# High prevalence of Silurus glanis papillomavirus 1 (SgPV1) detected among farmed wels catfish (*Silurus glanis* Linnaeus, 1758): a retrospective study

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**Data availabity statement**

The data that support the findings of this study are available from the corresponding author upon reasonable request.

**Conflict of interest**

The authors declare no conflict of interest.

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**Keywords:** fish papillomavirus, Silurus glanis papillomavirus 1, wels catfish, silurid herpesvirus 2, aquaculture

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## Introduction

The wels catfish (*Silurus glanis* Linnaeus, 1758), also known as the European catfish is the largest freshwater fish species in Europe. The species' native range is Central and Eastern Europe and Southwest Asia (Copp et al., 2009). Similar to other catfish species like the pangasius (*Pangasius bocourti* Sauvage, 1880), they are favoured fish for consumption, as their meat is white and boneless (Linhart et al., 2002). While most wels catfish are sourced from wild catches, aquaculture of this species is on the rise (Linhart et al., 2002). Modern intensive catfish farming methods increasingly rely on closed (recirculating aquaculture) systems to prevent ectoparasite outbreaks, however in high-density farming environments the risk of widespread infection by viruses and bacteria is notably higher than in natural environments. Scientific literature on viral infections or diseases in wels catfish is sparse. Infections caused by ranavirus (family *Iridoviridae*) and rhabdovirus (family *Rhabdoviridae*) are the most thoroughly described (Ahne et al., 1989; Békési et al., 1987; Borzym et al., 2015; Costa & Holmes, 2024; Fijan et al., 1984; Jeney et al., 1990). Other viral agents like herpes-, circo-, and papillomaviruses (PVs) have been reported occasionally in wels catfish, with or without accompanying clinical signs (Costa & Holmes, 2024; Saleh et al., 2021; Surján et al., 2021; Tarján et al., 2022). The precise role of these viruses in provoking diseases remain unclear; however, viral infections can be a limiting factor to fish farming in aquacultures (Zhang & Gui, 2015).

Fish herpesviruses (HVs) (alloherpesviruses) have been reported in at least 17 fish species over the past fifty years. At least 11 of them can cause extensive losses to aquaculture production by infecting economically important fish species from the families Acipenseridae, Salmonidae, Cyprinidae, Anguillidae (L. Hanson et al., 2011; L. A. Hanson et al., 2024). The most common signs of alloherpesvirus infections include skin and gill lesions, cell proliferation, haemorrhages, and occasionally, kidney and liver necrosis. These proliferative lesions are unsightly but rarely fatal. A novel alloherpesvirus, named silurid herpesvirus 2, was detected by PCR from *Silurus glanis* specimens showing clinical signs of carp pox-like disease in Hungary; however, the association between the lesions and the virus remains unclear (Tarján et al., 2022).

Papillomaviruses (PVs) are small, non-enveloped viruses with icosahedral capsids and double-stranded circular DNA genomes (Van Doorslaer et al., 2018). PVs have an extensive host range, including members of almost all vertebrate classes: mammals, reptiles, birds and fish (Kraberger et al., 2022; Saleh et al., 2021). PVs primarily infect epithelia, including both

mucosal and keratinized epithelia. So far, 18 fish PVs belonging to the genus *Alefpapillomavirus* have been identified from diverse host species (Blake et al., 2024; Ghasemi et al., 2023; Kraberger et al., 2022; López-Bueno et al., 2016; Surján et al., 2021; Tisza et al., 2020; Xi et al., 2023; Xue et al., 2024). One of these fish PVs was described from farmed wels catfish showing clinical signs of wels catfish herpesvirus disease, namely the Silurus glanis papillomavirus 1 (SgPV1) (Surján et al., 2021).

## Materials and Methods

### Samples and screening

Overall 64 wels catfish specimens were sent to the laboratory of the Veterinary Medical Research Institute from eight fish farms in Hungary between 2017 and 2021 for routine parasitological examinations, apparently healthy and diseased specimens as well (locations are shown in Figure 1.). At the necropsy of the fish, small parts of tissue were excised from the main internal organs (liver, spleen and kidney) and pooled, then stored at -20°C until further investigations. Tissue samples were homogenized and the DNA was extracted using the NucleoSpin® 'DNA RapidLyse' kit (Macherey-Nagel, Dueren, Germany) following the manufacturers' protocol. Subsequently, the presence of the viral DNA of SgPV1, SiHV-2, ECV, and CfCV was screened with conventional PCRs. For papillomavirus detection, an SgPV1 specific PCR, targeting the E2 gene of the virus (Surján et al., 2021) was employed. For the circovirus screening, a nested broad-spectrum PCR was applied (Halami et al., 2008), and the ECV was monitored by a PCR targeting ranaviral major capsid protein gene (Murali et al., 2002). For SiHV-2, a novel PCR was developed with primers targeting a 267 bp part of the DNA-dependent DNA polymerase gene (Supplementary Table 1.). PCR products were purified using a NucleoSpin® 'Gel and PCR Clean-up' kit (Macherey-Nagel). Sanger DNA sequencing with both the forward and reverse primers was performed using a BigDye Terminator v3.1 Cycle Sequencing Kit (Thermo Fisher Scientific, Waltham, MA, USA). Subsequently, the samples were sent to a commercial service provider for electrophoresis on an ABI Prism 3100 Genetic Analyzer (Thermo Fisher Scientific, Waltham, MA, USA). All PCR products in predicted size have been sequenced bidirectionally. The sequence identity was determined using with BLASTn algorithm on the NCBI website.

## Quantitative PCR analysis

Separated organs were available from four specimens originating from farm location No. 3. Two of them were used in former studies (Surján et al., 2021; Tarján et al., 2022), while the other two were investigated in this study. Quantitative PCR (qPCR) was performed on the gill, skin, brain, liver, spleen, kidney, and muscle tissues to examine the viral load of SgPV1 in different organs (Supplementary Table 1.).

## Statistical analysis

Statistical analysis of the qPCR ΔCt values in the examined organs of the specimens was performed using one-way ANOVA followed by Tukey post hoc test with OriginPro 2023b software (OriginLab Corporation, Northampton, MA, USA). P-values of 0.05 or below were considered statistically significant. Z-score outlier detection with $Z > 2$ parameter was also performed on the data of each specimen.

## Results

### Prevalence/epidemiology of *Silurus glanis* DNA viruses

Out of the 64 samples examined, 26 were positive for SgPV1, which is an overall 40.6% prevalence; 7 of the 8 locations had positive specimens (Table 1.). The prevalence in each location varied from 0% to 100%. Nine samples were positive for SiHV-2, with a 14.0% prevalence rate, and 50% of locations had positive samples (Table 1.). The number of fish with PV and HV co-infections was 6, which is a 66.66% co-infection rate for HVs and 23.0% co-infection rate for PVs.

The specimens examined showed no or different clinical signs, such as hyperplastic skin lesions, spiral swimming, hole-in-head disease, and so on (Table 2.). Nineteen individuals, 73.07% of SgPV1 positive samples showed no clinical signs. In the case of SiHV-2, 2 out of the 9 positive samples had clinical signs (fry mortality, muscle necrosis) resulting in a 77.8% asymptotic infection rate. Both SiHV-2 infected fish with clinical signs were co-infected with SgPV1. However, there were several co-infected specimens without any clinical signs, for example Location No. 2 from 2018, all SiHV-2 infections and half of the SgPV1 infections

proved to be co-infected. Detailed data on the clinical signs detected by location and asymptotic virus infection rates are shown in Table 2.

In the case of SiHV-2, no single nucleotide polymorphism was detected in this short region of the DNA polymerase gene (227 nt). The sequences showed 100% nucleotide identity to the formerly published one (GenBank Acc. No.: MW732127) (Tarján et al., 2022). The SgPV1 sequences of the E2 gene (180 nt) also matched 100% to the previously described isolate (GenBank Acc. No.: MN515404) (Surján et al., 2021), except one sample from the Location No. 4 which presented a single nucleotide polymorphism causing an amino acid change in the position 263 (valine to alanine).

No ECV and CfCV were detected in any studied populations.

## Tissue distribution of *Silurus glanis* papillomavirus 1

The wels catfish PV was present in all examined tissues, even the brain. The organ with the highest viral load and the burden in organs varied between specimens (Figure 2). There was no statistically significant difference between the viral loads in different organs.

## Discussion

Skin diseases resembling papillomas have been documented earlier in wels catfish and flatfish (Pleuronectiformes) populations (Costa & Holmes, 2024; Peters & Watermann, 1979; Saleh et al., 2021). The SgPV1 was described in *Silurus glanis* in Hungary showing clinical signs resembling the wels catfish herpesvirus disease (Surján et al., 2021). Although aquaculture of *S. glanis* is developing rapidly, the prevalence of viruses threatening the wels catfish production has not been previously evaluated in detail in Hungary.

In this study, we investigated the prevalence of some DNA viruses affecting wels catfish populations in specimens originating from Hungarian fish farms. Based on our findings, the high prevalence (40.6%) of papillomavirus infection and the lower, yet still significant presence of alloherpesvirus (14.0%) in the farmed populations of Hungarian *Silurus glanis*, SgPV1, and SiHV-2 could limit the wels catfish production by exerting continuous pressure. Although a high prevalence of SgPV1 was found, no clinical signs or mortality events could be attributed to the virus. Only one location was free of SgPV1, however, only one fish was examined from

this population. The majority (73%) of the SgPV1 infected catfish did not display any clinical signs, similar rate was found with the SiHV-2 as well (77%). Any (atypical) clinical signs (spiral swimming before fry mortality, hole in the head disease, renal haemorrhage, hole in the head disease, renal haemorrhage, muscle necrosis) were observed in only 27% of fish infected with SgPV1 and 23% of those infected with SiHV-2, respectively. The altered prevalence of SgPV1 in various locations may be a consequence of different sample sizes (sometimes small enough), however, the fact that almost all farms were infected draws attention to SgPV1 as a potential risk factor.

In our previous study, on the first detection of SgPV1, it remained unclear whether SgPV1 alone or only in co-infection with SiHV-2 can cause clinical signs or diseases. Co-infection of fish PVs with other large DNA viruses in gilthead seabream has already been reported; 8 out of 10 fish infected with lymphocystivirus, were positive for PV (López-Bueno et al., 2016). Out of the six wels catfish infected with herpesvirus (published by Tarján et al., 2022), four were infected with SgPV1 as well (Surján et al., 2021). No PV was detected alone in either the wels catfish or the gilthead seabream. Thus, the question has arisen whether some piscine PVs require the presence of helper viruses, like dependoparvoviruses (Berns, 1990). Later, in a large-scale screening, the co-infections involving lymphocystivirus (LCDV-Sa) and one or both papillomavirus (SaPV1), or polyomavirus (SaPyV1) were detected in almost all gilthead seabream with LCDV-diseased (98%). These co-occurring viruses rarely were found in asymptomatic fish (24%). This suggests a potential role for SaPyV1 and SaPV1 in exacerbating lymphocystis disease. Only 2.8% of the seabream carried the SaPV1 alone (Labella et al., 2019).

Based on the results of the current study, SgPV1 infection alone occurred in 77% of the positive samples, hence the answer is a probably no to the above-mentioned question of whether SgPV1 requires the presence of SiHV-2. Although, the occurrence of other large DNA viruses, such as adenoviruses, had not been excluded. On the other hand, SiHV-2 related clinical signs manifested only in co-infection with SgPV-1. However, the pathological aspects of these two DNA viruses require further study, especially given their high (~70%) asymptotic infection rate. The lack of detection of irido- or circoviruses suggests that these viruses are not so prevalent in Hungarian wels catfish aquaculture. It is also important to mention that while the specimens in this study were sent for routine parasitological examinations and apparently healthy and diseased specimens were both sent, this sampling method is not entirely

randomised, furthermore some locations only had very low sample numbers, so these results are not entirely representative to the Hungarian wels catfish aquaculture.

The different SgPV1 viral loads in the organs did not indicate pronounced tissue tropism, consistent with the results of a previous study on tongue soles infected with papillomavirus in China (Xue et al., 2024). The observation that the quantity of the viral DNA in specimen #2 exceeded that of the control gene in almost all tissue samples suggests that SgPV1 can cause high-level viremia. This fish was co-infected with SiHV-2 and displayed multiple papillomas on the skin (Surján et al., 2021). The significant presence of SgPV1 DNA in the brain tissue of all the examined specimens suggests that SgPV1 can enter the brain despite the blood-brain barrier (BBB), however, the exact mechanism is not evident and requires further study. The invasion or penetration of the virus into the brain in bony fish also could potentially be associated with aflatoxin B1 contaminated fish feed, which increases the permeability of the blood-brain barrier and can cause synaptic dysfunction resulting in hyperlocation of the fish (Baldissera et al., 2018). Infection of the brain by SgPV1 can activate defined immune responses that increase its impact as a limiting factor in aquaculture (Sun et al., 2022).

The sequence analysis of short gene fragments of the E2 gene from the SgPV-1 and of the DNA-dependent DNA polymerase gene from the SiHV-2 suggests that these regions are very well conserved so suitable targets for molecular diagnostics. Validation of the existing conventional and qPCRs or development of novel ones for these regions might help the early recognition of viral infections in fish farms.

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324 Tables

325 Table 1. Prevalence of SgPV1 and SiHV-2 in *Silirus glanis* in Hungarian fish farms

| Location | Number of samples | Number of SgPV1 positives | Number of SiHV-2 positives | Prevalence SgPV1/SiHV-2 |
|---|---|---|---|---|
| Location No.1 | 25 | 6 | 0 | 24% / 0% |
| Location No.2 | 17 | 10 | 5 | 58.82% / 29.41% |
| Location No.3 | 2 | 2 | 2 | 100% / 100% |
| Location No.4 | 7 | 3 | 0 | 42.86% / 0% |
| Location No.5 | 6 | 1 | 0 | 16.67% / 0% |
| Location No.6 | 3 | 3 | 1 | 100% / 33.33% |
| Location No.7 | 3 | 1 | 1 | 33.33% / 33.33% |
| Location No.8 | 1 | 0 | 0 | 0% / 0% |
| sum | 64 | 26 | 9 | 40.62% / 14.06% |

326

327 Table 2. Clinical signs and DNA virus infections detected in *Silurus glanis* from Hungarian fish farms

| Location | Date | Number of samples | Number of SgPV1 positives | Number of SiHV-2 positives | Clinical signs |
|---|---|---|---|---|---|
| **Location No.1** | 2014 | 8 | 2 | 0 | spiral swimming before fry mortality |
| | 2017-2018 | 5 | 4 | 0 | tissue proliferation on the gill cover |
| | 2020-2021 | 12 | 0 | 0 | no clinical signs |
| **Location No.2** | 2017 | 2 | 0 | 0 | malnutrition |
| | 2018 | 15 | 10 | 5† | no clinical signs |
| **Location No.3** | 2017 | 2 | 2 | 2 | no clinical signs |
| **Location No.4** | 2017 | 7 | 3 | 0 | no clinical signs |
| **Location No.5** | 2018 | 1 | 1 | 0 | hole in the head disease, renal haemorrhage |
| | 2019 | 5 | 0 | 0 | hyperplastic skin lesions |
| **Location No.6** | 2014 | 1 | 1 | 1 | fry mortality |
| | 2018 | 2 | 2 | 0 | |
| **Location No.7** | 2021 | 3 | 1 | 1 | muscle necrosis |

| | | | | | |
|---|---|---|---|---|---|
| **Location No.8** | 2017 | 1 | 0 | 0 | haemorrhagic rings on the skin all over the body |

† SgPVV1 and SiHV-2 co-infection

## Figures

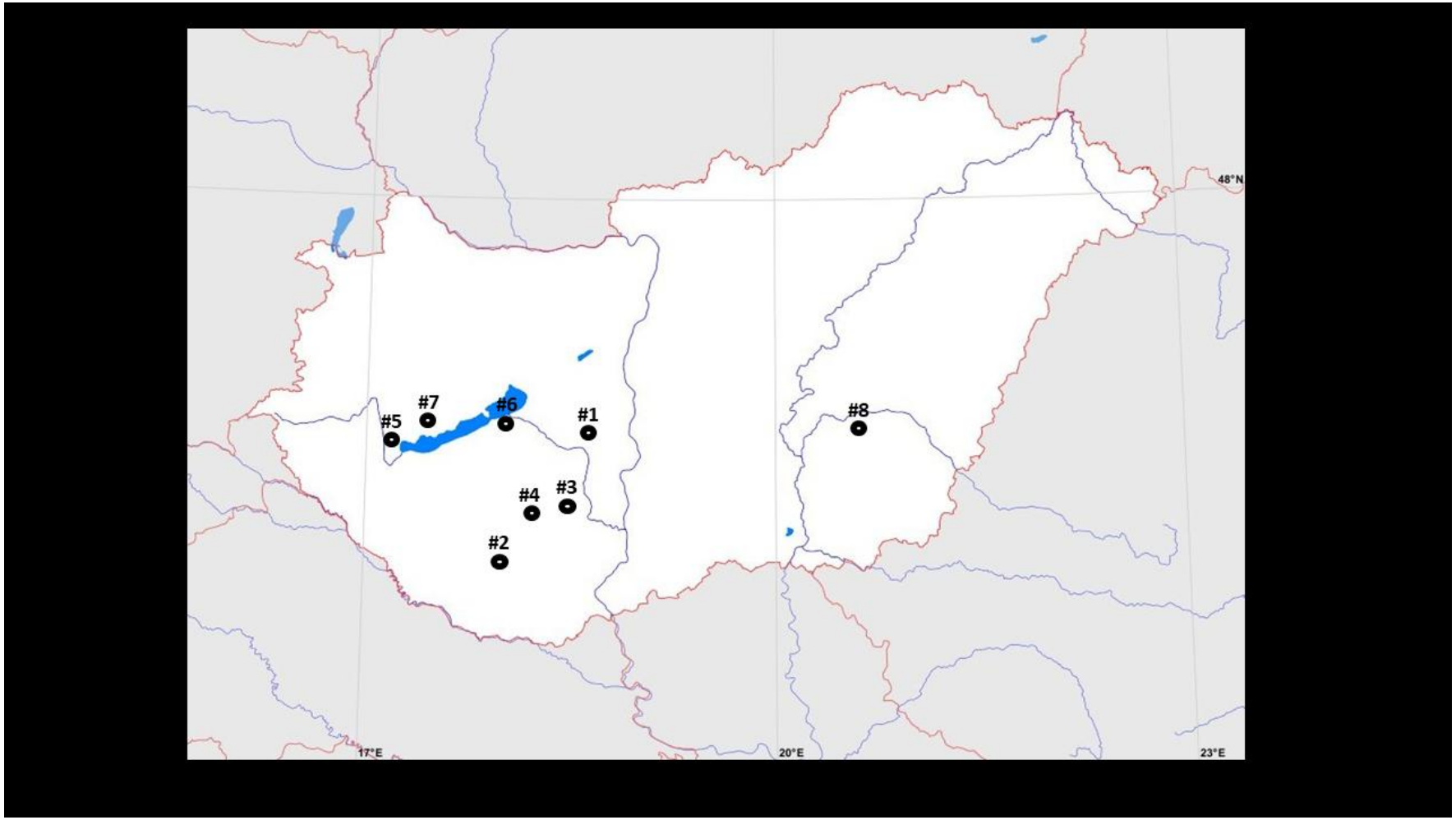
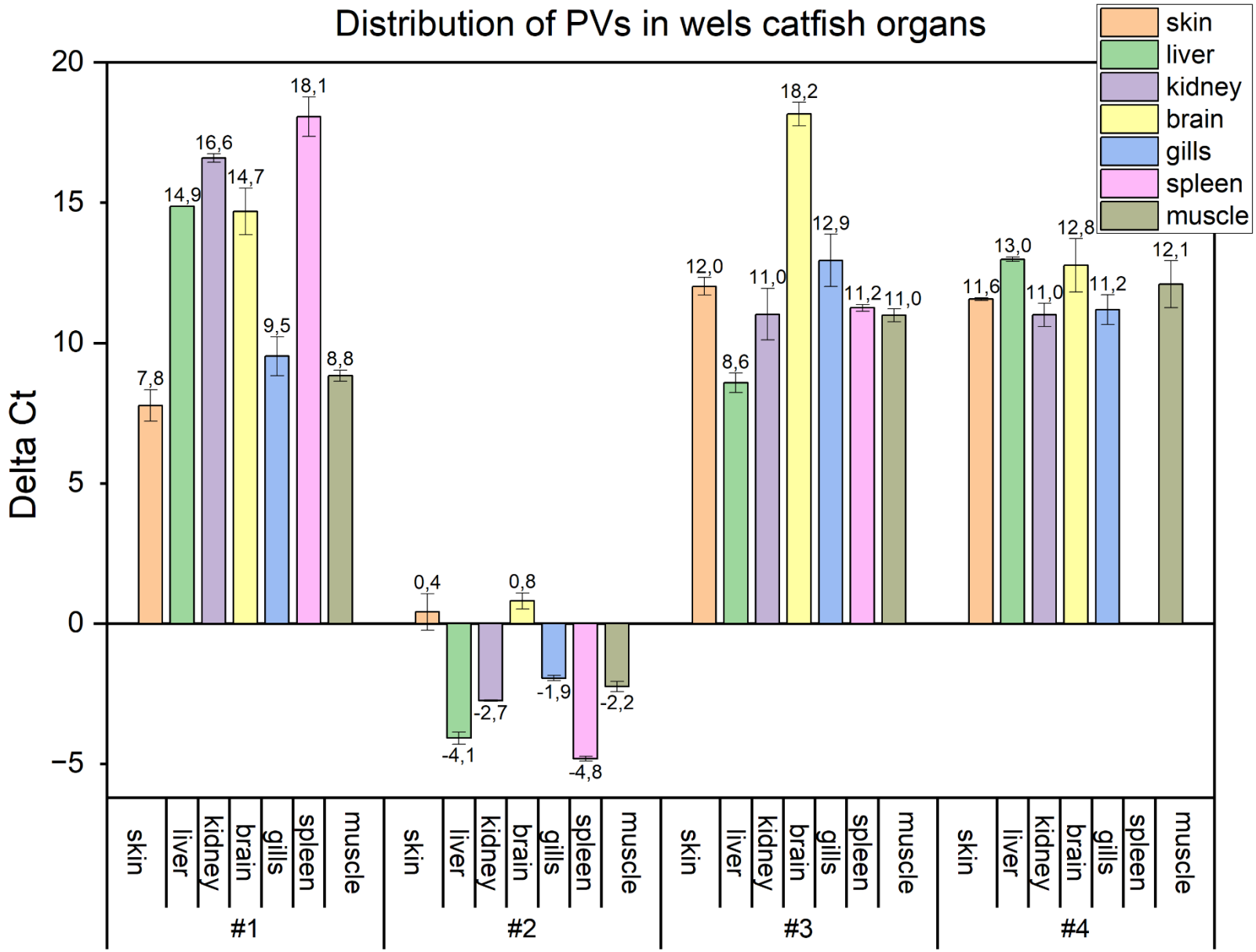



Figure 1. Location of fish farms in Hungary where specimens and samples were sent from. The figure is based on a hydrological map of Hungary ("Hungary Outline Map - A Learning Family", 2020).


Distribution of PVs in wels catfish organs
Delta Ct
20
15
10
5
0
−5
skin
liver
kidney
brain
gills
spleen
muscle
7,8
14,9
16,6
14,7
9,5
18,1
8,8
0,4
-4,1
-2,7
0,8
-1,9
-4,8
-2,2
12,0
8,6
11,0
18,2
12,9
11,2
11,0
11,6
13,0
11,0
12,8
11,2
12,1
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#3
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334

Figure 2. Distribution of SgPV1 in the skin, liver, kidney, brain, gills, spleen and muscle of four *Silurus glanis* specimens. The columns represent the mean ΔCt values of the viral load and the beta-actin housekeeping gene. Different tissues are indicated with disparate colours. Values are expressed as the mean +/- SEM.