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REPORT 25/2023

The surveillance programme for *Aphanomyces astaci* in Norway 2022 and evaluation of disease freedom in Buåa watercourse

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Content

Summary

This surveillance programme uses environmental DNA (eDNA) monitoring for species specific detection of *Aphanomyces astaci* spores directly from water filtrates. The presence/absence of eDNA from noble crayfish (*Astacus astacus)* and signal crayfish (*Pacifastacus leniusculus*) is also determined to supplement the results, and to evaluate the habitat status. Detection of noble crayfish eDNA, combined with the absence of eDNA from *A. astaci* and signal crayfish, substantiate the presence of non-infected noble crayfish which constitutes the desired habitat status. These analyses are part of the collaboration and coordination with the national surveillance programme for noble crayfish. The geographic focus of the surveillance programme in 2022 is the Halden watercourse and neighbouring risk areas; the Mosse watercourse, Glomma watercourse, River Mysenelva and areas in the Eidskog municipality including the Buåa watercourse and the rivers Vrangselva and Finnsrudelva. Additionally, extensive monitoring (eDNA sampling and cage trials) were conducted in Buåa watercourse in 2021 and 2022 in order to evaluate the watercourse for disease freedom from *A. astaci*.

- In the Halden watercourse, eDNA of *A. astaci* was detected in the southern part of Lake Rødnessjøen and in River Hølandselva, within the control zone. eDNA of signal crayfish was also detected in the south of Lake Rødnessjøen, where there is a known signal crayfish population. All water samples in the risk area of the Halden watercourse region were negative for eDNA from *A. astaci* and signal crayfish, while most samples were positive for noble crayfish eDNA. No eDNA from signal crayfish was detected in River Lierelva in 2022.
- In the Mosse watercourse, no eDNA from *A. astaci* or signal crayfish was detected, while noble crayfish eDNA was detected upstream of Lake Langen.
- In the Glomma watercourse, eDNA from *A. astaci* and signal crayfish were detected at Fossum bridge, where signal crayfish were discovered in 2020. No eDNA from *A. astaci*, signal crayfish or noble crayfish was detected at the remaining stations in Glomma.
- In River Mysenelva, eDNA from noble crayfish were detected at two locations. No eDNA from signal crayfish or *A. astaci* was detected.
- In Eidskog, all samples were negative for signal crayfish and *A. astaci*, while several samples were positive for noble crayfish eDNA in the rivers Vrangselva and Finnsrudelva.
- In Buåa water course, no eDNA of *A. astaci*, signal crayfish or noble crayfish was detected in the extended survey in 2021-2022. Neither was *A. astaci* detected in the cage trials conducted in 2021-2022.

In summary, eDNA from *A. astaci* was detected within the control zone in Lake Rødnessjøen and River Hølandselva in the Halden watercourse, and at Fossum bridge in the Glomma watercourse. Frequent detections of noble crayfish eDNA within the regulated *A. astaci* control zones of the Halden watercourse, Mosse watercourse, and the rivers Vrangselva and Finnsrudelva in Eidskog, suggests the presence of vital noble crayfish populations within *A. astaci* control zones. The absence of eDNA from *A. astaci* and signal crayfish, and absence of *A. astaci* using cage trials demonstrates that Buåa watercourse meets the criteria for declaration of disease freedom. Thus, the Norwegian Veterinary Institute (NVI) recommends that the Buåa watercourse is declared disease free.

Introduction

Aphanomyces astaci in Norway

The oomycete *Aphanomyces astaci*, the crayfish plague pathogen, is lethal to native European freshwater crayfish [1-3]. It is carried and transmitted by North American freshwater crayfish, which act as healthy carriers of the pathogen. *A. astaci* reproduces and spreads with swimming zoospores, the infective stage of the pathogen. It was accidentally introduced to Europe in the 1860s, and resulted in mass-mortalities of freshwater crayfish all over Europe. It was later re-introduced to Europe through many independent introductions of alien North American carrier crayfish [3], in particular signal crayfish.

Crayfish plague is a category F disease in Norway, according to the "*The animal health regulations" Chapter II, § 6"* FOR-2022-04-06-631.

Since 1971, nine water systems in Norway have been affected by crayfish plague outbreaks one or several times [4-6]. These include the Vrangselva watercourse and River Veksa (1971), the Glomma watercourse (1987 and 2003), Lake Store Le (1989), the Halden watercourse (1989, 2005 and 2014), River Lysakerelva (1998), Buåa watercourse (2010), Mosse watercourse (2016), and recently River Mysenelva (2021) [6]. In 2016, crayfish plague was confirmed in noble crayfish inhabiting the bordering watercourse Vrangselva and River Billa between Norway and Sweden (which is also called River Finnsrudelva on the Norwegian side), but the infection has not been detected on the Norwegian side. In addition, four more localities have been (or still are) subject to crayfish plague regulations due to illegally introduced and confirmed *A. astaci* positive signal crayfish [4]. These include Dammane (Vestfold and Telemark), Ostøya (Viken), The Fjelna watercourse (Trøndelag) and Lake Kvesjøen (Trøndelag) where signal crayfish were discovered in 2006, 2009, 2011 and 2013, respectively [4-7]. At two of these locations (Dammane and Ostøya), signal crayfish have been successfully eradicated and the areas were declared disease free [4].

Focus areas 2022

The focus areas of the 2022 surveillance programme for crayfish plague cover the

- Halden watercourse, including follow up in Lierelva (under regulation FOR-2015-05-26-592)
- Mosse watercourse (under regulation FOR-2016-12-13-1523)
- Glomma watercourse, including River Mysenelva (under regulation FOR-2005-06-20-652)
- Eidskog municipality, including Buåa watercourse, Vrangselva watercourse and River Finnsrudelva (under regulation FOR-2016-08-17-972)

Halden Watercouse

The Halden watercourse was first struck by crayfish plague in 1989, re-stocked with noble crayfish in the 1990s and the population successfully recovered until the crayfish plague returned in 2005 [8]. Immediate closure of the Ørje locks prevented upstream spread to Lake Rødenessjøen. Illegally introduced *A. astaci* positive signal crayfish were found in Lake Øymarksjøen in 2008 [9], leading to the permanent closure of the locks. This prevented further spread, until illegally introduced signal crayfish were found upstream of the locks in

2014. The re-established noble crayfish population in Lake Rødenessjøen was lost during the following plague outbreak [10]. In this period, the TARGET project (NRC- 243907) compared cage-based surveillance with environmental DNA (eDNA) monitoring [10]. The infection front was followed through analysis of water, and eDNA of *A. astaci* was sometimes detected in the water samples prior to crayfish mortalities in the cages. Noble crayfish and signal crayfish eDNA was also detected in the locations where the crayfish are known to occur [10]. After the main outbreak in Rødnessjøen and the spread of crayfish plague to the River Hølandselva in 2015, *A. astaci* was detected at the outlet of the river in 2016 and in 2019, and further upstream in 2021 ([6, 10]; **Figure 1**). Noble crayfish eDNA has been detected at Hølandselva and upstream from 2016-2021 ([7, 11-15]; **Figure 1**). In 2021 one sample was positive for signal crayfish eDNA in River Lierelva ([15];**Figure 1**).

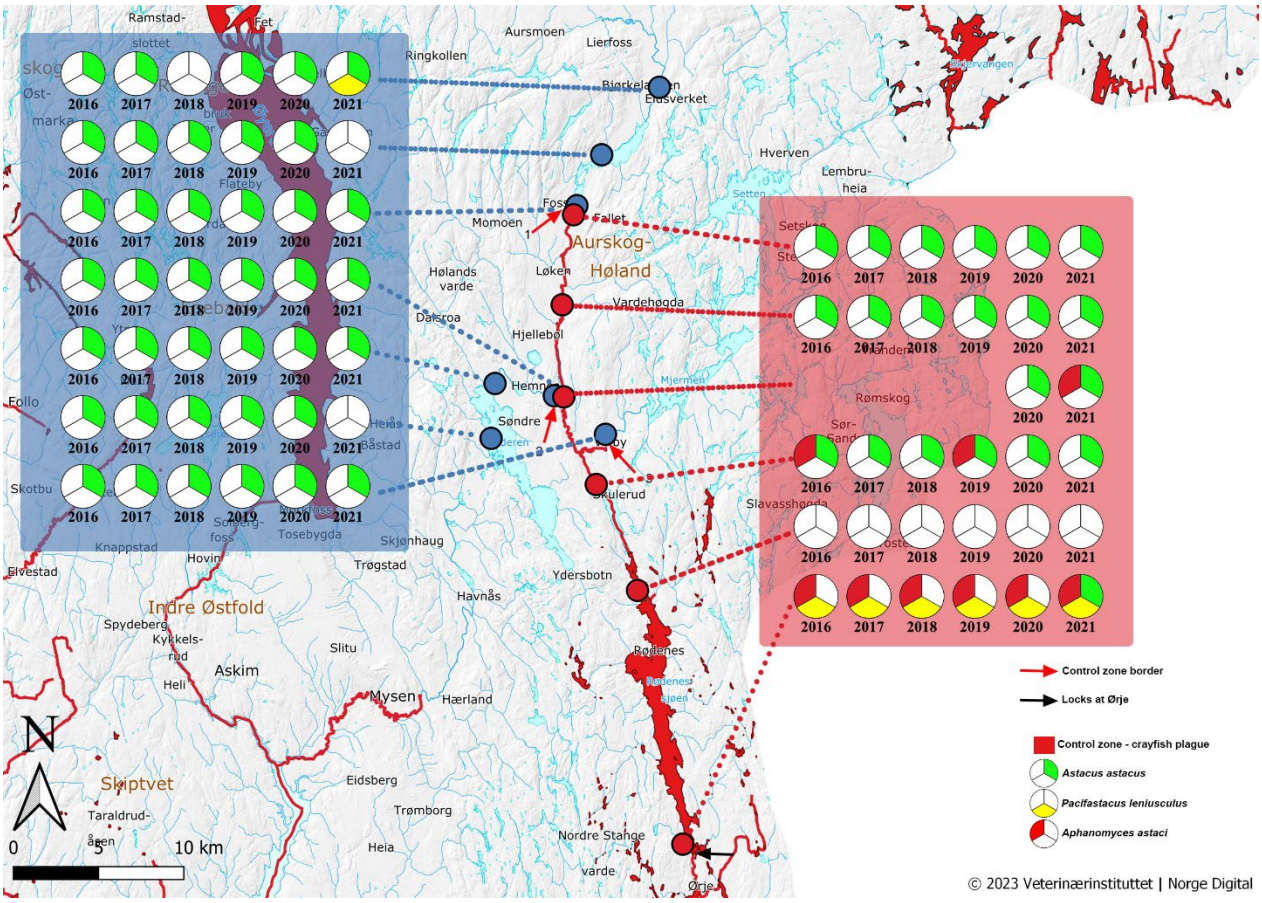

***Figure 1**. Recurring maps of the years 2016 – 2021, showing the stable detection of noble crayfish eDNA within the crayfish plague control zone from the middle part of River Hølandselva (stippled red line) up to the boarder of the control zone at Fosser dam (solid green line).*

The Mosse watercourse

The Mosse watercourse was struck by crayfish plague in 2016 [16]. When the crayfish season started in August 2016, the Norwegian Food Safety Authority (NFSA) received reports regarding possible absence of noble crayfish from Lake Mjærvann and River Hobølelva. No dead crayfish could be found, but eDNA-analyses of water from the small River Tangenelva upstream of Lake Mjærvann (Enebakk) conducted at the Norwegian Veterinary Institute (NVI) confirmed high levels of *A. astaci* eDNA, corresponding to an outbreak situation [16]. The NFSA established zone

regulations and initiated surveillance with cages in infected areas. In the cage upstream of the lower dam in the pond Steinkistedammen, the spread of crayfish plague was detected in December 2016, while the cage placed in Lake Våg was not affected in 2016 [12]. No *A. astaci* eDNA was detected in the Mosse watercourse in 2017, but there was a significant drop in eDNA detection of noble crayfish from June to August in Lake Våg [11]. A dead crayfish found in Lake Langen in 2018 was diagnosed with crayfish plague, confirming the upstream spread of crayfish plague in the watercourse [12]. No *A. astaci* was detected in the watercourse in 2019-2021 [13-15].

The Glomma watercourse

The Glomma watercourse was struck by crayfish plague in July 1987, from Kirkenær in Solør and further downstream including Lake Vingersjøen and Lake Storsjøen/River Oppstadåa [4]. Environment authorities and landowners cooperated to re-establish crayfish in the river system, but the plague struck again in 2003. Cage experiments combined with crayfish plague diagnostics confirmed active crayfish plague in the system from 2005 until 2015 [4, 5, 7]. The last detection was in the tributary Opstadåa in 2015. No *A. astaci* eDNA has been detected in the Glomma watercourse, the outlet of Lake Vingersjøen or Oppstadåa in the period 2016-2021 [7, 11-15], while noble crayfish eDNA was detected in River Oppstadåa in 2016 [7] and at Skarnes and Kongsvinger in 2019 [13]. Signal crayfish, and confirmed carriers of *A. astaci*, was discovered in Glomma at Fossum bridge, downstream Solbergfoss in 2020 [17, 18].

River Mysenelva (Hæra)

River Mysenelva (Hæra), which drains into Glomma was struck by crayfish plague in 2021 [6, 15]. *A. astaci* was detected on dead crayfish found in the river. The outbreak was limited to the river downstream of Rustadfossen.

The rivers Vrangselva and Finnsrudselva

The rivers Vrangselva and Finnsrudelva/Billa in Eidskog municipality that flow across the border into Sweden were struck by crayfish plague on the Swedish side of the border in 2016. The crayfish plague has been active and slowly spreading upstream in River Finnsrudelva/Billa on the Swedish side of the border in 2017 and 2018. However, no sign of crayfish plague has been detected on the Norwegian side of the border in either of these two watercourses in the period 2016-2021 [7, 11-15].

The Buåa watercourse

The Buåa system was struck by crayfish plague in 2010 caused by the presence of signal crayfish on the Swedish side of the river [19]. A barrier built to prevent the spread of signal crayfish did not stop the infection from spreading, but hopefully stopped the signal crayfish [4]. Cage experiments were conducted in the area until 2016 without revealing any active infection source [7]. eDNA analysis of samples for Buåa tested negative for *A. astaci* and signal crayfish in the period 2017-2021 [11-15].

The surveillance programme for *A. astaci* is commissioned by NFSA and conducted by NVI. Until 2015, surveillance of crayfish plague relied on cage experiments with live noble crayfish. In

2016, classical cage experiments were combined with eDNA monitoring [7]. Based on an overall assessment taking crayfish welfare and cost-benefit into account, the cage experiments were excluded from the surveillance programme in 2017 [11]. From 2018, the program has collaborated with the National surveillance programme for noble crayfish, commissioned by the Norwegian Environment Agency (NEA) and coordinated by the Norwegian Institute of Nature Research (NINA). This involves joint field work and joint exploitation of water samples and molecular results in overlapping surveillance areas. These synergies enable analyses of a slightly larger sample size than the NFSA-programme alone would allow.

Aims

This surveillance programme aims to

- Monitor the presence and spread of the crayfish plague pathogen *A. astaci* in areas regulated as a result of earlier detection of the pathogen (referred to as control zones[1]).
- Substantiate disease free waterbodies in neighbouring areas of the control zones (referred to as risk areas[2]).
- Alert the authorities of any eventual spread of the disease from control zones to risk areas.
- Continue to evaluate eDNA as a monitoring tool for *A. astaci* - alone and in combination with complementary eDNA targets including both the carrier and susceptible crayfish host species.
- Evaluate the Buåa watercourse with regards to declaration of disease freedom.

Materials and methods

Work plan

The surveillance programme is based on eDNA monitoring of water, where DNA from spores of *A. astaci* is detected directly from water filtrates. To complement information on the habitat status, eDNA from the native and susceptible noble crayfish and the alien carrier signal crayfish is monitored within the same water samples. The logistics and analyses are conducted in collaboration with the national surveillance of noble crayfish, funded by NEA, and coordinated by NINA (**Figure 2**). For the evaluation of Buåa watercourse with regards to declaration of disease freedom, additional sites for eDNA sampling and cage trials have been monitored.

[1] The «control zone» refers to the complete restriction zone covered by each of the regulations. For all practical purposes, a crayfish plague restriction zone does not differentiate between a protection zone and a surveillance zone.

[2] Risk area is not an official term according to the animal health regulations, but a term we have chosen to use for areas adjacent to or geographically close to the crayfish plague control zones covered by the regulations. These areas host healthy noble crayfish populations that face a high risk for spread of the infection from the control zones.

Surveillance sites

The main areas for surveillance include the Halden watercourse and surrounding areas, the Mosse watercourse, the Glomma watercourse, the River Mysenelva and Eidskog municipality including the Vrangselva watercourse, River Finnsrudelva and Buåa watercourse. Plotted locations for water sampling, in total 40 sites, as well as the crayfish plague zones, are displayed in **Figure 3**. Additionally, eight extra sites were sampled in Buåa as part of the evaluation of disease freedom (**Figure 4**). Supplementary details are summarised in Appendix 1 (**Table S1-S8**).

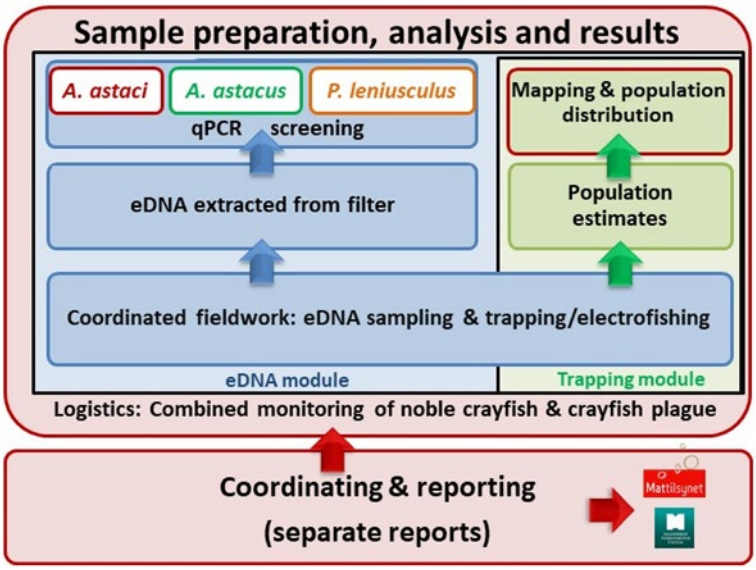

Figure 2. *Work plan: The Norwegian Veterinary Institute (NVI) coordinates the project, and organises the eDNA water sampling and qPCR screenings in collaboration with the national surveillance of noble crayfish (Funded by the Norwegian Environment Agency (NEA).*

Halden watercourse: The control zone was monitored at a total of 5 sites from Lake Fossersjøen to the south of Lake Rødenessjøen (Ysterud). Previous detection of noble crayfish eDNA within the crayfish plague control zone from the middle part of river Hølandselva (**Figure 1**) suggests that the upper parts of the system so far has escaped an outbreak. However, one sample was positive for eDNA from signal crayfish in 2021. To follow up, two more sites were monitored in Lierelva. In total, 9 sites were monitored in the risk area (**Table S3**, Appendix 1).

Mosse watercourse: The control zone was monitored from Lake Sværsvann and Lake Bindingsvann and downstream to River Hobølelva, in total 10 sites (**Table S4**, Appendix 1).

Glomma watercourse: The control zone comprises the main passageway downstream Braskereidfoss in Våler. Seven sites within the control zone were monitored. (**Table S5**, Appendix 1).

River Mysenelva: The control zone comprises the main passageway downstream Rustadfossen in Mysen. One site in the risk area and two sites within the control zone were monitored (**Table S6**, Appendix 1).

Eidskog: The control zone (defined by the municipality border) was monitored in the Vrangselva watercourse (4 sites), Buåa watercourse (10 sites, see below) and River Finnsrudelva (2 sites) (**Table S7**, Appendix 1).

Buåa: In addition to the two regular sites in Buåa, and additional eight sites were sampled for eDNA, resulting in a total of five sites in the River Buåa and five sites in Lake Harstadsjøen (**Figure 4**). These additional sites were sampled in June 2021, September 2021, June 2022 and September 2022 (**Table S8**, Appendix 1) as part of evaluating the Buåa watercourse with regards to declaration of disease freedom.

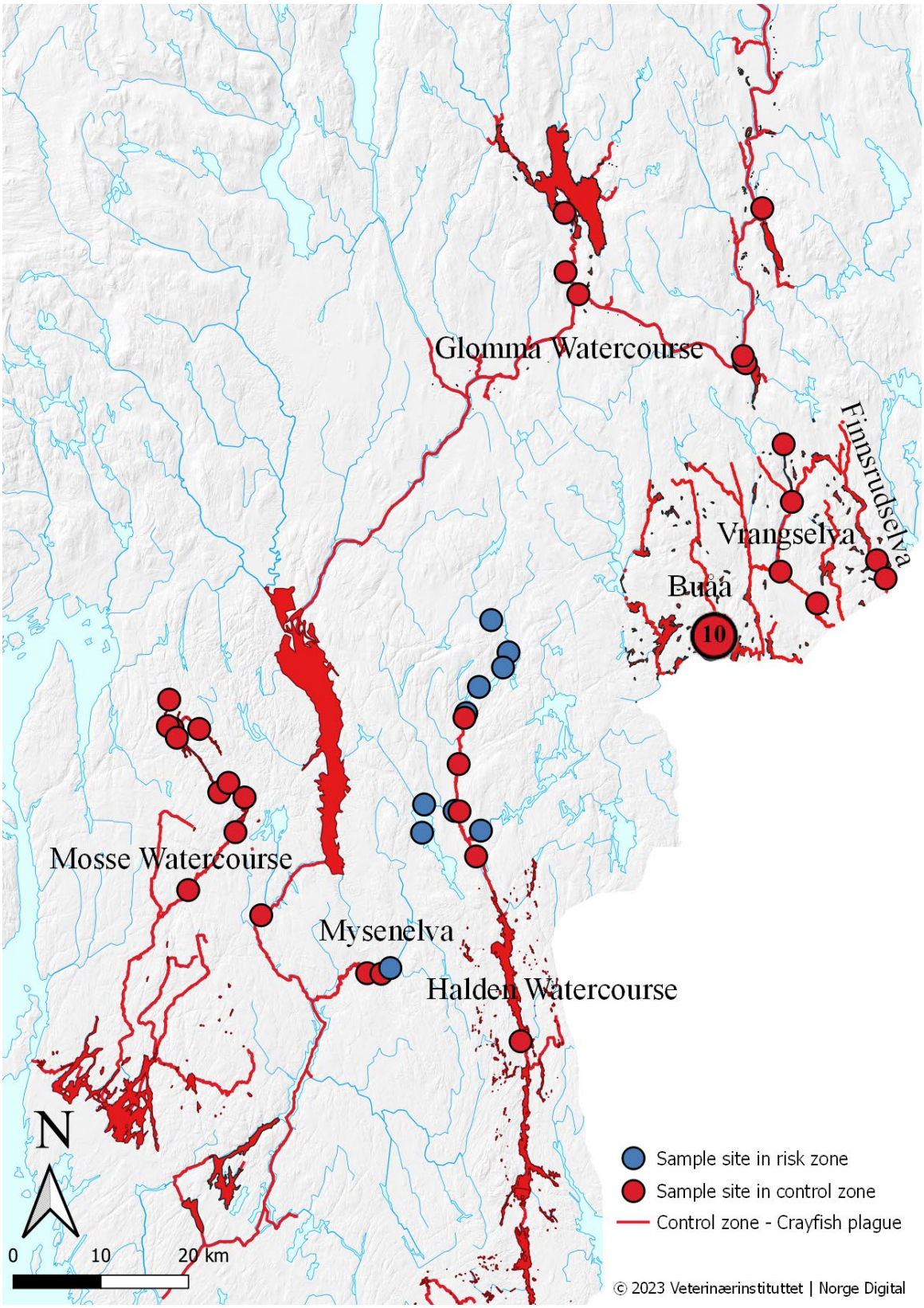

Figure 3. *Surveillance sites in South-Eastern Norway 2022. Water samples (circles) were collected in June and September. Regulated areas (crayfish plague control zones) are marked in red. Note: For Glomma, the control zone is an approximation.*

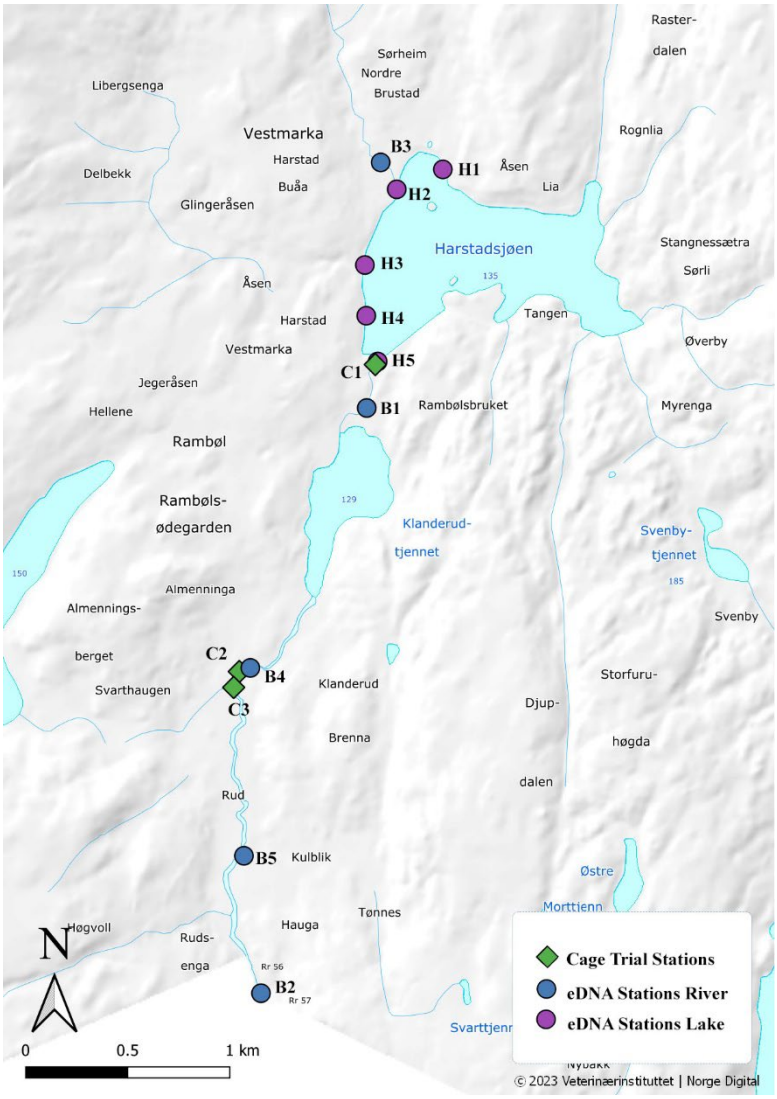

***Figure 4**. Surveillance sites in Buåa in 2021-2022. Water samples were collected in June and September both years from Lake Harstadsjøen (purple circles) and River Buåa (Blue circles). Cage trials were conducted at three locations (green diamonds).*

eDNA sampling

The filter samples were collected in May/June and August/September. From each site, two samples of up to ~5 L water were filtered through sterile glass fibre filters on-site [10]. From the Buåa sites (sampled in 2021 and 2022), two samples of up to 5 L was filtered from per river site, while one sample of up to 5 L was filtered per lake site. Ideally, 5 L was to be filtered per filter sample, but due to high turbidity or clay particles, the total filtered volume was sometimes lower.

The filters were transferred with a clean forceps to a 15 ml falcon tube with ATL-buffer. DNA was extracted using a NucleoSpin Plant II Midi kit (Marcherey-Nagel) protocol [20, 21]. The extracted DNA samples were screened by qPCR for three DNA targets: the species-specific qPCR assay for *A. astaci* [10, 22] and two crayfish species specific qPCR assays for noble crayfish and signal crayfish developed [23]. **Figure 5** presents an overview of the eDNA monitoring procedure.

***Figure 5**. Water samples of ~5 L each were filtered on-site through glass fibre filters using a portable peristaltic pump (Masterflex E/S portable sampler). Each filter was carefully transferred to a 15ml tube with buffer and stored there, until further processing in the laboratory. DNA was isolated with a large volume extraction procedure and presence/absence of eDNA from all target organisms was analysed using qPCR. Figure modified from Vrålstad et al. 2016 [7].*

Cage trials

Three cages were placed in Buåa from Lake Harstadsjøen Outlet and downstream (C1-3, **Figure 4**) from June to October 2021 and June to October 2022. Each cage contained ten noble crayfish and the cages were monitored weekly by local landowners for any crayfish mortality.

Result and Discussion

eDNA monitoring in the Halden watercourse

In the Halden watercourse region, 56 water samples representing a total of ~227 L water were collected during the sampling in May and August 2022. In the control zone, *A. astaci* eDNA was detected in one water sample (one in May) at the Southern part of Lake Rødenessjøen where signal crayfish were confirmed to be present by positive eDNA results in a total of four water samples (two in May, two in September; **Figure 6**, **Table S3**). eDNA from *A. astaci* was also detected at low intensity in one sample in August at the middle station in the River Hølandselva. This indicates that *A. astaci* is still present in the river system. The positive detections of noble crayfish eDNA in samples from River Hølandselva and upstream (within the control zone), as observed in the previous years (**Figure 1**), support the presence of live noble crayfish inhabiting the northern part of the Halden watercourse control zone. In total, noble crayfish eDNA was detected in 13 water samples from River Hølandselva and upstream (within the control zone).

All water samples from the risk area surrounding the Halden watercourse were negative for *A. astaci* eDNA, while most samples were positive for noble crayfish eDNA (**Figure 6**, **Table S3**), demonstrating the presence of noble crayfish within most of the monitored risk area. The single detection of signal crayfish eDNA at River Lierelva in 2021 was not confirmed by the sampling (three eDNA stations and samples) in May and August 2022. Only eDNA from noble crayfish was detected in 10 of 12 samples from River Lierelva in May and August. The presence of noble crayfish in River Lierelva was also confirmed by baited traps in the national surveillance of freshwater crayfish 2022 (S. Johnsen pers. med.).

eDNA monitoring in the Mosse watercourse

In the Mosse watercourse, 40 water samples representing a total of ~164 L water were analysed. None of the analysed samples showed any sign of *A. astaci* or signal crayfish eDNA (**Figure 7**, **Table S4**). eDNA from noble cayfish was detected in nine samples (five in May and four in August) at three stations upstream of Lake Langen. This suggests that crayfish plague has not spread upstream from Lake Langen where crayfish plague was confirmed in 2018, after detection in one dead crayfish found at Kilevika [12].

eDNA monitoring in the Glomma watercourse

In the Glomma watercourse, 28 water samples representing a total of ~123 L water were analysed. No sign of *A. astaci* or signal crayfish was found through eDNA analysis (**Figure 8**, **Table S5**) at the stations upstream of Solbergfoss. One sample was positive for *A. astaci* and signal crayfish eDNA downstream of Solbergfoss at the station Fossum (**Figure 9**, **Table S5**), where signal crayfish was discovered in 2020 [17, 18]. The results cannot verify any active *A. astaci* infection or infection source from the monitored sites in the Glomma, upstream of Solbergfoss.

eDNA monitoring in River Mysenelva

In River Mysenelva, 12 water samples representing a total of ~56 L water were analysed. None of the samples tested positive for eDNA from *A. astaci* or signal crayfish (**Figure 9**, **Table S6**). However, two samples from the station in the risk area, upstream Rustadfossen, amplified after the detection limit for *A. astci* and with skewed curves. This suggest unspecific amplification, or could indicate very low amounts of *A. astaci* in the samples, below the detection limit. These results do not meet the criteria for positive detection. The four samples (two in May and two in August) from the station in the risk area and one of the samples (in August) from the control zone were positive for noble crayfish eDNA (**Figure 9**, **Table S6**).

eDNA monitoring in the rivers Vrangselva and Finnsrudselva

In the Eidskog municipality, 24 water samples representing a total of ~105 L water were analysed. All samples were negative for eDNA from *A. astaci* or signal crayfish (**Figure 8**, **Table S7**). In the Vrangselva watercourse, five samples from Åbogen to Skotterud were positive for noble crayfish eDNA (3 in June, 2 in September), suggesting that the river stretch is still inhabited by noble crayfish. Positive visual detection of noble crayfish at Åbogen and S. Åklangen was done in 2022 during the eDNA sampling. In River Finnsrudelva, eight samples were positive for noble crayfish eDNA (4 in June and 4 in September, **Figure 8**, **Table S7**).

eDNA monitoring and cage trials in the Buåa watercourse (2021 and 2022)

In River Buåa and Lake Harstadsjøen, a total of 70 water samples representing a total of ~231 L of water were analysed in 2021-2022. All samples were negative for the presence of eDNA from noble crayfish, signal crayfish and *A. astaci* (**Figure 10**, **Table S8**). A few mortalities were observed in the cages during the trials in 2021 and 2022. These were not ascribed to crayfish plague, but to moulting and competition in the cages.

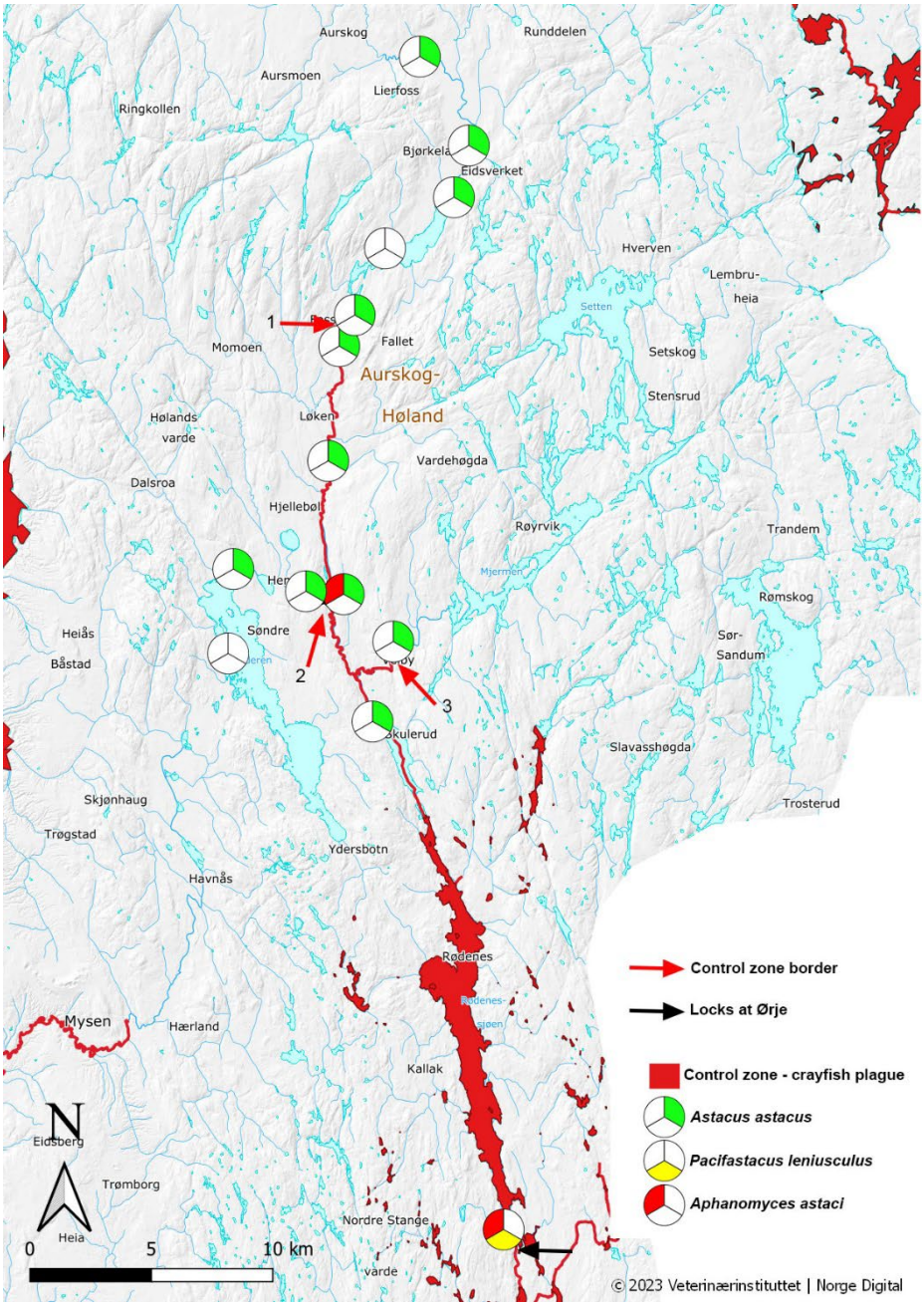

Figure 6 *Overview map of the surveyed part of the Halden watercourse region in 2022, starting from the Ørje locks (black arrow) in the south where signal crayfish is present. The control zone is indicated by red colour on involved lakes and rivers, and ends at Fosserdam, Daltorpsfoss and Lundfoss (red arrows 1, 2 and 3 respectively), where dams acts is artificial barriers for further spread. The pie chart indicates presence (colour) or absence (white) of* A. astaci *(red), signal crayfish (*P. leniusculus*; yellow), and noble crayfish (*A. astacus*; green). Presence is listed if at least one of the tested water samples yielded a positive eDNA result.*

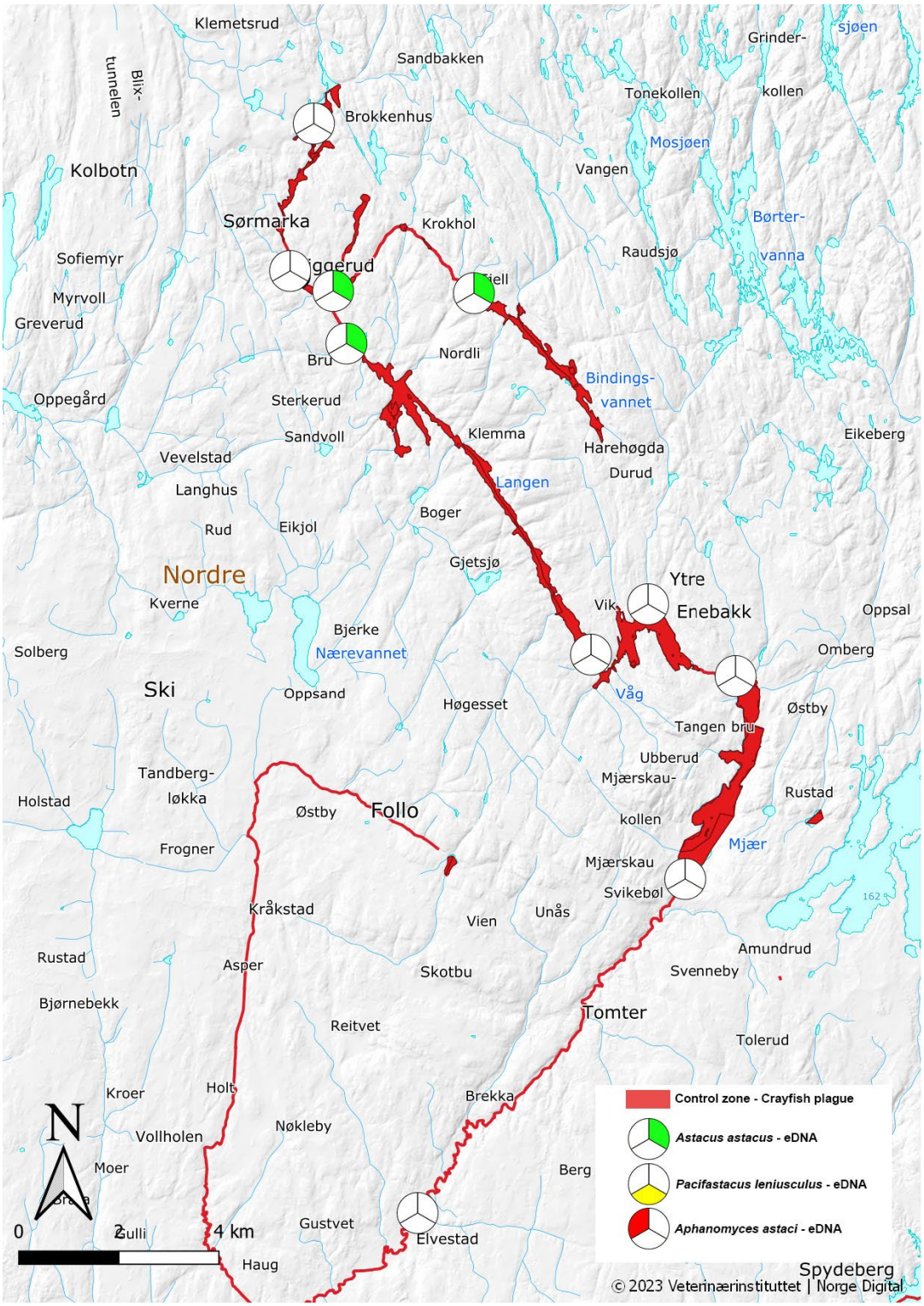

***Figure 7**. Overview map of the surveyed part of the Mosse watercourse in 2022. The control area is represented by red colour. The pie chart indicates presence (colour) or absence (white) of* A. astaci *(red), signal crayfish (*P. leniusculus*; yellow), and noble crayfish (*A. astacus*; green). Presence is listed if at least one of the tested water samples yielded a positive eDNA result. No eDNA of A. astaci and signal crayfish was detected.*

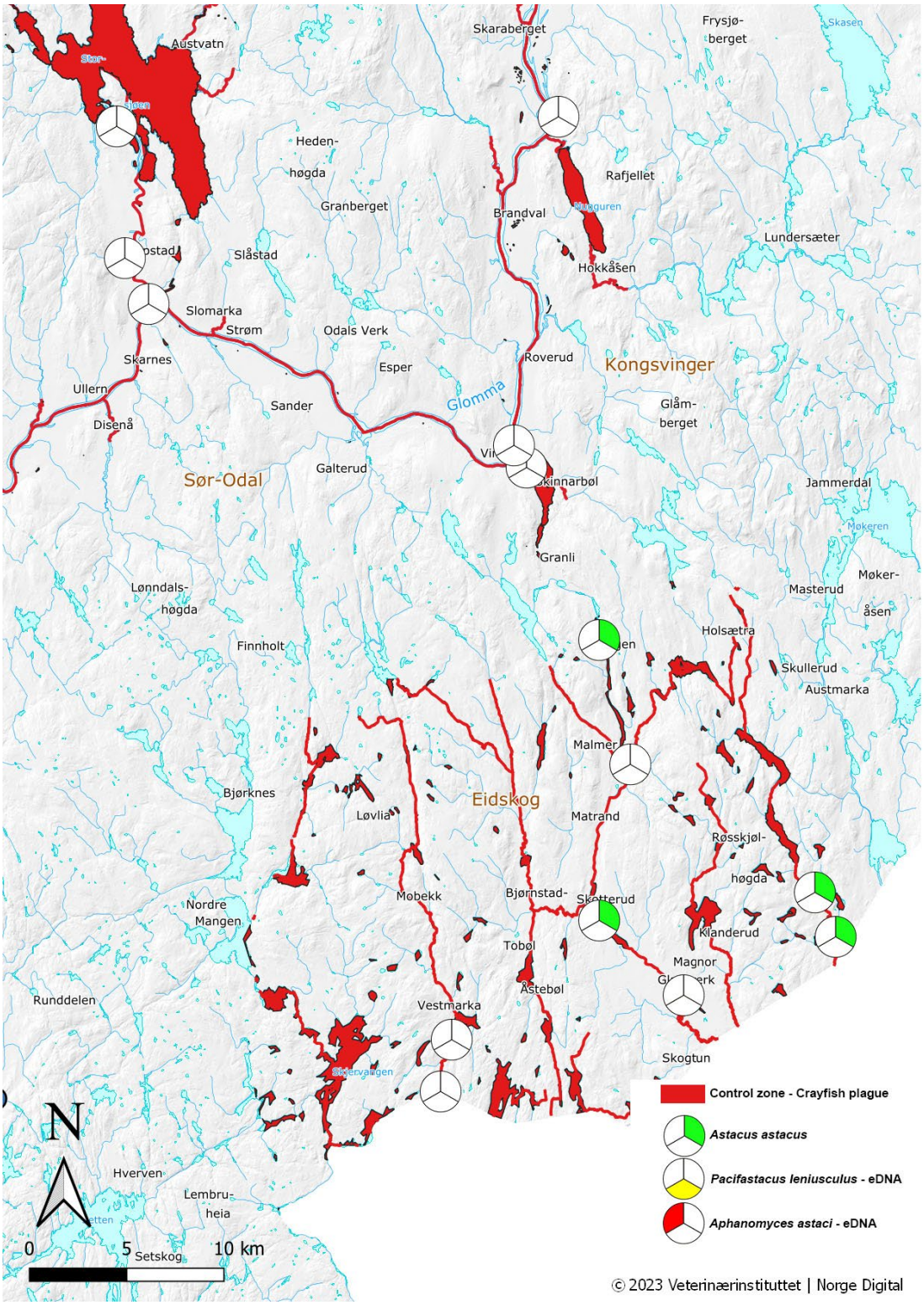

***Figure 8**. Overview map of the surveyed part of the Glomma watercourse region and Eidskog municipality in 2022. Regulated areas (crayfish plague control zones) are marked in red. For each location site, the pie chart indicates presence (colour) or absence (white) of* A. astaci *(red), signal crayfish (*P. leniusculus*; yellow), and noble crayfish (*A. astacus*; green). Presence is listed if at least one of the tested water samples yielded a positive eDNA result.*

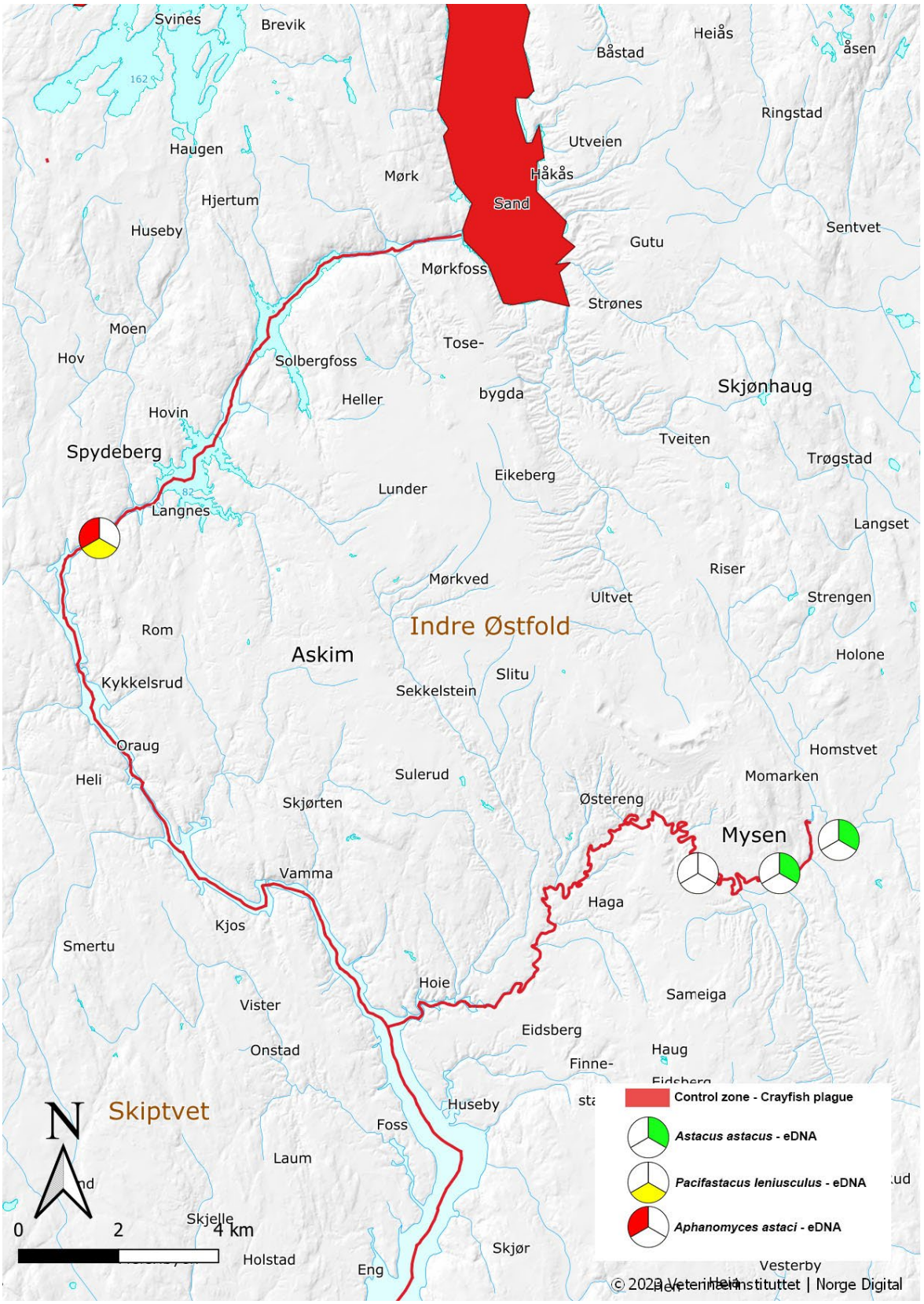

***Figure 9**. Overview map of the surveyed part of the southern part of Glomma watercourse and River Mysenelva in 2022. Regulated areas (crayfish plague control zones) are marked in red. For each location site, the pie chart indicates presence (colour) or absence (white) of* A. astaci *(red), signal crayfish (*P. leniusculus*; yellow), and noble crayfish (*A. astacus*; green). Presence is listed if at least one of the tested water samples yielded a positive eDNA result.*

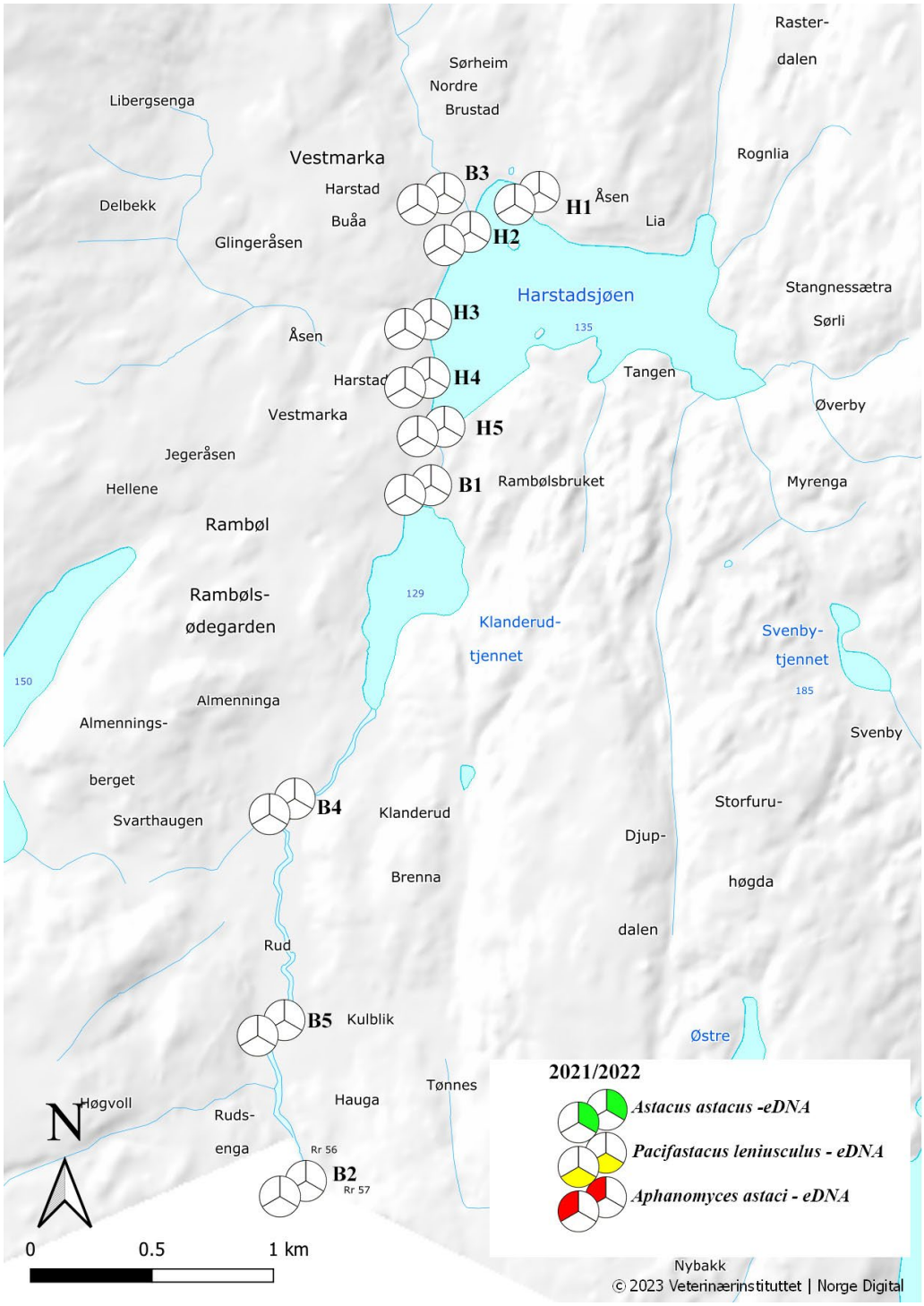

***Figure 10**. Overview map of the Buåa watercourse with results from 2021-2022. Regulated areas (crayfish plague control zones) are marked in red. For each location site, the pie chart indicates presence (colour) or absence (white) of* A. astaci *(red), signal crayfish (*P. leniusculus*; yellow), and noble crayfish (*A. astacus*; green). Presence is listed if at least one of the tested water samples yielded a positive eDNA result.*

Evaluation of disease freedom

Background

The Buåa watercourse drains from Norway to Sweden. In 2004, signal crayfish were discovered in the Buåa watercourse on the Swedish side of the border. Extensive trapping was carried out on both side of the border to investigate the distribution of signal crayfish in Buåa, after the discovery in 2004. The distribution was mainly limited to the lower parts of River Høgseterelva (Swedish name of the river Buåa) where it flows into Lake North Sea, and the trapping demonstrated that there was a low density of signal crayfish [24]. In order to prevent the spread of signal crayfish and crayfish plague into Norway, the NEA financed the construction of a migration barrier on the Swedish side of the border. This barrier was completed in 2007 [24]. However, crayfish plague wiped out the crayfish population in Buåa upstream the barrier in 2010, and *A. astaci* was detected in dead crayfish from cage trial in the watercourse. Diving surveys and trapping from the migration barrier up to Klanderudtjern and electro-fishing directly upstream of the migration barrier yielded no catch or observation of noble crayfish or signal crayfish in 2010. Control area regulation were imposed in the River Buåa and Lake Harstadsjøen in 2011 to combat crayfish plague and to reduce the risk of spread to other areas.

Guidelines for declaration of disease freedom from *Aphanomyces astaci*

No clear guidelines have been prepared for when a watercourse can be declared disease-free after a crayfish outbreak [8]). In the manual of diagnostics test for aquatic animals, WOAH 2021 [25], the only advice for wild crayfish stocks with regard to declaration of disease freedom is: “As movements of both finfish and crayfish stocks from infected waters present a risk of disease transmission, monitoring the status of crayfish populations to confirm that they remain healthy, is necessary”. Different countries have different practices, and in many European countries North American crayfish, which carry crayfish plague, are so widespread that declaration of disease freedom is not realistic [26]. In 2010, guidelines were proposed for declaration of disease freedom for crayfish plague locations, after eradication of signal crayfish at two smaller locations in Norway (see Johnsen et al., [26]). Based on these guidelines [26], the following guidelines is recommended in order to declare the Buåa watercource disease free from crayfish plague (*A. astaci*):

- No capture of signal crayfish during the 5-6 years after the outbreak of crayfish plague
- Cage trials using noble crayfish show no signs of illness or death due to crayfish plague during this period. It is sufficient to monitor with cages for three years, of which two years must be in the last years before declaration of disease freedom.
- eDNA investigations do not detect eDNA from *A. astaci* and/or signal crayfish during the 5-6 years after the outbreak of crayfish plague.
- Both cage trials and eDNA surveys must have been carried out during the last two years.

Surveillance in Buåa (2015-2022)

Until 2016, the Buåa water course was monitored by cages for more than 5 years. No crayfish plague has detected in these cage trials since 2011 [7]. Since 2016, environmental DNA monitoring has been conducted at two stations in Buåa, where water is analysed for the presence of eDNA from *A. astaci* (crayfish plague), signal crayfish and noble crayfish [11-15]. In

the period 2016 to 2020, no *A. astaci* or signal crayfish eDNA has been detected in Buåa, while eDNA from noble crayfish was detected only in 2019 [14]. Trapping was carried out in 2017 and 2020, and in 2020 one noble crayfish was caught in Buåa [19]. In 2021 and 2022, extensive eDNA sampling in combination with cage trial did not reveal any presence of *A. astaci* or signal crayfish (**Figure 10**, **Table S8**).

***Table 1**. Summary of the results from the various monitoring conducted in Buåa watercourse. CP = crayfish plague, SC = signal crayfish and NC = noble crayfish. "+/-" indicates detections/no detection.*

	2015			2016			2017			2018			2019			2020			2021			2022		
	CP	*SC*	*NC*	*CP*	*SC*	*NC*	*CP*	*SC*	*NC*	*CP*	*SC*	*NC*	*CP*	*SC*	*NC*	*CP*	*SC*	*NC*	*CP*	*SC*	*NC*	*CP*	*SC*	*NC*
Cage trials	-			-															-			-		
Trapping								-	-								-	+						
eDNA sampling				-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-

Recommendation for declaration of disease freedom

None of the eDNA samples collected in Buåa during the surveillance period 2016-2020 or during the extensive eDNA survey in 2021-2022 were positive for signal crayfish or *A. astaci* eDNA (**Table 1**). No signal crayfish were caught in the trapping conducted in 2017 and in 2020 [19]. Additionally, the cage trials in 2011-2016 and 2021-2022 did not detect *A. astaci* in the watercourse. Thus, the absence of detection of signal crayfish and *A. astaci* in the monitoring period 2011-2020 and in the expanded monitoring effort in 2021-2022, strongly suggest the total absence of both organisms in River Buåa and Lake Harstadsjøen.

The guidelines and criteria for declaration of disease freedom from *A. astaci* (crayfish plague) is met. NVI therefore recommend that the Buåa watercourse (River Buåa and Lake Harstadsjøen) is declared free from infection of the crayfish plague pathogen *A. astaci*.

Conclusion

In the Halden watercourse, combined eDNA monitoring of *A. astaci*, noble crayfish and signal crayfish confirmed that signal crayfish present in Lake Rødenessjøen release detectable, but low concentrations of *A. astaci* to the water. The detection of *A. astaci* in the middle part of River Hølandselva indicates that the pathogen is still present there in low abundance, and that it is slowly spreading upstream. There was no detection of *A. astaci* in the northern part of River Hølandselva or in any of the stations in the neighbouring risk areas indicating that the outbreak is limited to the lower part of River Hølandselva. This is also supported by detection of noble crayfish eDNA at most of the stations upstream. The single positive detection of signal crayfish eDNA in Lierelva in 2021 was not confirmed in 2022 in spite of increased sampling (three sites) in the river.

No eDNA samples were positive for *A. astaci* in the Mosse watercourse in 2022. While the crayfish plague reached Lake Langen in 2018, detection of noble crayfish eDNA upstream of the lake indicates no further spread.

In the Glomma watercourse, no *A. astaci* or signal crayfish eDNA was detected upstream Solbergfoss. The status is still uncertain, given many years of recurrent crayfish plague detection in cage experiments up until 2015. However, the results indicate that our sampling effort has not been sufficient to reveal a suspected infection source in the watercourse upstream Solbergfoss. eDNA from *A. astaci* or *signal crayfish* eDNA was detected at Fossum bridge, at the location where signal crayfish were discovered in 2020 downstream of Solbergfoss. Positive eDNA results for noble crayfish were obtained in 2016 and in 2021 (at Opstadåa) and 2019 (at Skarsnes and Vingersnoret), but not in 2017, 2018, 2020 or 2022.

Noble crayfish eDNA was detected in the risk area in River Mysenelva, and at one site in the control zone. No eDNA from *A. astaci* or signal crayfish were detected in the river. However, the unspecific amplification below the detection limit of one samples, raises concern about the possibility of the upstream spread of *A. astaci* (into the risk area) and this should be followed up in 2023.

We found no sign of *A. astaci* in any of the monitored sites in Eidskog municipality. Similar to the results of 2017-2021, noble crayfish eDNA was detected at several of the monitored sites in the Vrangselva watercourse and River Finnsrudelva. This supports the view that the crayfish plague has still not yet entered the Norwegian side of these river systems and suggests the presence of live noble crayfish in both systems. The expanded survey in Buåa in 2021 or 2022 did not reveal any *A. astaci* or signal crayfish, supporting the recommendation of declaring disease freedom in the watercourse.

In summary, eDNA from *A. astaci* was not detected anywhere else than in the control zone within the Halden watercourse and the Glomma watercourse. The follow up of River Lierelva did not detect any eDNA from signal crayfish. The frequent detections of noble crayfish eDNA within the regulated *A. astaci* control and risk areas of the Halden watercourse, Mosse watercourse, and the rivers Vrangselva and Finnsrudelva in Eidskog, suggest the presence of vital noble crayfish populations within several of the *A. astaci* regulated and restricted zones.

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Appendix

Supplementary information to the report "The surveillance programme for *Aphanomyces astaci* in Norway 2022" - Tables S1 – S8.

***Table S1**. Agreed areas and locations of the "NOK A. astaci 2022" program.*

Location	Watercourse[1]/ municipality, county[2]	Location infection status	# water samples (site X samples X visits)
Halden watercourse			**Total samples 56**
Rødenessjøen	HW/Marker, V	Control zone	4 (1 x 2 x 2)
Hølandselva	HW/Aurskog-Høland, V	Control zone	12 (3 x 2 x 2)
Fossersjøen	HW/Aurskog-Høland, V	Control zone, outbreak expected	4 (1 x 2 x 2)
Fosserdam	HW/Aurskog-Høland, V	Risk area/control zone border	4 (1 x 2 x 2)
Bjørkelangen	HW/Aurskog-Høland, V	Risk area	4 (1 x 2 x 2)
Lierelva	HW/Aurskog-Høland, V	Risk area	12 (3 x 2 x 2)
Lundsfoss	HW/Aurskog-Høland, V	Risk area	4 (1 x 2 x 2)
Dalstorpsfoss	HW/Aurskog-Høland, V	Risk area	4 (1 x 2 x 2)
Hemnessjøen	Lake/Aurskog-Høland, V	Risk area	8 (2 x 2 x 2)
Glomma watercourse			**Total samples 28**
Oppstadåa	GW/Sør-Odal, I	Control zone	8 (2 x 2 x 2)
Skarsnes	GW/ Sør-Odal, I	Control zone	4 (1 x 2 x 2)
Vingersnoret	GW/ Sør-Odal, I	Control zone	4 (1 x 2 x 2)
Vingersjøen	GW/ Sør-Odal, I	Control zone	4 (1 x 2 x 2)
Glomma, Fossum	GV/Indre Østfold, V	Control zone	4 (1 x 2 x 2)
Glomma, Strategic	GV/Indre Østfold, V	Control zone	4 (1 x 2 x 2)
River Mysenelva			**Total samples 12**
Upstream Rustadf.	RM/Indre Østfold, V	Risk area	4 (1 x 2 x 2)
Downstr. Rustadf.	RM/Indre Østfold, V	Control zone	4 (1 x 2 x 2)
Downstr. Susebakk.	RM/Indre Østfold, V	Control zone	4 (1 x 2 x 2)
Eidskog			**Total samples 24**
Buåa	BW/Eidskog, I	Control zone	See Table S2
Finnsrudelva	RF/Eidskog, I	Control zone	8 (2 x 2 x 2)
Vrangselva	VW/Eidskog, I	Control zone	16 (4 x 2 x 2)
Mosse watercourse			**Total samples 40**
Hobølelva	MV/Enebakk, V	Control zone	4 (1 x 2 x 2)
Mjær	MV/Enebakk, V	Control zone	4 (1 x 2 x 2)
Tangenelva	MV/Enebakk, V	Control zone	4 (1 x 2 x 2)
Våg	MV/Enebakk, V	Control zone	4 (1 x 2 x 2)
Langen	MV/Enebakk, V	Control zone	8 (2 x 2 x 2)
Upstream Langen	MV/Enebakk, V	Control zone	16 (4 x 2 x 2)
Total			**160**

[1] HW = Halden watercourse, GW = Glomma watercourse, MW = Mosse-watercourse, BW = Buåa watercourse, RF = River Finnsrudelva, VW = Vrangselva watercourse
[2] I = Innlandet, V = Viken

Table S2. *Agreed areas and locations for evaluation of disease freedom in Buåa, 2021 and 2022.*

Location	Watercourse[1]/ municipality, county[2]	Location infection status	# water samples (site X samples X visits)
Buåa (B) og Harstadsjøen (H)			**Totalt 30 vannprøver**
B1 – v/ Montessori	B, Eidskog, I	Control zone	4 (1 x 2 x 2)
B2 – v/ National border	B, Eidskog, I	Control zone	4 (1 x 2 x 2)
B3- v/ bru oppstrøms Harstadsjøen	B, Eidskog, I	Control zone	4 (1 x 2 x 2)
B4 – v/ bru nedstrøms Klanderudtjern	B, Eidskog, I	Control zone	4 (1 x 2 x 2)
B5 – v/ Kulblik	B, Eidskog, I	Control zone	4 (1 x 2 x 2)
H1 – v/Brustadvika	H, Eidskog, I	Control zone	2 (1 x 1 x 2)
H2 – nord for utløp Buåa	H, Eidskog, I	Control zone	2 (1 x 1 x 2)
H3 – v/ Judinbakken	H, Eidskog, I	Control zone	2 (1 x 1 x 2)
H4 – v/ badehuset	H, Eidskog, I	Control zone	2 (1 x 1 x 2)
H5 – v/ utløp sør	H, Eidskog, I	Control zone	2 (1 x 1 x 2)

[1]B = Buåa, H = Harstadsjøen, I = Innlandet

Table S3. *Locations for water sampling in the Halden watercourse area with corresponding location and sample information. eDNA results are listed for crayfish plague, noble crayfish and signal crayfish.*

Location[1]	Location details			Water samples[2]		# eDNA positive samples[3]					
						May			August		
	ID	S[1]	GPS coordinates	#	L	*CP*	*SC*	*NC*	*CP*	*SC*	*NC*
Lierelva, Lierfoss		R	59°55'04"N 11°32'18"E	4	20,0	0	0	2	0	0	2
Lierelva, Bjørkelagen	HA1	R	59°53'8"N 11°34'29"E	4	14,0	0	0	2	0	0	2
Lierelva, Utløp		R	59°52'12"N 11°33'53"E	4	11,5	0	0	2	0	0	0
Bjørkelangen	HA2	R	59°50'55"N 11°31'5"E	4	20,0	0	0	0	0	0	0
Fosserdam	HA3	R	59°49'17"N 11°29'27"E	4	18,6	0	0	0	0	0	1
Fossersjøen	HA4	C	59°48'58"N 11°29'32"E	4	9,5	0	0	1	0	0	2
Lundsfoss	HA5	R	59°42'7"N 11°32'14"E	4	19,5	0	0	2	0	0	2
Hemnessjøen pier	HA6	R	59°41'47"N 11°25'7"E	4	18,5	0	0	0	0	0	0
Hemnessjøen outlet	HA7	R	59°43'31"N 11°25'11"E	4	15,0	0	0	2	0	0	2
Daltorpsfoss	HA8	R	59°43'13"N 11°28'49"E	4	15,8	0	0	2	0	0	2
Hølandselva north	HA9	C	59°46'7"N 11°29'8"E	4	13,5	0	0	2	0	0	2
Hølandselva middle	HA14	C	59°43'13"N 11°29'31"E	4	14,9	0	0	0	1	0	2
Hølandselva outlet	HA10	C	59°40'30"N 11°31'50"E	4	16,6	0	0	2	0	0	2
Rødenessjøen Ysterud	HA12	C	59°29'17"N 11°38'23"E	4	20,0	1	2	0	0	2	0
Total				56	227,4	1	2	17	1	2	19

[1] C = Crayfish plague control zone, R = risk area
[2] # = Total number of water samples (May & August summarized), L = total water volume summarized for all samples
[3] Number of samples in May and August with positive detection of eDNA from crayfish plague (CP), noble crayfish (NC), and signal crayfish (SC).

***Table S4**. Locations for water sampling in Mosse-watercourse area with corresponding location and sample information. eDNA results are listed for crayfish plague, noble crayfish and signal crayfish.*

Location	Location details			Water samples[2]		# eDNA positive samples[3]					
						May			August		
	ID	S[1]	GPS coordinates	#	L	*CP*	*SC*	*NC*	*CP*	*SC*	*NC*
Bindingsvann, outlet	MO11	C	59°47'22.1"N 10°57'17.6"E	4	14,3	0	0	2	0	0	2
Tangentjern, inlet, bridge on brusagav.	MO12	C	59°47'18.2"N 10°54'02.9"E	4	12,0	0	0	2	0	0	2
Sværsvann	MO8	C	59°49'03.2"N 10°53'25.3"E	4	18,5	0	0	0	0	0	0
Tangentjern, inlet, bridge on Hareveien	MO10	C	59°47'25.7"N 10°53'27.5"E	4	16,0	0	0	0	0	0	0
Langen, inlet, bridge on Bru-fjellv.	MO9	C	59°46'44.7"N 10°54'38.6"E	4	8,1	0	0	1	0	0	0
Langen, bridge on Skiveien	MO1	C	59°43'33.3"N 11°00'12.1"E	4	20,0	0	0	0	0	0	0
Våg	MO2	C	59°44'10.2"N 11°01'14.7"E	4	17,0	0	0	0	0	0	0
Tangenelva, bridge on Tomterveien	MO5	C	59°43'19.9"N 11°03'18.9"E	4	20,0	0	0	0	0	0	0
Mjær, outlet	MO6	C	59°41'10.2"N 11°02'27.6"E	4	19,5	0	0	0	0	0	0
Hobølelva, Elvestad	MO7	C	59°37'26.5"N 10°57'09.2"E	4	19,0	0	0	0	0	0	0
Total				40	164	0	0	5	0	0	4

[1] C = Crayfish plague control zone, R = risk area
[2] # = Total number of water samples (May & August summarized), L = total water volume summarized for all samples
[3] Number of samples in May and August with positive detection of eDNA from crayfish plague (CP), noble crayfish (NC), and signal crayfish (SC).

***Table S5**. Locations for water sampling in the Glomma region with corresponding location and sample information. eDNA results are listed for crayfish plague, noble crayfish and signal crayfish*

Location	Location details			Water samples[2]		# eDNA positive samples[3]					
						May			September		
	ID	S[1]	GPS coordinates	#	L	*CP*	*SC*	*NC*	*CP*	*SC*	*NC*
Vingersnoret	GL1	C	60°11'36.3"N 12°01'54.5"E	4	13,9	0	0	0	0	0	0
North of Vingersnoret	GL2	C	60°11'39.7"N 12°01'41.2"E	4	20,0	0	0	0	0	0	0
Storsj. Ringåsvn. pier	GL5	C	60°20'18.4"N 11°38'36.5"E	4	16,5	0	0	0	0	0	0
Oppstadåa south	GL9	C	60°16'40.3"N 11°39'06.9"E	4	17,0	0	0	0	0	0	0
Glomma, Skarnes	GL10	C	60°15'20.8"N 11°40'49.4"E	4	20,0	0	0	0	0	0	0
Glomma, Hvebergåa		C	60°21'11.5"N 12°03'06.0"E	4	15,8	0	0	0	0	0	0
Glomma, Fossum		C	59°36'09.9"N 11°06'11.6"E	4	20,0	0	0	0	1	1	0
Total				28	123	0	0	0	1	1	0

[1] C = Crayfish plague control zone
[2] # = Total number of water samples (May & September summarized), L = total water volume summarized for all samples
[3] Number of samples in June and September with positive detection of eDNA from crayfish plague (CP), noble crayfish (NC), and signal crayfish (SC).

***Table S6**. Locations for water sampling in the River Mysenelva with corresponding location and sample information. eDNA results are listed for crayfish plague, noble crayfish and signal crayfish.*

Location	Location details			Water samples[2]		# eDNA positive samples[3]					
						May			August		
	ID	S[1]	GPS coordinates	#	L	*CP*	*SC*	*NC*	*CP*	*SC*	*NC*
Mysenelva Ramstad	MY1	R	59°33'22.1"N 11°22'09.0"E	4	16,4	0	0	2	0	0	2
Mysenelva Susebakkefossen	MY2	C	59°32'59.3"N 11°21'07.7"E	4	19,5	0	0	0	0	0	1
Mysenelva Kapellveien	MY3	C	59°32'58.8"N 11°19'23.8"E	4	20,0	0	0	0	0	0	0
Total				12	55,9	0	0	2	0	0	3

[1] C = Crayfish plague control zone
[2] # = Total number of water samples (May & August summarized), L = total water volume summarized for all samples
[3] Number of samples in May and August with positive detection of eDNA from crayfish plague (CP), noble crayfish (NC), and signal crayfish (SC).

***Table S7**. Locations for water sampling in the Eidskog region with corresponding location and sample information. eDNA results are listed for crayfish plague, noble crayfish and signal crayfish.*

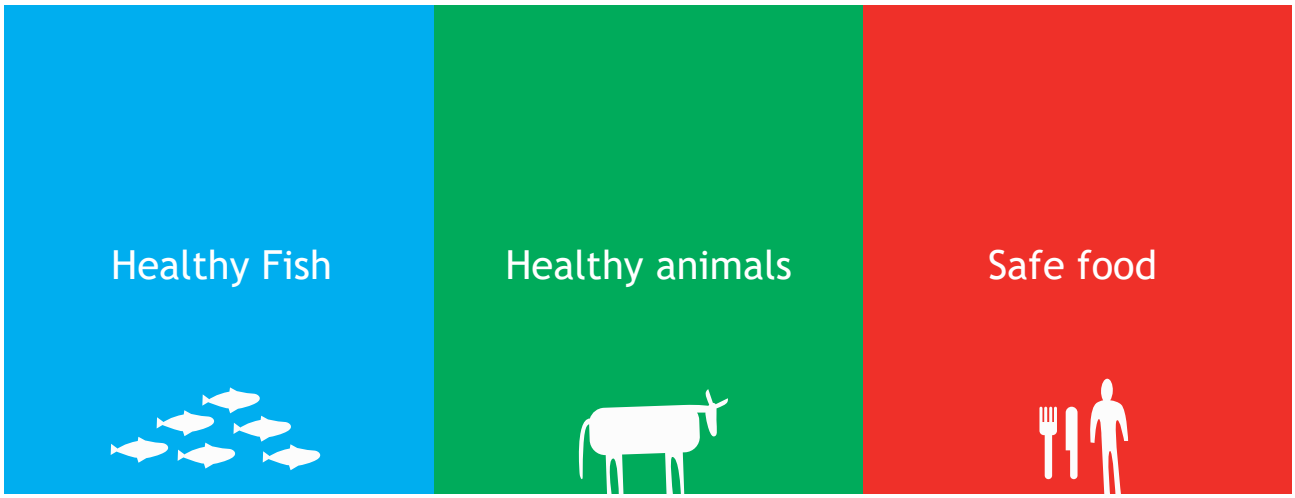
Location	Location details			Water samples[2]		# eDNA positive samples[3]					
						June			September		
	ID	S[1]	GPS coordinates	#	L	*CP*	*SC*	*NC*	*CP*	*SC*	*NC*
Vrangselva, Åbogen	VR1	C	60°06'43.6"N 12°07'01.0"E	4	20,0	0	0	2	0	0	2
Søndre Åklangen, Badeplass	VR2	C	60°03'12.8"N 12°08'20.8"E	4	20,0	0	0	0	0	0	0
Vrangselva, Skotterud	VR3	C	59°58'53.8"N 12°07'19.1"E	4	12,7	0	0	1	0	0	0
Vrangselva, Magnor bad	VR4	C	59°57'02.7"N 12°11'58.8"E	4	12,2	0	0	0	0	0	0
Finnsrudelva, Finnsrudvegen	FR1	C	59°59'50.7"N 12°19'05.4"E	4	20,0	0	0	2	0	0	2
Finnsrudelva, Billavegen	FR2	C	59°58'44.9"N 12°20'14.2"E	4	20,0	0	0	2	0	0	2
Total				24	105	0	0	7	0	0	6

[1] C = Crayfish plague control zone
[2] # = Total number of water samples (June & September summarized), L = total water volume summarized for all samples
[3] Number of samples in June and September with positive detection of eDNA from crayfish plague (CP), noble crayfish (NC), and signal crayfish (SC).

***Table S8**. Locations for water sampling in River Buåa and Lake Harstadsjøen with corresponding location and sample information. eDNA results are listed for crayfish plague, noble crayfish and signal crayfish.*

Location	Location details			Water samples[2]		# eDNA positive samples[3]						# eDNA positive samples[3]					
						June 2021			September 2021			June 2022			September 2022		
	ID	S[1]	GPS coordinates	#	L	*CP*	*NC*	*SC*	*CP*	*NC*	*SC*	*CP*	*NC*	*SC*	*CP*	*NC*	*SC*
Harstadsjøen, North	H1	C	59°56'10.0"N 11°59'55.5"E	6,00	23,3	0	0	0	0	0	0	0	0	0	0	0	0
Harstadsjøen, Brustadvika	H2	C	59°56'04.8"N 11°59'43.3"E	7,00	24,3	0	0	0	0	0	0	0	0	0	0	0	0
Harstadsjøen, Judinbakken	H3	C	59°55'53.3"N 11°59'33.9"E	6,00	21,8	0	0	0	0	0	0	0	0	0	0	0	0
Harstadsjøen, Badehytta	H4	C	59°55'44.9"N 11°59'35.0"E	6,00	21,8	0	0	0	0	0	0	0	0	0	0	0	0
Harstadsjøen, Utløp Os	H5	C	59°55'37.8"N 11°59'39.3"E	6,00	19,9	0	0	0	0	0	0	0	0	0	0	0	0
Buåa, Innlet Harstadsjøen	BU3	C	59°56'09.3"N 11°59'37.2"E	7,00	34,2	0	0	0	0	0	0	0	0	0	0	0	0
Buåa, Montesorri	BU1	C	59°55'31.1"N 11°59'37.0"E	8,00	38,1	0	0	0	0	0	0	0	0	0	0	0	0
Buåa, Klanderud	BU4	C	59°54'48.3"N 11°59'03.2"E	8,00	16,5	0	0	0	0	0	0	0	0	0	0	0	0
Buåa, Kulblikveien	BU5	C	59°54'19.4"N 11°59'04.3"E	7,00	12,2	0	0	0	0	0	0	0	0	0	0	0	0
Buåa, Riksgrense	BU2	C	59°53'56.4"N 11°59'12.0"E	9,00	18,6	0	0	0	0	0	0	0	0	0	0	0	0
Total				70	230,7	0	0	0	0	0	0	0	0	0	0	0	0

[1] C = Crayfish plague control zone
[2] # = Total number of water samples (June & September summarized), L = total water volume summarized for all samples
[3] Number of samples in June and September with positive detection of eDNA from crayfish plague (CP), noble crayfish (NC), and signal crayfish (SC).



Scientifically ambitious, forward-looking and collaborative- for one health!

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