater fish species and it is widespread worldwide (Fijan, 2006). In this study, 34 cases were recorded, making ichthyophthiriasis the most common disease in the Aquatika tanks (Figure 1). Other authors reported similar results. Gjurčević et al. (2006) reported that 40% of inspected goldfish *Carassius auratus* from pet shops were infected with the parasite *lchtyophtirius multifiliis*, the pathogen that causes ichthyophthiriasis. Shariff (1986) described that 58 fish species from 20 families were infected with lerneosis in a public aquarium in Malaysia.

The most common bacterial and fungal diseases were erythrodermatitis and saprolegniosis. Saprolegniosis was found in Turskyi dace *Telestes turskyi* at the time of spawning. In other fish, it occurred during lower temperatures, on fish-bearing small wounds, and as a characteristic secondary infection (Fijan, 2006). Two fish species, sensitive to stress from the appearance and noise of numerous visitors, Danubian roach *Rutilus virgo* and tench *Tinca tinca*, displayed physical injuries: contusions of the head, and lesions and lacerations all over the body. Five European grayling *Thymallus thymallus* caught with an electrofishing device from the wild did not survive the stress caused by transport from the river to the aquarium and the presence of numerous people passing by the public display tank.

145

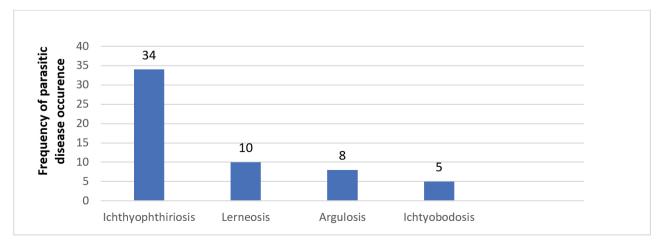


Fig 1. Graphical presentation of occurrence of parasitic diseases in fish in Aquatika - Freshwater Aquarium Karlovac between October 2016 and December 2019

**Table 1.** Occurrence of diseases during the period from October 2016 to December 2019 in endemic and non-endemic fish speciesfrom 10 families in Aquatika- Freshwater Aquarium Karlovac

Family	Species		Number of fish affectedby the disease		- Endemic
	Latin Name	Common Name	Species	Individual fish	Endemic
Acipenseridae	Acipenser baerii	Siberian sturgeon	1	2	No
Acipenseridae	Acipenser transmontanus	White sturgeon	1	1	No
Leucicidae	Abramis brama	Bream	2	6	No
Leucicidae	Alburnoides bipunctatus	Spirlin	1	7	No
Leucicidae	Alburnus alborella	White bleak	3	6	Yes
Leucicidae	Alburnus alburnus	Bleak	1	52	No
Leucicidae	Alburnus neretvae	Neretva bleak	1	1	Yes
Cyprinidae	Alopyge huegelii	Dalmatian barbelgudgeon	2	6	Yes
Cyprinidae	Barbus barbus	Barbel	1	4	No
Cyprinidae	Barbus plebejus	Adriatic barbel	6	9	Yes
Cyprinidae	Carassius carassius	Crucian carp	1	53	No
Cyprinidae	Carrasius gibelio	Prussian (gibel) carp	1	6	No
Leucicidae	Chondrostoma kneri	Dalmatian nase	1	1	Yes
Leucicidae	Chondrostoma nasus	Nase	2	6	No
Xenocyprinidae	Ctenopharyngodon idella	Grasscarp	3	16	No
Cyprinidae	Cyprinus carpio	Common and koi carp	7	25	No
Leucicidae	Delminichthys krbavensis	Krbava minnow	2	4	Yes
Gobionidae	Gobio obtusirostris	Danube gudgeon	1	1	Yes
Leucicidae	Leuciscus idus	Ide	2	4	No
Leucicidae	Rutilus aula	Adriatic roach	1	1	Yes
Leucicidae	Rutilus basak	Neretva roach	3	10	Yes
Leucicidae	Rutilus rutilus	Roach	1	1	No
Leucicidae	Rutilus virgo	Danubian roach	3	8	Yes
Leucicidae	Scardinius erythrophthalmus	Rudd	1	2	No
Leucicidae	Scardinius dergle	Bulldog rudd	2	7	Yes
Leucicidae	Scardinius plotizza	Adriatic rudd	1	2	Yes
Leucicidae	Squalius cephalus	Chub	4	16	No
Leucicidae	Squalius illyricus	Illyrian chub	1	6	Yes
Leucicidae	Squalius squalus	Cavedano chub	7	40	No
Leucicidae	Squalius zrmanjae	Zrmanja chub	1	6	Yes
Leucicidae	Telestes croaticus	Croatian minnow	2	1	Yes

Family	Species		Number of fish affectedby the disease		
	Latin Name	Common Name	Species	Individual fish	Endemic
Leucicidae	Telestes karsticus	Karst dace	1	5	Yes
Leucicidae	Telestes polylepis	Croatian riffle dace	2	7	Yes
Leucicidae	Telestes tursky	Tursky dace	3	8	Yes
Tincidae	Tinca tinca	Tench	4	12	No
Leucicidae	Vimba vimba	Vimba	1	1	No
Esocidae	Esox lucius	Pike	2	4	No
Gobidae	Padogobius bonelli	Marten's goby	1	2	Yes
Percidae	Sander lucioperca	Pikeperch	1	7	No
Salmonidae	Hucho hucho	Huchen	1	2	Yes
	Oncorhynchus mykiss	Rainbow trout	2	13	No
	Salmo trutta	Brown trout	1	4	No
	Salvelinus alpinus	Arctic char	1	3	No
	Thymallus thymallus	European grayling	1	3	No

#### Continued. Table 1.

Ind. - individual fish

All fish names were harmonized with A review of extant Croatian freshwater fish and lampreys - Annotated list and distribution (Ćaleta et al., 2019).

They died within seven days after being moved from the quarantine/adaptation tank to the aquarium tank. One rainbow trout *Oncorhynchus mykiss* immediately began to dominate other fish of the same species in the aquarium and killed two other rainbow trout, so it was translocated to another tank.

The number of diseases among the fish in the aquarium was the highest in the initial months of aquarium operation (October, November and December 2016) with 29 cases,

which was 31.52% of all fish diseases in the entire threeyear period (92 cases in total). This can be explained by the stress due to environmental factors (noise by the visitors). Despite daily warnings, groups of children, pupils, students and senior citizens often express their excitement towards the fish by shouting or tapping on the aquarium glass. It is well known that noise is a significant negative stress factor for fish health, through repetitive activation of the startle response (Stoskof, 2010).

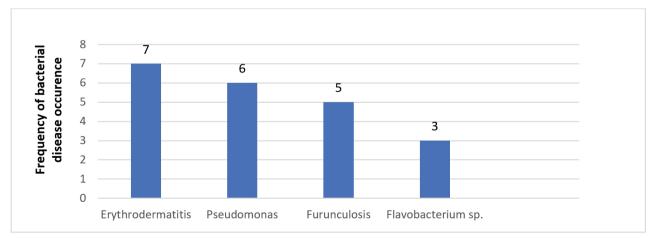


Fig 2. Graphical presentation of occurrence of bacterial diseases in fish in Aquatika - Freshwater Aquarium Karlovac between October 2016 and December 2019

© 2022 Author(s). This is an open access article licensed under the Creative Commons Attribution-NonCommercial-NoDerivs License 147 (http://creativecommons.org/licenses/by-nc-nd/3.0/)

Over the next three years, the number of diseases in the aquarium roughly stabilised in line with the number of visitors (Table 2). The two most common affected fish species were Cavedano chub Squalius squalus with seven occurrences of diseases at the species level and 40 affected individuals; carp Cyprinus carpio with seven cases and 25 individuals; and Italian barbel Barbus plebejus with six cases on species level and seven affected individuals. Considering the most common diseases overall, it should be taken into consideration that Cavedano chub and Italian barbel were caught in the Mirna River, below the sewage outlet of the local spa pools, which were highly polluted waters with a strong chlorine odour. During electrofishing on that river, we observed a high number of fish with symptoms of various diseases. Carp was one of the most numerous fish species in our aquarium and it was reasonable to expect that a high number of diseases would be detected in this species.

**Table 2.** Number of visitors and disease in fish between the endof October 2016 and December 2019 in Aquatika - KarlovacFreshwater Aquarium

Year	Visitors at Aquatika	Diseases	Percent overall (%)
2016 (two months) (November/December)	26.318	29	31.87
2017	91.369	20	21.74
2018	71.005	21	22.83
2019	73.222	22	23.91

In this survey, we tried to determine whether disease incidence was more prevalent in (non-) endemic fish in the aquarium. For the rare and endemic fish species in Croatia, the level of threat was determined for each species, and a protection strategy is proposed for each. This issue has been prescribed under the Nature Protection Act (Official Gazette 80/13) and the Ordinance on strictly protected species (Official Gazette 144/13) which proclaims the strictly protected species in Croatia (Ćaleta et al., 2015).

Endemic fish account for 36.5% (31/85) of all freshwater fish species in our aquarium. The study of the occurrence of disease by species and individuals showed that endemic fish species (43.2%) were not more susceptible than nonendemic species (56.8%). However, when analysing data at the individual level, we did find nearly the same pattern: endemic individuals (34.65%), non-endemic (65.35%). (Table 3). Despite the thesis that endemic fish species are particularly vulnerable and commonly have little ability to adapt to changes in external factors, their behaviour in the aquarium proves the opposite.

Fish diseases always have a serious negative effect on public aquariums. When some of the conditions for the fish in the aquarium are not optimal, stressors can accumulate, and disease can lead to the death of the fish. **Table 3.** Disease occurrence by species and affected individualsof fish in relation to endemic and non-endemic species betweenOctober 2016 and December 2019 in Aquatika-KarlovacFreshwater Aquarium

Disease Occurrence	Total	Endemic Species	Non-endemic Species
Incidence by species	44	19 (43.2%)	25 (56.8 %)
Incidence by individuals	381	132 (34.65 %)	249 (65.35%)

Even if they do not cause death, fish with visible symptoms are not suitable for aquarium public display. That is the main reason why preventive fish medicine is extremely important for effective aquarium management. The results of this study are of great value for the evaluation of risks and measures to minimise the risk of introduction and the spread of pathogens in the aquarium.

#### CONCLUSIONS

The detection of diseases in aquarium fish highlights the importance of a comprehensive health monitoring programme, including quarantine systems, control of feed and environmental parameters as well as regular fish observation.

The most common disease in freshwater aquarium fish in our research was ichthyophthiriasis, with the same incidence level recorded for both endemic and nonendemic species. Further studies are required to better understand the impact of this disease on fish and to develop successful prevention measures. As the Aquatika aquarium is only five years old, the results of this survey provide some general information on the health of aquarium animals. Further research is needed to provide more data on occurrence, diagnostics and potentially more effective measures that would improve the health and well-being of aquatic animals.

#### ACKNOWLEDGMENT

The authors thank the editor and two anonymous reviewers for their constructive comments, and Linda Zanella for proofreading the manuscript.

#### UPRAVLJANJE ZDRAVSTVENOM ZAŠTITOM ENDEMSKIH I NEENDEMSKIH RIBA U AQUATICI - SLATKOVODNOM AKVARIJU KARLOVAC

#### SAŽETAK

U ovoj studiji prikazani su podaci o redovitom zdravstvenom praćenju riba u Aquatici - slatkovodnom

akvariju Karlovac u razdoblju od listopada 2016. do prosinca 2019. U akvariju Aquatika smješteno je 85 različitih slatkovodnih vrsta riba, od kojih je 31 endemska vrsta za Hrvatsku. Ova je studija uključivala evaluaciju rezultata programa praćenja zdravlja akvarija. Utvrđeno je koje su najčešće bolesti riba u akvariju (na razini vrste i jedinke), te jesu li endemske ili neendemske slatkovodne ribe podložnije bolestima i poremećajima. Programom redovitog zdravstvenog praćenja utvrđene su različite bolesti i poremećaji kod endemskih i neendemskih vrsta riba. Tijekom monitoringa analizirano je 3104 jedinki. Najčešća bolest bila je ihtioftirijaza, koja se sa sličnom učestalošću javljala u endemskih i neendemskih vrsta riba. Provedeni rezultati su vrijedni za procjenu rizika i mjera za minimiziranje rizika od unošenja i širenja patogena u akvarij. Preventivna medicina za ribe iznimno je važna za učinkovito upravljanje akvarijima. Sveobuhvatni program praćenja zdravlja, uključujući karantenske sustave, kontrolu hrane i parametara okoliša, zajedno s redovitim promatranjem riba, ključni su za rano otkrivanje ribljih bolesti.

**Ključne riječi:** vodene životinje, praćenje zdravlja, endemske riblje vrste, ihtioftirijaza

#### REFERENCES

- Aganović, M. (1979): Salmonidne vrste riba i njihov uzgoj. IKGRO "Svjetlost", Sarajevo, Bosna i Hercegovina [In Bosnian].
- Chadfield, C., Clayton, L. (2011): Fish Quarantine: Current Practises in Public Zoos and Aquaria. Journal of Zoo and Wildlife Medicine, 42, 4, 641-650.
- Cusack, R., Cone, D.K. (1986): A review of parasites as vectors of viral and bacterial diseases of fish. Journal of Fish Diseases 9, 196-171.
- Ćaleta, M., Buj, I., Mrakovčić, M., Mustafić, P., Zanella, D., Marčić, Z., Duplić, A., Mihanjač,T., Katavić I. (2015): Endemic Fishes of Croatia. Croatian Environmental Agency, Zagreb, 116 pp.
- Ćaleta, M., Marčić, Z., Buj, I., Zanella, D., Mustafić, P., Duplić, A., Horvatić, S. (2019): A review of extant Croatian freshwater fish and lampreys - Annotated list and distribution. Croatian Journal of Fisheries, 77, 136-232.
- Fairfield, T. (2000): A Commonsense Guide to Fish Health. Barron's, New York, USA.
- FAO and WHO (2020): Code of Practice for Fish and Fishery Products. Rome. https://doi.org/10.4060/cb0658en
- Fijan, N. (1975): Hranidba riba, Zavod za biologiju i patologiju riba i pčela Veterinarskog fakulteta Zagreb, Hrvatska [In Croatian].
- Fijan, N. (2006): Zaštita zdravlja riba. Poljoprivredni fakultet, Osijek, Hrvatska. 392 pp [In Croatian].
- Floyd, F. R., Beleau, M. H. (1986): Aquarium Fish Health Management, International Association for Aquatic Animal Medicine, 1986. Conference Proceedings. USA.

- Gjurčević, E., Petrinec, Z., Matašin, Ž., Kozarić, Z. (2006): Nametnici utvrđeni kod zlatnog karasa (*Carassius auratus* L), Ribarstvo, 64, 19-26 [In Croatian].
- Grozdanić Begović, T. (2013): Idejni projekt rekonstrukcije (prenamjena i dogradnja) nedovršenog autokampa u Turistički i sportsko – rekreacijski centar "Slatkovodni akvarij – Kaquarium"u Karlovcu. VODOTEHNIKA – PROJEKT, Zagreb, Croatia. 35 pp. [In Croatian].
- Hadfield, C. (2021): Clinical Guide to Fish Medicine. Edited by Catherine Hadfield, Wiley-Blackwell, 2021. 608 pp.
- Hadfield, C., Clayton, L.A. (2011): Fish Quarantine: Current Practices in Public Zoos and Aquaria. Journal of Zoo and Wildlife Medicine. 42, 4, 641-650.
- Jakovlić I., Piria, M., Šprem, N., Tomljanović, T., Matulić, D., Treer, T. (2014): Distribution, abundance and condition of invasive Ponto-Caspian gobies *Ponticola kessleri* (Gunter, 1861), *Neogobius fluviatilis* (Pallas, 1814), and *Neogobius melanostomus* (Pallas, 1814) in the Sava River basin, Croatia. Journal of Applied Ichthyology. 31, 5, 888-894.
- Jakšić, G. (2019): The Freshwater Fish of Aquatika, Aquatika – Karlovac Freshwater Aquarium, Public Institute, Croatia. 182 pp.
- Johansen, R., Needham, J. R., Colquhoun, D. J., Poppe, T. T., Smith, A. J. (2006): Guidelines for health and welfare monitoring of fish used in research. Laboratory Animals. 40, 323-340.
- Johnson, E. L. (2006): Koi Health and Disease, Lulu Publishing, USA.
- Leal, J., Neves, M.G.P.M.S., Santos, E.B.H., Esteves, V.I. (2018): Use of formalin in intensive aquaculture: Properites, application and effects on fish and water quality. Reviews in Aquaculture. 10, 2, 281-295.
- Mihinjač T., Oraić, D., Špelić, I., Jelić, D. (2016): Priručnik za edukaciju djelatnika Javne ustanove Aquatika – Slatkovodni Akvarij Karlovac, Karlovac, Croatia (Public Institution Aquatika -Freshwater Aquarium Karlovac internal edition). [In Croatian].
- Mrakovčić, M., Brigić A., Buj I., Ćaleta M., Mustafić P., Zanella D. (2006): Red Book of Freshwater Fish of Croatia. Ministry of Culture, State Institute for Nature Protection, Zagreb, Republic of Croatia.
- Noga, E. J. (2010): Fish Disease: Diagnosis and Treatment, 2<sup>nd</sup> Edition. A John Wiley & Sons, Inc., Publication. 520 pp.
- National Research Council (2011): Nutrient Requirements of Fish and Shrimp. Washington, DC: The National Academies Press. https://doi.org/10.17226/13039 (Accessed: 18.11.2021).
- OIE, The World Organisation for Animal Health (2021): OIE - Aquatic Animal Health Code. Link: https://www.oie. int (Accessed: 18.11.2021).
- Plumb, J.A. (1999): Health Maintenance of Cultured Fishes: Principal Microbial Diseases, University Press, Ames, Iowa State, USA 344 pp.
- Shariff, M., Kabata, Z., Sommerwille, C. (1986): Host susceptibility to *Lernea cyprinacea* L. and it's treatment

in a large aquarium system. Journal of fish diseases. 9, 5, 399-401. Trivedi, S. P. (2020): Aquarium Fish ar (accessed:18.11.2021.).Link:https site/writeaddata/sitecontent/20

- Florida, USA. 397 pp.
- Stoskopf, K. M. (2010): Fish Medicine, Art Sciences LLC, North Carolina, USA. 626 pp.
- Trivedi, S. P. (2020): Aquarium Fish and Their Maintenance (accessed:18.11.2021.).Link:https://www.ikouniv.ac.m/ site/writeaddata/sitecontent/20004150935214277/ sptrivedi
- Webster, C. D., Lim, C. (2001): Nutrition and Fish Health. The Haworth Press. Inc. 365 pp.