West Pomeranian University of Technology in Szczecin

Faculty of Food Sciences and Fisheries

DETECTION OF HERPESVIRUS ANGUILLAE (AngHV-1) IN NORTH - WESTERN POLAND -A NEW CHALLENGE FOR THE EXISTING STOCK MANAGEMENT RECOMMENDATIONS FOR THE EUROPEAN EEL *(Anguilla anguilla)*

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Results described in this doctoral dissertation are included in the published original scientific papers listed below;

1. **Nguyen T.T.**, Kempter J., Panicz R., 2016.
Monitoring of herpesvirus anguillae (AngHV-1) infections on European eel in north-west Poland. Veterinary Medicine, 72 (9), 564-566.

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2. **Nguyen T.T.,** Kempter J., Panicz R.,2016.
Dynamics of herpesvirus anguillae (AngHV-1) transmission by the native ichthyofauna of north-western Poland. EJPAU 2016 Volume1 Issue 4.

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3. **Nguyen T. T.**, Yeonhwa J., Kiełpińska J., Bergmann S.M., Lenk M., Panicz R. 2017.
Detection of herpesvirus anguillae (AngHV-1) in European eel *Anguilla anguilla* originating from northern Poland – assessment of sustainbility of selected diagnostic methods.
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1. INTRODUCTION

The European eel *(Anguilla anguilla)* is an important species due to the taste quality and the high price of its meat. The stocks of this species have been constantly monitored in the context of the eel stock management plan implemented in Poland since 2010. In Poland, the material for eel pre-rearing is obtained directly from exporters, i.e. those countries in which capturing glass eel or juvenile eel in their coastal waters is possible. The purchased juveniles in Poland are used for pre-rearing conducted as part of stocking schedules. The quantity of material is estimated by ichthyologists and the size of stocks is included in, e.g., fishery documentation. An essential element in determining the effectiveness of these procedures is the assessment of survival of the stocking material. The effectiveness of activities associated with eel management in Poland is described in a report by the Institute of Inland Fisheries of the year 2008. Apart from the analysis of catches, it also includes assessment of the health status of eel. This aspect is of particular importance as diseases of fish, including viral diseases, can significantly reduce the survival of fish of all ages, from nursery fish to sexually mature fish. Disturbances in the age structure of the population can be a sign of viral infections, especially taking into account the fact that juvenile fish are particularly vulnerable to high mortality. In eel, however, there is the serious problem of verification of the condition of the species, as part of its developmental cycle takes place in the Sargasso Sea, and no effective and commercially feasible breeding technology for this species has been established to date. The lack of data on the mortality of eel in the natural environment is due to the kills of the fish that immediately fall to the bottom and are decomposed, providing additional biomass to the reservoir. Unfortunately, monitoring the mortality of eel is only possible at pre-rearing centres, hence the available data are very scarce.

1.1 Global catches and aquaculture of the European eel

According to the FAO statistics (Tab. 1), global catches of eel have decreased systematically over the past ten years. The catches in 2015 amounted to only half of the quantity caught a decade earlier. The biggest decrease in the catches was reported in Denmark, Sweden, France and Germany. Only in Great Britain, the catches have been maintained at a relatively constant level. Eel aquaculture has been conducted in several European countries, in particular: Spain, Germany, Italy, Denmark and the Netherlands (in the order of produced quantity) (Tab. 2). In these countries, a significant decrease reaching 30% has been noted as well. However, it is difficult to establish the reason for this phenomenon since, on the one hand, new facilities for eel rearing are being established, and on the other hand, breeders may have difficulty obtaining the stocking material due to the systematic decrease in the catches from the natural environment. In light of the eel restoration programmes implemented in the European Union states, breeders can have restricted access to the stocking material, which may further cause lower production size.

Tab. 1. Catches of the European eel according to the FAO statistics (FAO. 2017. Fishery and Aquaculture Statistics. Global aquaculture production 1950-2015 (FishstatJ). In: FAO Fisheries and Aquaculture Department [online]. Rome. Updated 2017.)

Country	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	Sum
Ireland	120	94	108	0	0	0	0	0	0	0	322
Albania	193	119	98	70	59 F	48	10	10 F	10	46	663
Spain	86	50	60	81	64	62	65	97	65	47	677
Italy	64	109	75	87	64	48 F	43 F	49 F	73 F	76 F	688
Hungary	90	34	52	92	235	26	17	67	155	10	778
Norway	296	194	211	69	32	0 0	0 0	0 0	0 0	0 0	802
Poland	184	181	160	161	168	120	119	141	124	111	1469
Germany	303	294	328	305	298	278	256	307	203	198	2770
Netherlands	316 F	258 F	256 F	253 F	307	372	341 F	321	328	329	3081
Denmark	580	531	457	467	422	371	328	331	331	262	4080
United Kingdom	405	486	416	463	461	460	418	428	400	355	4292
Sweden	730	698	666	518	523	440	336	361	320	245	4837
France	759 F	679 F	581 F	564 F	580	578	339	401	330	292	5103
Totals - tonnes	4348	3923	3660	3304	3410	2959	2416	2660	2494	2115	31289

" F " = FAO estimate from available sources of information

Tab. 2. Aquaculture of the European eel according to the FAO statistics (FAO. 2017. Fishery and Aquaculture Statistics. Global aquaculture production 1950-2015 (FishstatJ). In: FAO Fisheries and Aquaculture Department [online]. Rome. Updated 2017.)

Country	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	Sum
Sweden	191	175	172	0	0	90	93	92	64	104	981
Greece	385	454	489	428	372	289	322	250	285	322,1 F	3274
Spain	403	479	534	488	423	434	374	305	361	372	4173
Germany	567	440	447	385	398	660	460	471	643	1147	5618
Italy	807	1000	551	567	647	510	738	642	572	545 F	6033
Denmark	1699	1614	895	1659	1532 F	1154 F	1061	712	789	1232	9661
Netherlands	5000	4000	3700	2800	3000	2050	1800	2885	2335	2300 F	27570
Totals	9105	8218	6856	6379	6402	5199	4854	5359	5177	6027	57311

" F " = FAO estimate from available sources of information

1.2 Viral diseases in eel

Viral diseases in eel are a problem not only due to the difficult-to-diagnose epidemiological factor and high mortality, but predominantly due to the dramatic decline in the abundance of eel over the past years. The cause of the deteriorating condition and the increasingly observed mortality of the European eel *(Anguilla anguilla)* is thought to be the lack of resistance to viral diseases such as Eel Virus European (EVE) Anguillid herpesvirus 1 (AngHV-1), Eel Virus American (EVA) or Eel Virus European X (EVEX).

Eel Virus European is an infective agent likely to have been carried to Europe with the imported Japanese eel, in which this type of virus was first diagnosed in 1969. The clinical signs associated with EVE infection are primarily unnatural body shape, numerous hyperaemic areas of the fins, skin and gills, and frequent secondary bacterial and fungal infections associated with a decrease in the general immunity of the fish. Unfortunately, in many cases, viral diseases of fish do not give specific clinical signs that would allow a preliminary identification of the pathogen. The signs are usually suggestive of advanced bacterial or fungal infections. Therefore, it is a secondary factor, not the primary one, that is diagnosed in practice. The use of antimicrobial or antifungal agents often further weakens the already weak fish. As reported by Castric (1980), EVE is capable of inducing clinical signs typical of infectious pancreatic necrosis (IPN) observed in the rainbow trout. However, these observations are contradictory to those reported by Sano (1981). The latter author suggests that EVE does not induce any clinical signs in the rainbow trout, even during the exposure of the fish to the virus in a bath for 40 days at a temperature of 10°C. An interesting observation is made by van Buerden (2012), who believes that EVE seems to be pathogenic to the rainbow trout, but does not always cause a clinical disease in juvenile eel. This would mean that the viral genome undergoes debilitating changes in the course of evolution due to tropism, i.e. the affinity for selected cells or tissues. This process unfortunately allows extending the spectrum of hosts to other ichthyofauna species, and consequently allows a faster transmission of the virus in the environment.

Herpesvirus anguillae (HVA) or Anguillid herpesvirus (AngHV-1) belongs to Alloherpesviridae, the most abundant group of DNA viruses that infect fish. Due to the rapid replication and causing mass kills within a very short period of time, this group constitutes a real epidemiological risk. The clinical image of AngHV-1 infection includes primarily apathy, heterogeneous ecchymoses on the skin, fins and gills, occasionally observed hepatic necrosis and pale spleen (Haenen et al., 2002), but these are non-specific symptoms that do not enable even a preliminary diagnosis. During the mass kills of fish in Germany, the observed signs included lethargy and light discolouration of the skin with visible local ecchymoses on fins and in the abdominal area (Scheinert and Baath, 2004). According to Chang (2002), the intensity of the described signs is strongly correlated with the level of stress.

As reported by Sano (1990), AngHV-1 was isolated in 1985 in reared eel in Japan, and a few years later also in Taiwan (Ueno et al., 1992).

In the Netherlands, the virus was diagnosed in 1986 in a European eel with signs of skin peeling (Bekesi et al., 1986). In the following years, the presence of AngHV-1 was systematically confirmed in Japan, Taiwan, Italy, France and Germany (van Gineken et al., 2004, Jakob et al., 2009, Haenen et al., 2010, Van Beurden et al., 2012). In 2011 (Varvarigos et al.), the presence of combined virus infection (HVA and IPN) in eel from breeding farms in Greece was confirmed. A year later (Kim et al., 2012), a Korean group conducted a study of the molecular characteristics of their isolates of AngHV-1 obtained from reared Japanese eel *(A. japonica).* In Poland, the first detection of AngHV-1 was revealed in 2014 (Kempter et al.). The authors confirmed the presence of the virus in the European eel originating from both natural waters and exported from Denmark for pre-rearing and stocking. The same authors were also the first to confirm the presence of AngHV-1 in the American eel *(A. rostrata).* The molecular characteristics of this pathogen suggest that it contains a conserved and stable genome that is not prone to mutations. At the same time, no isolates common for the various eel species have been found (Ueno et al., 1992). This is probably associated not only with the morphological stability of the virus, but also with eel development cycle that guarantees a uniform environment of origin for all individuals. As reported by Environmental Agency (www.environmental-agency.gov.uk), HVA is the only one of the three known and widely diagnosed eel viruses hat has been observed in wild fish. The virus is also known as the factor causing mass kills, reducing the ability of the fish to swim, and capable of limiting eel migration to the Sargasso Sea for reproduction.

Eel Virus European X (EVEX) and Eel Virus American (EVA) belong to rhabdoviruses whose complete genome was sequenced in 2012 (Galinier et al., 2012). The two viruses share high genetic and serological similarity, as well as physico-chemical properties (von Beurden, 2011), therefore are frequently reported in the literature as synonymous. The viruses were isolated for the first time in Japan in the 1970s from the American eel imported from Cuba (Sano, 1976). A year later, Sano (1977) reported the isolation of a virus structurally very similar to EVA from the European eel imported from France. In 2014, Caruso et al. described a case of an outbreak of the disease in an eel farm in Italy, which was caused by EVEX. According to the authors, the isolated and amplified virus was phylogenetically very similar to the already known isolate of the virus, obtained from wild eel. The clinical signs of EVEX infection described to date are preliminarily the characteristic bending of the head by infected individuals and the presence of ecchymoses on the fins and the abdomen. As with the majority of viral diseases, non-specific signs that may result from secondary infections occur simultaneously. The recorded mortality of the disease observed in the American eel is approximately 60%. As reported by van Beurden (2012), EVEX can be pathogenic for the American and European eel, but also for juvenile rainbow trout. In the latter case, stock mortality can reach 100%.

In the relatively recent reports of JEECV (Japanese Eel Endothelial Cells-infecting Virus), it was diagnosed in the Japanese eel *(A. japonica)* originating from natural habitats (Okazaki et al., 2015). JEECV was diagnosed in wild juveniles (elvers) caught in Japan (Okazaki et al., 2016) and in adult eel caught

in Mariana Trench in 2013 (Okazaki–Terashima et al., 2016). The disease caused by JEECV is referred to as Viral Endothelial Cell Necrosis of Eel (VECNE) and results in significant economic loss for eel aquaculture in Japan (Naoi et al., 2017). In the cited paper, local researchers presented a complete genomic sequence of JEECV derived from reared Japanese eel.

1.3 Pathogen, carrier status and immunity

Viral diseases are characterised by a specific selection of the host. In the viral diseases of fish, particular problem is posed by rapid and mass kills of infected fish, as well as progressive secondary infections, both fungal and bacterial. General weakening of the organism caused by viral replication also causes non-specific signs that are difficult to diagnose, particularly in open waters in which observation of infected fish is virtually impossible. As regards the identification of the tropism of the virus to selected cells or tissues, a process aimed to increase the spectrum of hosts occurs, which enables faster and easier spreading of the virus in the environment. It is possible on the occurrence of mutations in the viral genome. As a result, it is possible for the virus to incorporate and replicate into species other than that recognized as the specific host species. This process has been observed in the Koi Herpes Virus (KHV) that can replicate and cause pathological signs not only in the Koi, but also in the carp and crucian carp hybrids (Bergmann et al., 2010). Therefore, carrier status is an evolutionary form of a waiting room—an intermediate state between a virus specific for the given host species and a form invasive for other species, not always phylogenetically close. Genetic factors largely determine the phenotypic characteristics of resistance to a certain viral infection (Siwicki, 1994). Differences in the resistance against certain viruses can be present not only between species, but even between individuals from specific populations or lineages. The trait of increased resistance is most often the selection threshold in the formation of resistant herds. This type of selection studies is also currently conducted at the Institute of Ichthyobiology and Fishery Management in Gołysz in order to characterise and locate the fragment of the genome conferring resistance to Koi Herpes Virus. It has been confirmed experimentally that differences in the survival and susceptibility to pathogens of genetically different carp lineages indicate the existence of genetically controlled differences in the functioning of the immune mechanisms (Pilarczyk et al., 2002). It should be stressed, however, that the most efficient, but also the most costly form of protection of selected fish populations or herds is the use of vaccines, including those of the latest generation—DNA vaccines.

1.4 Horizontal and vertical transmission

The transmission or transfer of the virus in the aquatic environment is the key element in epidemiological analysis. This applies not only to the presence in the environment of species constituting the route of transmission, but also to the number of species that allow such a “transfer.” In viral diseases of fish, the most common of possible transmission types is horizontal transmission, i.e. transfer of viruses from one

fish to aquatic invertebrates and then to another fish, and alternatively via an “unspecified environmental factor” to another fish.

In the case of “fish-to-fish” transfer, the virus can be transmitted not only between ill individuals with an ongoing and irreversible replication of the virus, but also between individuals of the “carrier” group, which is more dangerous. This last case poses a very serious threat to fish farms, because it allows introduction of the virus by asymptomatic individuals to an area in which the rearing conditions can affect the outbreak of the disease. This is associated mainly with stress and non-natural density of stocks. As has been observed in studies of experimental cohabitation (Kempter et al., 2008), effective infection of the target species for the given virus (the carp) is possible through the presence of other fish species (carp, tench, vimba bream) constituting “carriers” and presenting no clinical signs of the disease. This poses questions: whether the same mechanism can take place in the case of AngHV-1? Whether species phylogenetically distant, but occupying a common ecological niche, can be vectors directly involved in the transmission of AngHV-1? Whether isolates obtained from eel are varied, form different genotypes, and if so, how they may present a risk to the indigenous ichthyofauna, for example, during the stocking of natural waters in Poland with eel? The second option, “fish-to-aquatic invertebrates-to-fish” is rather complex due to the variety of species of invertebrate fauna, among which are bivalves, gastropods and crustaceans. Unfortunately, the confirmed carrier status for viruses specific to fish in this group of animals greatly increases the risk of transfer of the virus to fish ponds, but also to closed circuits, if they are supplied with water from rivers or lakes. As reported by Giraud et al.(1994), the level of contamination of the natural environment associated with infected trouts (WHS/IHN) is relatively low, but this does not exclude the possibility of virus transmission by wild individuals. A group from Germany (Peters and Neukirch, 1996) has also determined the possibility of transmission of pathogenic viruses to fish by the grey heron. They demonstrated that herons can constitute a mechanical vector for IPN, VHS and SVC, which in turn means that herons are potential vectors and can actively contribute to the spread of viral diseases in fish.

Vertical transmission is defined as the possibility of transmitting a virus from individuals undergoing spawning directly to the hatch. This type of spread of viral diseases in fish is particularly dangerous in hatcheries in which no systematic diagnostics are conducted. Infected spawning fish can be asymptomatic carriers, while eggs and then hatch are vectors and can be a means of entry of viral diseases into natural waters. This process was studied in Canada in 1990s (Bootland et al., 1991). Researchers attempted an experimental infection of one-year-old brook trout individuals *(Salvelinus fontinalis)*. They established that the phenomenon of vertical transmission of IPNV excludes the assessment of the effectiveness of immunization of spawning herds by hatch examinations. The knowledge of vertical transmission is very fragmented, as such hypotheses are difficult to verify. This is due to the difficulty in distinguishing mechanical transfer of viral particles on egg envelope or in seminal plasma from active transfer inside the

eggs or sperm. Epidemiological studies are also designed to exclude the environmental factor, i.e. to prove that the virus was transferred by the spawning fish and not, e.g., by viral particles present in the water supplied to the spawning chambers. According to spoken information (Bergmann, 2017), this type of transmission has been confirmed for IPNV and SAV. Unfortunately, no publications concerning such experiments are available.

2. AIM OF WORK

The aim of this PhD thesis was to analyse the degree of the threat to the European eel *(Anguilla anguilla)* associated with the viral disease caused by the AngHV-1 pathogen.

Detailed objectives constituting the components of the primary task were:

1. Detection of Herpesvirus anguillae (AngHV-1) in north-western Poland in order to assess the degree of the threat.
2. Determination of vectors of the virus among the indigenous ichthyofauna as potential routes of spread of AngHV-1 in the aquatic environment.
3. Assessment of the effectiveness of selected diagnostic methods and the usefulness of selected tissues in the determination of efficient diagnostic methods of AngHV-1 in the course of eel stocking programmes.
4. Development of guidelines for entities involved in eel pre-rearing and stocking centres.

3. METHODOLOGY OF VIROLOGICAL TESTS

Among the methods allowing detection and verification of viruses in fish are those that directly indicate the presence of viral particles, such as electron microscopy, and those that demonstrate the presence of these pathogens in an indirect manner. The second group of methods includes antibody identification, in situ hybridisation, PCR, real-time PCR, rtPCR and multiplication of viruses in cell cultures. Optimization of virological diagnostics is conducted by identifying a method characterised by a high sensitivity and speed. The studies are also aimed to determine the optimal test that guarantees the smallest error. The methods listed in this section are characterised by a different level of sensitivity, which means that the positive result is dependent on the detection threshold. Detection threshold is defined as the smallest quantity of viral particles that is detectable by the diagnostic method. A major role is also played by the type of the biological material sampled for tests. Generally, some methods are excluded due to the source material (spawning fish, reared fish, stocking fish). For example, it is not possible to use an effective method of detection of viral DNA from the gills, spleen or skin using PCR if animals must be kept alive (e.g., spawning fish or valuable Koi individuals). In such cases, only non-lethal methods can be used, such as aggressive swab from the gills, skin scraping in recesses under the fins, or analysis of antibodies obtained from blood. Such restrictions do not exist in the case of samples from reared or stocking material at hatching or juvenile stages, when the availability of material is unlimited. Of great importance is also the knowledge of the tropism of each virus. This property of viruses causes different rates of their replication depending on the tissue or organ in which they occur. It is vital in the diagnostics at the stage of sampling due to the possibility of obtaining false-negative results when inappropriate tissue fragments are collected. Tropism is the affinity only to selected tissues/organs, and it means that infected fish may not have viral particles in tissues for which there is no tropism. The consequence of false negative results is certification of the material as “free from virus”, e.g., in fish intended for stocking open waters. This results in an increased risk of carrying the pathogen into the environment, which generates considerable loss and the risk of further spread of the disease to open waters and fish farms supplied with water from rivers or lakes. Determining the appropriate organ or tissue is necessary for proper diagnostics and conducted every time for individual viruses and species they infect.

4. RESULTS OF STUDIES

4.1 Infection status in eel

In 2014, 86 eel that originated from a location in north-western Poland were caught (as summarised in Table 3).

Tab. 3 Comparison of the number of samples containing AngHV-1 according to method of DNA isolation (Med. Weter. 2016, 72 (9), pp. 565)

Sampling site No. of samples	Sample type	No. of positive samples vs. total samples (percentage of eel with positive PCR results) Column method	 DNAzol
Stocking material— Centre 1 (n=6)	Gills Mix	0 (0%) 0 (0%)	0 (0%) 0 (0%)
Lake Dąbie (n=20)	Gills	6 (30%)	5 (25%)
Szczecin Lagoon (Trzebież) (n=15)	Gills	6 (40%)	4 (27%)
Denmark (n=9)	Gills	6 (66%)	4 (44%)
Stocking material— Centre 2 (n=31)	Gills Mix	29 (94%) 22 (71%)	19 (61%) 15 (48%)
RSD Dolna Odra (n=5)	Gills Mix	5 (100%) 3 (60%)	3 (60%) 1 (20%)

According to the conducted studies, the quantity of tested samples in which the presence of AngHV-1 genome was confirmed is epidemiologically significant. The most important problem associated with eel management in Poland is the confirmation of AngHV-1 carrier status in fish imported directly from Denmark in this study. Before the test, they had not had any contact with water or other fish in Poland. This means that a vertical transmission of AngHV-1 is probable. Unfortunately, the lack of availability of glass eel juveniles in our studies did not allow the final confirmation of this hypothesis. Surprisingly, there is a discrepancy in the degree of detection of viral genome between two different, although commonly used, methods of isolation of viral DNA. As can be seen in the above list, the isolation method involving DNAzol should be discontinued in commercial diagnostics. It is a low-efficiency method that does not guarantee isolation of sufficient quantities of DNA when its original quantity is too small. However, it is interesting that in the comparison table, in the cases where the column method showed the presence of AngHV-1 genome even at minimal quantities (Lake Dąbie, with the method efficiency at 30%), even the DNAzol method demonstrated positive results of isolation at 25%. More disturbing from diagnostic perspective are the proportions of detection of viral DNA in samples with a higher degree of detection (94 and 100% for material derived from Centre 2 and RSD Dolna Odra, respectively). In these cases,

the use of DNAzol yielded much lower results—61% and 60%, respectively. This allows to conclude that, in commercial diagnostics, isolation using DNAzol should be abandoned and that using the column method should be widely implemented. The latter gives a greater chance of determining the degree of viral infection in the analysed groups that usually correspond to the collection of catch sites, as well as eel pre-rearing centres. Details of the above cited study, methodology used, as well as detailed results and discussion can be found in the publication from 2016 by Nguyen T.T., Kempter J., Panicz R.: Monitoring of herpesvirus anguillae (AngHV-1) infections on European eel in north-west Poland. The study was published in the Medycyna Weterynaryjna journal.

4.2 Transmission of the virus in the environment

In 2015, experiments in 332 individuals representing 20 species from 20 locations in north-western Poland were conducted. Analyses aimed to detect AngHV-1 genome demonstrated its presence in 17 individuals representing 5 species. Due to their presence among the natural waters ichthyofauna or at rearing centres, particular attention should be paid to the potential AngHV-1 transmission by these individuals. The species include: Prussian carp (2), European perch (2), zander (2), sterlet (5) and round goby (6). Particularly dangerous seems to be the confirmed fact that an invasive species, such as the round goby, can be a vector in natural waters. As it has been confirmed that this species dominates the native species of Gobiidae, as well as the viviparous eelpout and the European flounder, AngHV-1 is transmitted automatically along with this invasive species. Bearing in mind that the species has increasingly less natural enemies, such as zanders, pikes or eel, in the environment, it constitutes a considerable risk not only due to the large uncontrolled growth of its population, but also in the context of epidemiological threat. Details of this study along with the discussion of the results and the literature can be found in the publication of 2016: Nguyen T.T., Kempter J., Panicz R. Dynamics of Herpesvirus anguillae (AngHV-1) transmission by the native ichthyofauna of north-western Poland. The study was published in the EJPAU.

4.3 Diagnostic capabilities and limitations

Following the recommendations of the World Organization for Animal Health (OIE) urging the member states of the European Union to seek and develop diagnostic methods to minimise the false-negative results, experiments aimed to optimise the diagnostic methods for the detection of AngHV-1 in eel were conducted. In 2016, 15 individuals of the European eel were used as the source of organs for the assessment of tropism, and thus the usefulness of tissues for AngHV-1 diagnostics. In the study, the sensitivity of conventional PCR, nested PCR and in-situ hybridisation was compared. It was found that conventional PCR used by laboratories for virological diagnostics of fish without the subsequent cycle of nested PCR is burdened with a highly risk of low detectability of viral DNA. Therefore, it is necessary to use a reaction yielding a shorter product, but based on the product of standard PCR. The results showed that such a procedure increases the number of positive samples from 53.3% to 93.3%.

Observation of the cytopathic effect (CPE) was possible after the first two subcultures. As shown by the subsequent sequencing of all obtained products, their molecular similarity was between 88% and 99%. The sample used as the positive control was a sequence derived from the European eel regarded as standard in OIE tests. The results of this third phase of studies demonstrated the usefulness only of gill and skin sections of eel for commercial diagnostics, deprecating the very frequent use of mixed samples (pools) in tests aimed to ensure low costs and shorten the time of analysis. Details of this study along with the discussion of the results and the literature can be found in the publication of 2017: Nguyen T. T., Yeonhwa J., Kiełpińska J., Bergmann S.M., Lenk M., Panicz R. Detection of herpesvirus anguillae (AngHV-1) in European eel *Anguilla anguilla* originating from northern Poland—assessment of suitability of selected diagnostic methods. The article was published in the Journal of Fish Diseases.

5. Conclusions

1. It is recommended to abandon the DNAzol method of isolation for viral DNA and replace it with the column method for the isolation of AngHV-1 from the tissues of the European eel.
2. It is recommended to use fragments of the gills and the skin only for the AngHV-1 diagnostics, and to exclude the method involving mixed samples that is often used in commercial diagnostics.
3. The presence of AngHV-1 in eel obtained directly from the importer from Denmark, which constitutes the starting material for pre-rearing and has a degree of infection of 66%, encourages introduction of mandatory diagnostics of the virus.
4. The Prussian carp, European perch, zander, sterlet and round goby are vectors potentially involved in the horizontal transmission of AngHV-1.
5. As to determine the presence of AngHV-1 in eel, the nested PCR method is recommended due to the high degree of false-negative results in conventional PCR.
6. The degree of infection of the stocking material can be considered alarming, therefore, as part of a reasonable and rational restitution programme, every batch of the stocking material should be tested for the carrier status of AngHV-1 and other viruses potentially dangerous to eel.

6. Summary

As reported by Lirski and Myszkowski (2016) in their analysis based on RRW-22 questionnaire, total production of eel in Poland in 2015 was 100 kg, while sold production was 350 kg. The value of eel production in Poland is estimated at approx. 22 thousand PLN. According to the same source, production of stocking material in 2015 was 0.4 metric tonnes, while the estimated production of juvenile eel in hatcheries and pre-rearing centres in the same year was approx. 83,000 individuals.

Excellent sensory characteristics, fat content and taste, especially after cold smoking, along with the decreasing catches (section 1.1), resulta in very high prices. As regard price per unit among 30 fish and crustacean species, eel is ranked second, reaching the price of 63.01 PLN/kg, just after crayfish (132.86 PLN/kg).

Data from 2017 (Lirski and Myszkowski, 2017) indicate that the sales of eel as stocking and introduction material in 2015 was: 0 kg of juveniles, 209 kg of autumn juveniles and 400 kg of combined autumn and spring juveniles. According to dr. S. Robak (spoken report, 2017), fish farmers rear the stocking material of eel using a trial and error approach, non-cyclically, importing it along with eel for fattening. European data show that approximately 1,5 metric tonnes of glass eel are imported to Poland and used for stocking in open waters after pre-rearing. However, there have been cases in which farmers bought juvenile eel meant for stocking, reared the fish to a size adequate for consumption, and sold mainly to China and Japan. These quantities are not seen in the aquaculture data, as they are not reported or recorded.

In summary, an important element of eel management is primarily the introduction of a permanent monitoring programme for the health of the stocking material. It is extremely important, since it is known that this species does not have the ability for self-restoration of the population from juveniles that are subject to natural migrations from the Sargasso Sea. This means that a successful stabilization of eel stocks in the environment depends only on the effectiveness of implementation of stocking programmes. Therefore, it is the rearing centres of stocking material that take full responsibility for the quality and health condition of eel, and it is their duty to maintain the quality of the material introduced into open waters.

7. References

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