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The surveillance programme for *Aphanomyces astaci* in Norway 2024

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The surveillance programme for *Aphanomyces astaci* in Norway 2024

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SEM photo of *Aphanomyces astaci* hyphae and spore ball.
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Content

Summary

This surveillance programme for *Aphanomyces astaci* uses environmental DNA (eDNA) monitoring for detection of A. *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 evaluate the habitat status. Detection of noble crayfish eDNA, combined with the absence of eDNA from A. *astaci* and signal crayfish, support the presence of non-infected noble crayfish which constitutes the desired habitat status. In 2024 eDNA from A. *astaci* was detected within the restriction zone in Lake Rødnessjøen in the Halden watercourse, and within the restriction zone in River Hæra. The active outbreak of crayfish plague in River Hæra spread upstream into River Dalselva during the summer, but remained within the restriction zone. Illegally introduced signal crayfish was discovered in Lake Øyern, a large lake in the Glomma watercourse, after notification from the locals. Tissue samples from the signal crayfish was analysed and confirmed A. *astaci* positive. Frequent detections of noble crayfish eDNA within the regulated A. *astaci* restriction zones of the Halden watercourse, Mosse watercourse, and the rivers Vrangselva and Finnsrudelva in Eidskog, suggest the presence of vital noble crayfish populations within A. *astaci* restriction zones.

Sammendrag

Overvåkningsprogrammet for krepsepest benytter miljø-DNA overvåking for påvisning av A. *astaci* sporer (krepsepest agens) direkte fra filtrerte vannprøver. Tilstedeværelse/fravær av miljø-DNA fra edelkreps (*Astacus astacus*) og signalkreps (*Pacifastacus leniusculus*) undersøkes også for å evaluere habitat status. Påvisning av miljø-DNA for edelkreps, kombinert med fravær av miljø-DNA fra A. *astaci* og signalkreps, støtter tilstedeværelsen av ikke-infisert edelkreps som utgjør ønsket habitatstatus. I 2024 ble det påvist miljø-DNA fra A. *astaci* innenfor restriksjonssonen i Rødnessjøen i Haldenvassdraget, og innenfor restriksjonssonen i Hæra. Det aktive krepsepestutbruddet i Hæra spredte seg oppstrøms til Dalselva i løpet av sommeren, men forble innenfor restriksjonssonen. Etter varsling fra lokalbefolkningen ble ulovlig utsatt signalkreps funnet i Øyeren, en stor innsjø i Glommavassdraget. Det ble påvist A. *astaci* i vevsprøver fra signalkrepsen. Flere påvisninger av miljø-DNA fra edelkreps innenfor de regulerte restriksjonssonene for bekjempelse av krepsepest i Haldenvassdraget, Mossevassdraget og elvene Vrangselva og Finnsrudelva i Eidskog, tyder på tilstedeværelse av vitale edelkrepsbestander innenfor restriksjonssonene.

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 horizontally via 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 (*Pacifastacus leniusculus*), red swamp crayfish (*Procambarus clarkii*) and spiney-cheek crayfish *(Faxonius limosus*).

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 River Vrangselva 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 Hæra (2021). In 2016, crayfish plague was confirmed in noble crayfish inhabiting the bordering River 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, five 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 (Telemark), Ostøya (Akershus), The Fjelna watercourse (Trøndelag), Lake Kvesjøen (Trøndelag) and Lake Østersjøen (Innlandet) where signal crayfish were discovered in 2006, 2009, 2011, 2013 and 2023 respectively [4, 5, 7, 8]. At two of these locations (Dammane and Ostøya), signal crayfish have been successfully eradicated, and the areas were declared disease free [4]. In addition, Illegal A. *astaci* positive signal crayfish have also been discovered at numerous locations in the Halden and Glomma watercourses in recent years (see below).

Halden Watercouse (under regulation FOR-2015-05-26-592)

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 [9]. Immediate closure of the Ørje water locks prevented upstream spread to Lake Rødenessjøen. Illegally introduced A. *astaci* positive signal crayfish were found in Lake Øymarksjøen in 2008 [10], 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 [11]. By august 2015 the outbreak had also spread throughout the adjacent Lake to the outlet of River Hølandselva. Repeated eDNA monitoring in the period 2016-2023 [6, 7, 12-17] has revealed that A. *astaci* is still present in low amounts in River Hølandselva, but has not spread upstream the restriction zone (Appendix A, Figure S1). Illegal translocation of signal crayfish within the watercourse has occurred, and signal crayfish is present in Lake Rødnessjøen, Lake Øymarksjøen, Lake Aremarksjøen, Lake Aspern and Lake Femsjøen [18].

The Mosse watercourse (under regulation FOR-2016-12-13-1523)

The Mosse watercourse was struck by crayfish plague in 2016 and eDNA analyses pinpointed an active outbreak to River Tangenelva upstream of Lake Mjærvann and River Hobølelva [7]. No A. *astaci* eDNA was detected in the Mosse watercource in 2017, but there was a significant drop in eDNA detection of noble crayfish from June to August in Lake Våg [12]. A dead crayfish found in Lake Langen in 2018 was diagnosed with crayfish plague,

confirming the upstream spread of crayfish plague in the watercourse [13]. No A. *astaci* has been detected in the watercourse in 2019-2023 [6, 14-17].

The Glomma watercourse (under regulation FOR-2005-06-20-652)

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 returned in 2003. Cage experiments combined with crayfish plague diagnostics confirmed active crayfish plague in the system from 2005 until 2015 [4, 5, 7]. No A. *astaci* eDNA has been detected at the monitored locations in the Glomma watercourse from Hvebergåa to Skarsnes, nor in the outlet of Lake Vingersjøen or Oppstadåa in the period 2016-2023 [6, 7, 12-17]. A. *astaci* positive signal crayfish was discovered in Glomma at Fossum bridge, downstream Solbergfoss in 2020 [18, 19] and A. *astaci* positive signal crayfish was discovered in Lake Østersjøen in 2023 [17]. Lake Østersjøen is connected to the Glomma watercourse via the outlet River Gjesa, River Julussa and River Rena into River Glomma.

Eidskog municipality (under regulation FOR-2016-08-17-972)

The Buåa system was struck by crayfish plague in 2010 caused by the presence of signal crayfish on the Swedish side of the river [20]. A barrier built to prevent the spread of signal crayfish did not stop the infection from spreading, but hopefully stopped the signal crayfish [4]. There has been no detection of A. *astaci* with cage experiments (2011-2016 [7]) or eDNA monitoring (2017-2023 [6, 12-15, 17]). Neither has signal crayfish been detected on the Norwegian side of the river using eDNA or trapping [16]. 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 2016-2023 [6, 7, 12-17], nor during the extensive monitoring during 2021 and 2022 to evaluate the watercourse for disease freedom [16].

River Hæra (under regulation FOR-2005-06-20-652)

River Hæra, which drains into Glomma was struck by crayfish plague in 2021 [6]. A. *astaci* was detected molecularly from dead crayfish found in the river. The outbreak was limited to the river downstream of Rustadfossen, where a constructed dam could act as a barrier. In august 2022 , two eDNA samples amplified for A. *astaci* at the station upstream Rustadfossen in the risk area, but below the detection limit [16]. In June 2023, A. *astaci* eDNA was detected in water samples collected at one station upstream Rustadfossen, indicating a crayfish plague outbreak. Additional trapping and eDNA sampling in 2023 confirmed an active crayfish plague outbreak in the river, with a slow upstream spread during 2023 [17].

Aims

The surveillance programme for A. *astaci* is based on eDNA monitoring and 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 restriction zones[1]).
- Substantiate disease free waterbodies in neighbouring areas of the restriction zones (referred to as risk areas[2]).
- To evaluate the habitat status of the monitored areas by screening all collected samples for noble crayfish and signal crayfish eDNA.
- Alert the authorities of eventual spread of the disease from restriction zones to risk areas.

The surveillance programme cooperates with the National surveillance programme for noble crayfish and spread of signal crayfish that is commissioned by the Norwegian Environment Agency (NEA) and coordinated by the Norwegian Institute of Nature Research (NINA).

Materials and methods

Surveillance sites

The main areas for surveillance include the Halden watercourse and surrounding areas, the Mosse watercourse, the Glomma watercourse, River Gjesa and River Julussa downstream Lake Østersjøen, River Hæra, and rivers in Eidskog municipality, including the Buåa watercourse, River Vrangselva and River Finnsrudelva. A map of the 46 water sampling stations, as well as the crayfish plague restriction zones, are displayed in Figure 1. Supplementary details are summarised in Appendix 1 (Table S1-S6).

eDNA sampling

Water filter samples were collected in June and August or September. From each site, two samples of up to 5 L water were filtered through sterile glass fibre filters on-site [11]. 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 single-use, sterile forceps to separately marked 15 ml falcon tubes with ATL-buffer. DNA was extracted using a NucleoSpin Plant II Midi kit (Marcherey-Nagel) protocol [21, 22]. The extracted DNA samples were screened by qPCR for three DNA targets: the species-specific qPCR assay for A. *astaci* [11, 23] and two crayfish species-specific qPCR assays for noble crayfish and signal crayfish [24].

[1] The «restriction 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 restriction zones covered by the regulations. These areas host healthy noble crayfish populations that face a high risk for spread of the infection from the restriction zones.

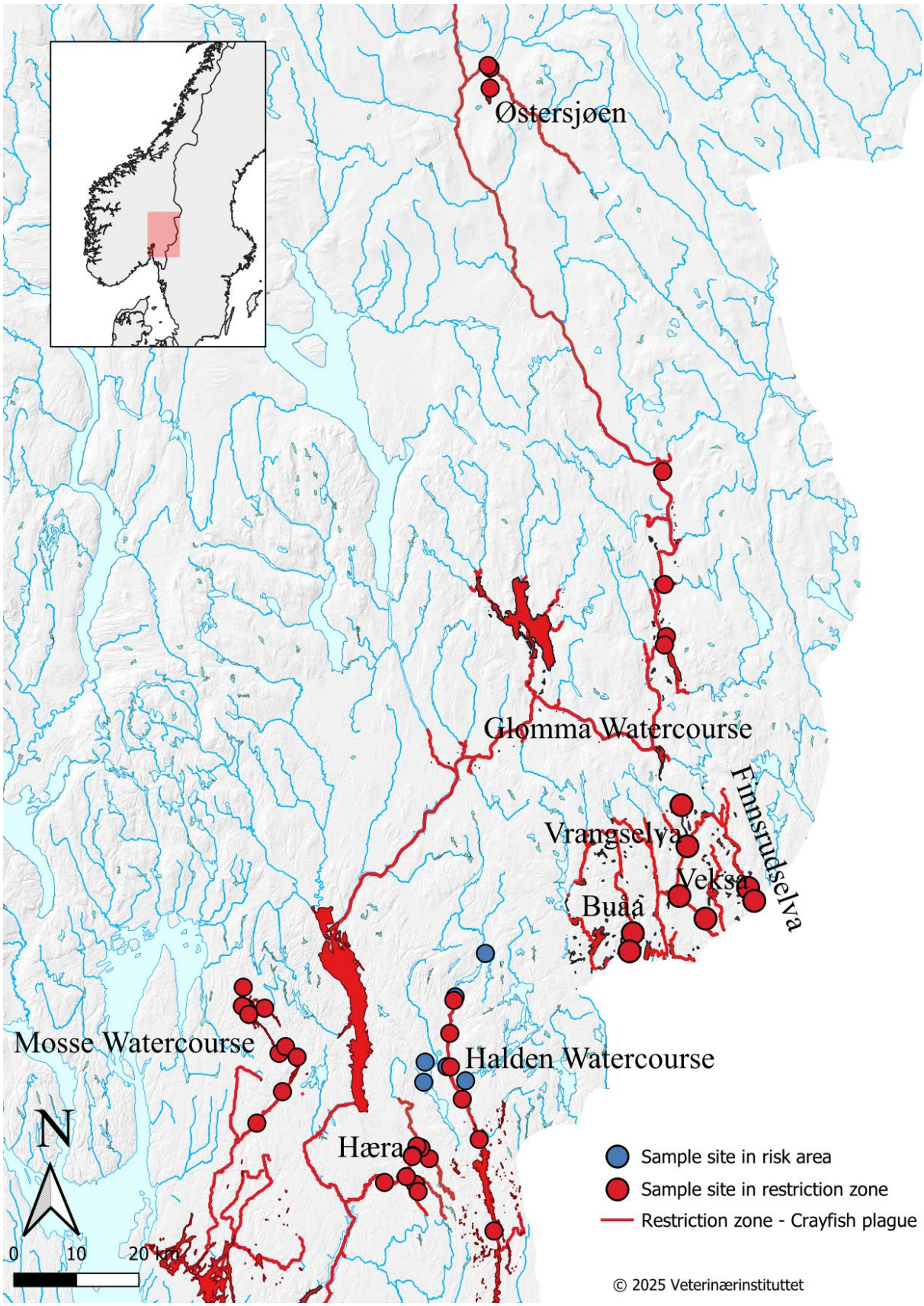

Figure 1. Surveillance sites in South-Eastern Norway 2024. Water samples (circles) were collected in June and August or September. Regulated areas (crayfish plague restriction zones) are marked in red. Note: For Glomma, the restriction zone is an approximation.

Result and Discussion

eDNA monitoring in the Halden watercourse

From the Halden watercourse region, a total of 48 water samples were collected from 12 stations during the sampling in June and August 2024. In the restriction zone, eDNA from A. *astaci* and signal crayfish was detected at the Southern part of Lake Rødenessjøen (Figure 2, Table S2). eDNA from A. *astaci* was detected at low levels in the River Hølandselva in June. Noble crayfish eDNA was detected in 15 water samples from River Hølandselva and upstream (within the restriction zone). The positive detections of noble crayfish eDNA in samples from River Hølandselva and upstream (within the restriction zone) (Figure 2) support the presence of live noble crayfish inhabiting the northern part of the Halden watercourse restriction zone. All water samples from the six stations in the risk area surrounding Halden watercourse were negative for A. *astaci* eDNA and signal crayfish, while most samples (17 samples) were positive for noble crayfish eDNA (Figure 2, Table S2), suggesting the presence of infection free noble crayfish population within most of the monitored risk area. While signal crayfish eDNA was detected in one sample from River Lierelva in 2021, this detection has not been confirmed by the extended sampling in 2022 [16] or during the survey in 2023 [17] and 2024.

eDNA monitoring in the Mosse watercourse

From the Mosse watercourse, a total of 40 water samples were analysed from 10 stations. None of the analysed samples showed any sign of A. *astaci* or signal crayfish eDNA (Figure 3, Table S3). eDNA from noble cayfish was detected from two 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 [13]. Noble crayfish eDNA was also detected at Elvestad in River Hobølelva.

eDNA monitoring in the Glomma watercourse

From the Glomma watercourse, 16 water samples were analysed from four stations. No sign of A. *astaci* or signal crayfish was detected from any of the monitored stations (Figure 4, Table S4). eDNA from noble crayfish was detected at the station where tributary River Flisa meets River Glomma. Lake Østersjøen is connected to the Glomma watercourse, via the outlet River Gjesa, River Julussa and River Rena into River Glomma. Downstream Lake Østersjøen, 12 water samples were analysed from three stations. All samples were negative for eDNA from A. *astaci*, signal crayfish and noble crayfish (Figure 4, Table S4). Signal crayfish was discovered in the southern part of Lake Øyern, the largest lake in the Glomma watercourse in August. The signal crayfish was confirmed as positive carriers of A. *astaci* after analysis at the Norwegian Veterinary Institute.

eDNA monitoring in the rivers Buåa, Vrangselva and Finnsrudselva

In Eidskog municipality, 32 water samples were analysed from eight stations. All samples were negative for eDNA from A. *astaci* and signal crayfish (Figure 5, Table S5). In the Vrangselva watercourse, eDNA from noble crayfish was detected at Åbogen and at Søndre Åklangen, demonstrating that the river stretch is still inhabited by noble crayfish. In River Finnsrudelva, eDNA from noble crayfish was detected at both stations (Figure 5, Table S5). In River Buåa, all samples were negative for eDNA from all three target organisms (Figure 5, Table S5).

eDNA monitoring in River Hæra

From River Hæra and the tributary River Dalselva and River Dugla, a total of 28 water samples were analysed from nine stations. From the June samples, eDNA were positive for A. *astaci* from four samples, from two

stations upstream and downstream Krokstad Bruk in Hæra (Figure 6, Table S6). In August, A. *astaci* was detected in eight samples from four stations, including one station in tributary Dalselva. eDNA from noble crayfish was detected at several of the stations (Figure 6, Table S6), signal crayfish-eDNA was not detected in any of the samples (Figure 6, Table S6).

Conclusion

In the Halden watercourse, signal crayfish present in Lake Rødenessjøen release detectable concentrations of A. *astaci* to the water. Additionally, A. *astaci* was detected at very low level in the middle part of River Hølandselva, within the restriction zone, suggesting that A. *astaci* is still present in the river system. 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 likely 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.

No eDNA samples were positive for A. *astaci* in the Mosse watercourse in 2024. While the crayfish plague reached Lake Langen in 2018, the lack of A. *astaci* detection, together with the detection of noble crayfish eDNA upstream the lake indicates no further spread. The positive eDNA detection of noble crayfish at Elvestad in River Hobølelva may suggest an early natural re-establishment in this river.

In the Glomma watercourse, no A. *astaci* or signal crayfish eDNA was detected at the sampled locations. eDNA from noble crayfish was detected at the confluence of River Flisa and River Glomma, suggesting the presence of noble crayfish in River Flisa. No A. *astaci* were detected at the three stations downstream Lake Østersjøen, where signal crayfish was discovered in 2023, suggesting that the infection pressure downstream from the signal crayfish inhabiting this lake is very low. The A. *astaci*-positive signal crayfish in Lake Øyern is the third discovery of illegal introduction of this species in connection to the Glomma watercourse. This introduction pose an increased risk for spreading the crayfish plague to several noble crayfish populations near Lake Øyeren.

We found no sign of A. *astaci* or signal crayfish in any of the monitored sites in Eidskog municipality. Similar to the results of 2017-2023, noble crayfish eDNA was detected at several of the monitored sites in the Vrangselva watercourse and River Finnsrudelva. This supports the tentative conclusion of limited (or no) dispersal of crayfish plague to the Norwegian side of these river systems, and suggests the presence of live noble crayfish in both systems. In Buåa, none of the samples were positive for A. *astaci*, signal crayfish or noble crayfish.

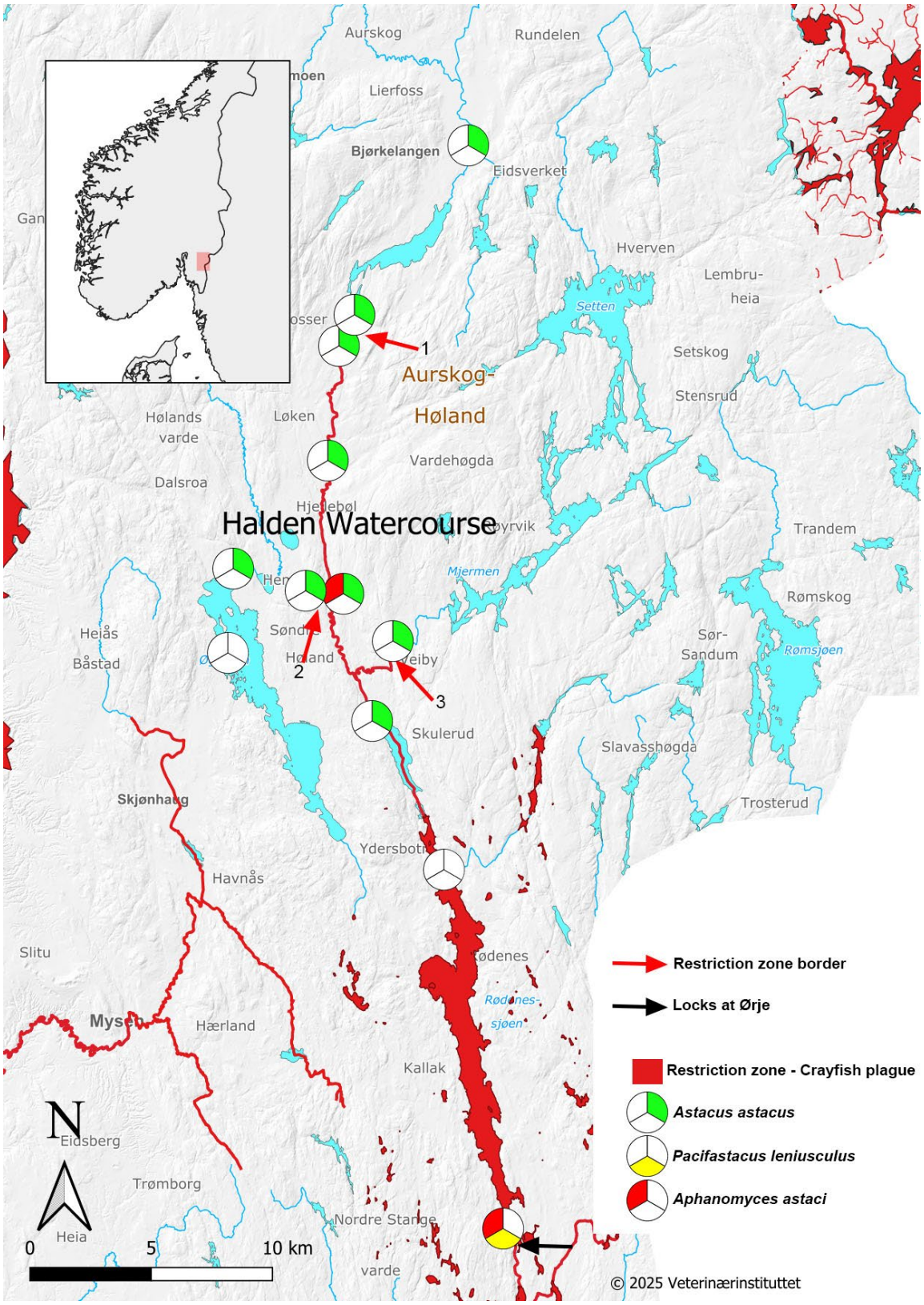

Figure 2. Overview map of the surveyed part of the Halden watercourse region in 2024, starting from the Ørje locks (black arrow) in the south where signal crayfish is present. The restriction zone is indicated by red colour on involved lakes and rivers, and ends at Fosserdam, Daltorpsfoss and Lundfoss (red arrows 1, 2 and 3 respectively), where dams act as 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.

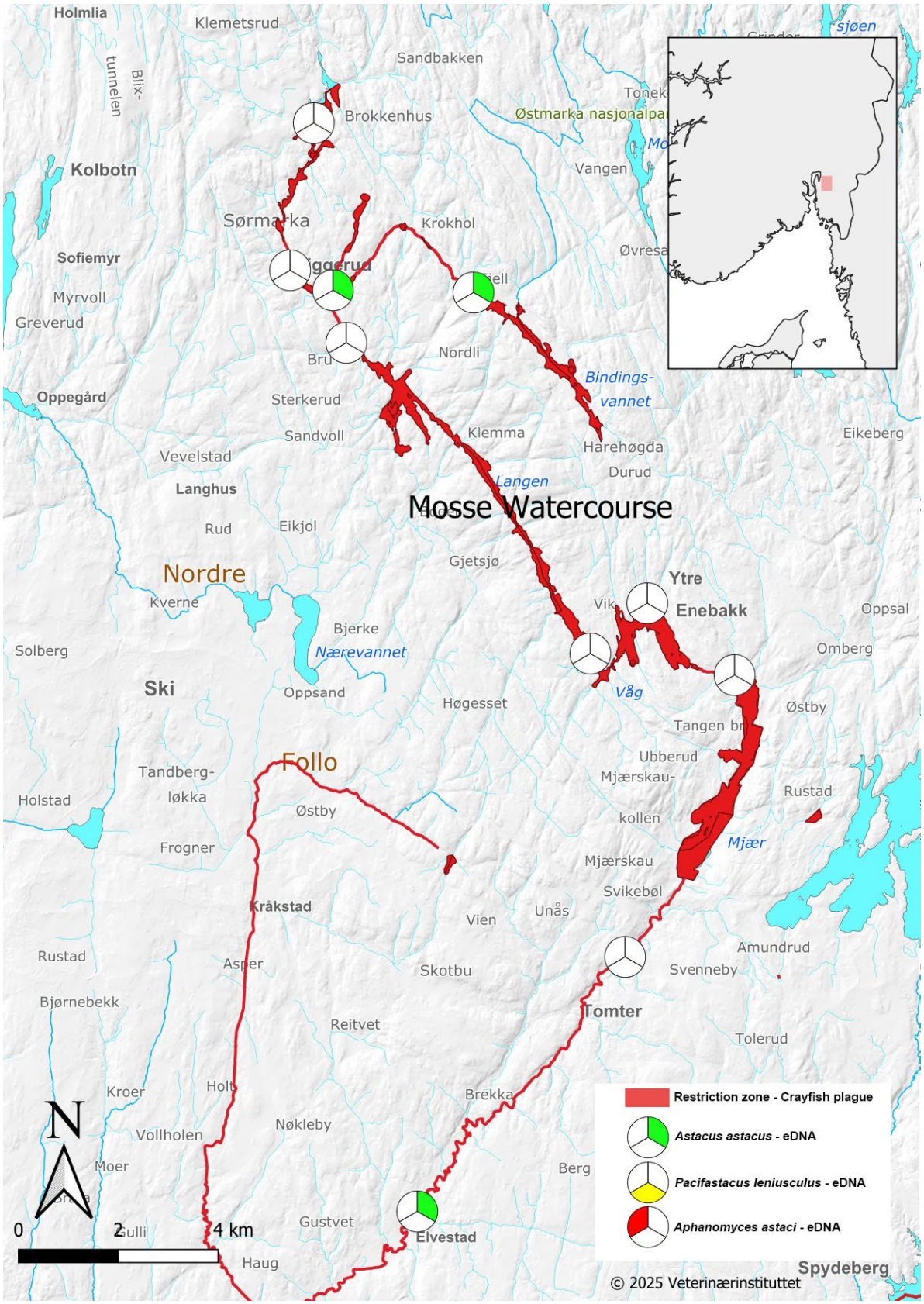

Figure 3. Overview map of the surveyed part of the Mosse watercourse in 2024. The restriction zone is represented by red colour. The pie chart indicates presence (colour) or absence (white) of A. *astaci* (red), signal crayfish (P. *leniusculus*; yellow), and noble crayfish (A. *astacus*; green). Presence is listed if at least one of the tested water samples yielded a positive eDNA result.

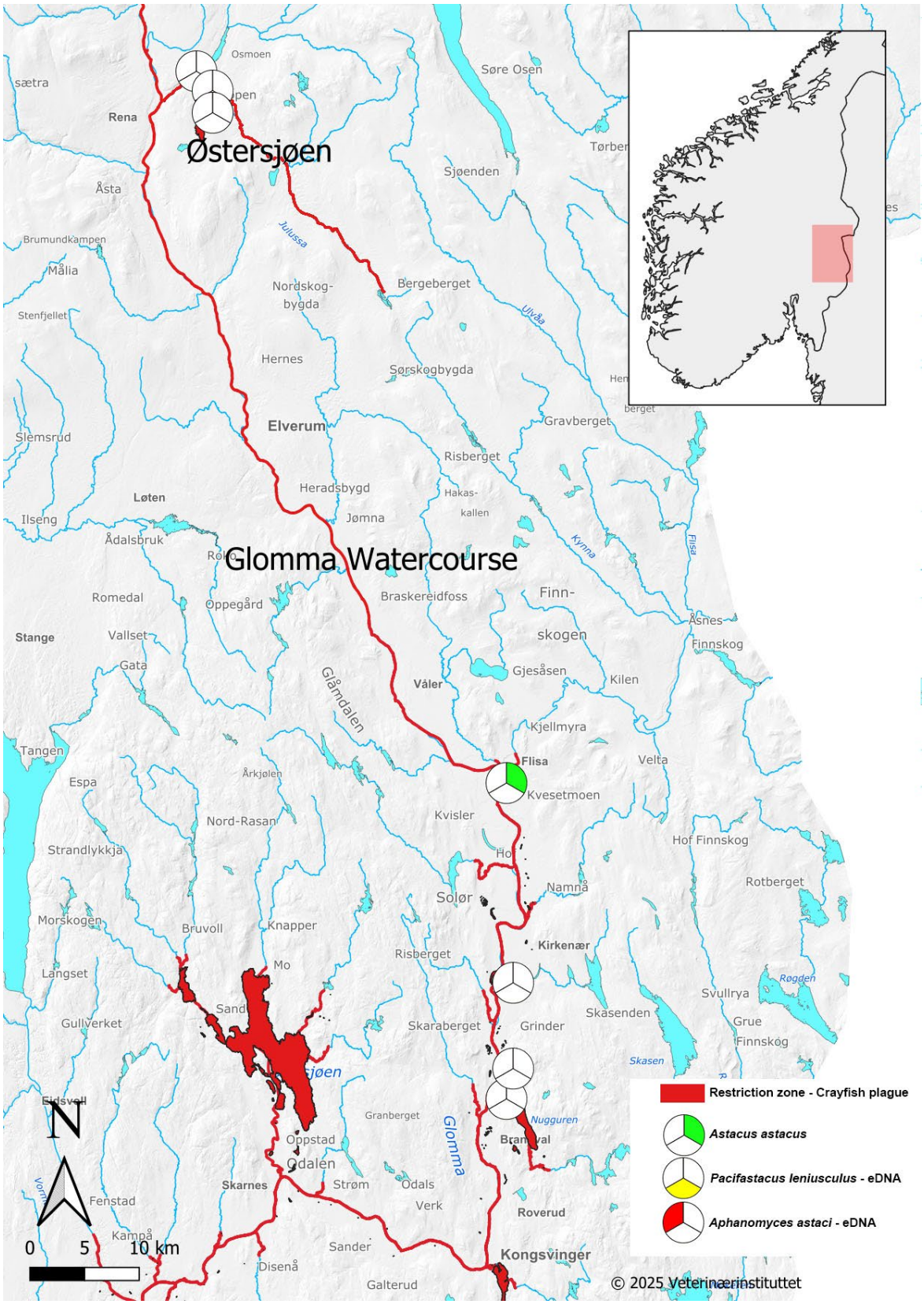

Figure 4. Overview map of the surveyed part of the Glomma watercourse region and downstream Lake Østersjøen in 2024. Regulated areas (crayfish plague restriction zones) are marked in red. For each location site, the pie chart indicates presence (colour) or absence (white) of A. *astaci* (red), signal 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.

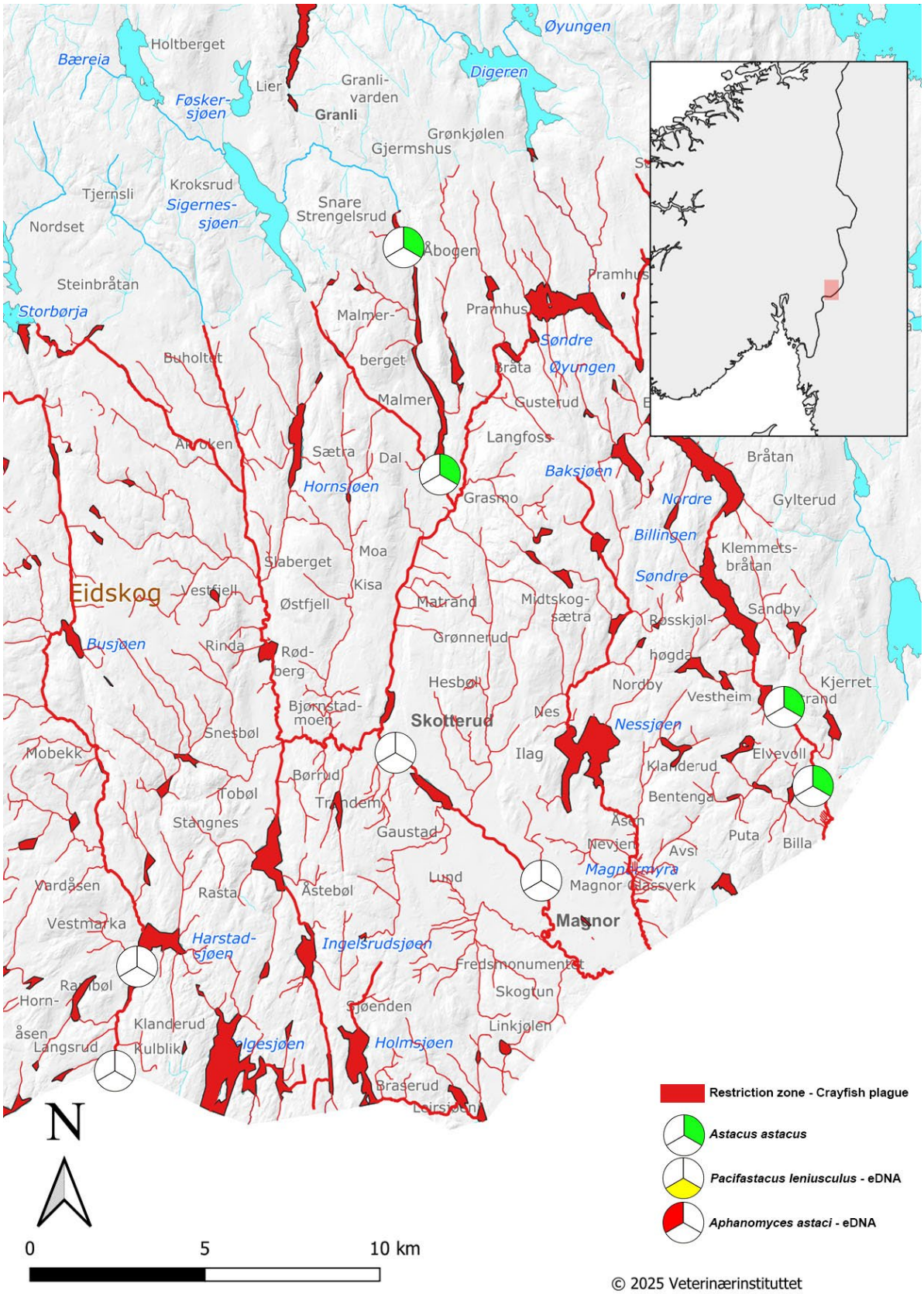

Figure 5. Overview map of the surveyed rivers in Eidskog municipality in 2024. Regulated areas (crayfish plague restriction zones) are marked in red. For each location site, the pie chart indicates presence (colour) or absence (white) of A. *astaci* (red), signal crayfish (P. *leniusculus*; yellow), and noble crayfish (A. *astacus*; green). Presence is listed if at least one of the tested water samples yielded a positive eDNA result.

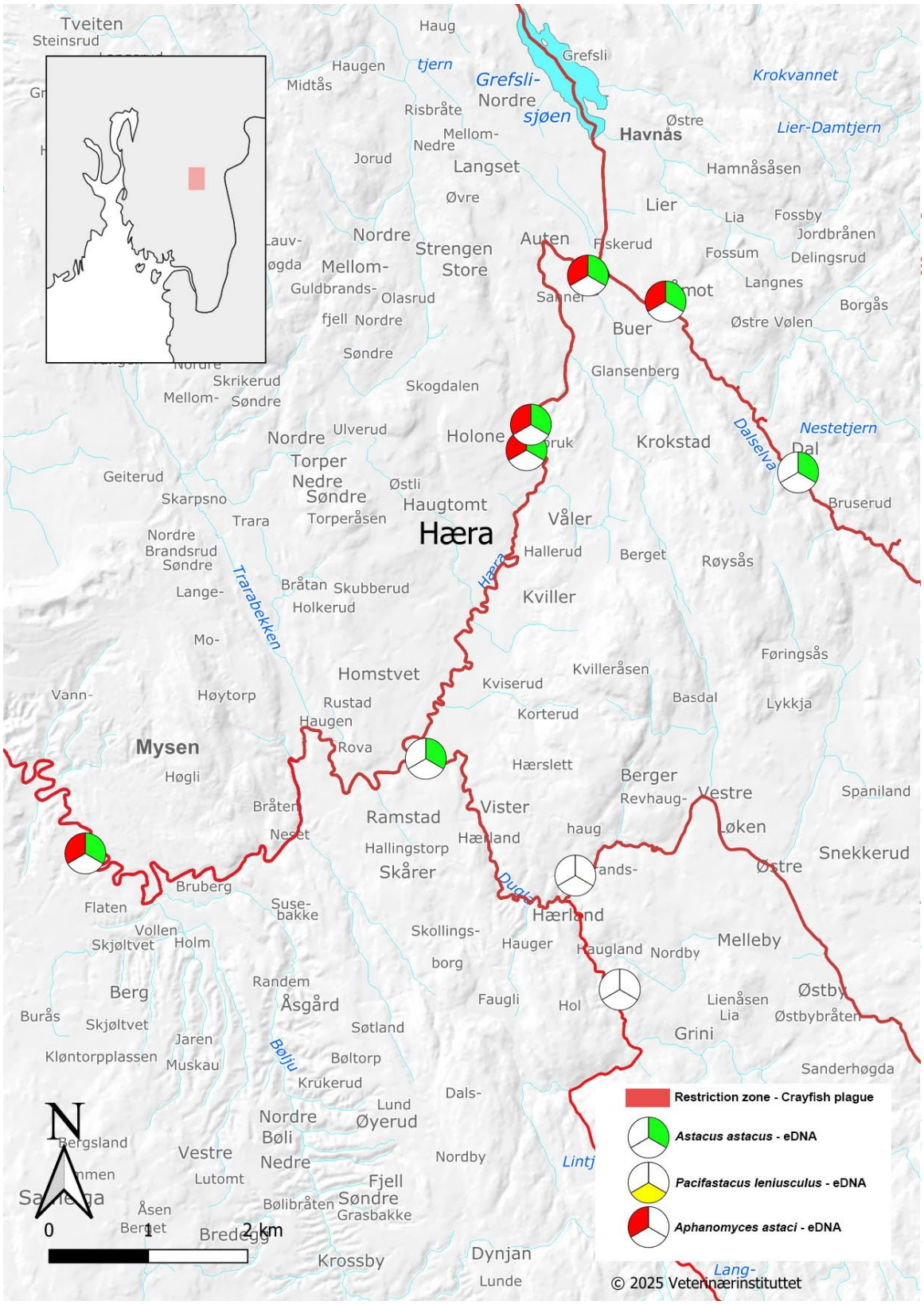

Figure 6. Overview map of the surveyed part of River Hæra in 2024. Regulated areas (crayfish plague restriction zones) are marked in red. For each location site, the pie chart indicates presence (colour) or absence (white) of *A. astaci* (red), signal crayfish (*P. leniusculus*; yellow), and noble crayfish (*A. astacus*; green). Presence is listed if at least one of the tested water samples yielded a positive eDNA result.

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Appendix

Supplementary information to the report "The surveillance programme for *Aphanomyces astaci* in Norway 2024" - Tables S1 – S6.

Table S1. Agreed areas and sample numbers for the surveillance programme for 2024.

	Water course/River	County[1]	No. stations	No. samples	(Stations x samples x visits)
NOK *A. astaci* Prog. 2024	Halden watercouse	A, Ø,	12	48	(12 x 2 x 2)
	Glomma watercouse	I, A, Ø	7	28	(7 x 2 x 2)
	River Vrangselva	I,	4	16	(4 x 2 x 2)
	River Finnsrudselva	I,	2	8	(2 x 2 x 2)
	River Buåa	I,	2	8	(7 x 2 x 2)
	Mosse watercourse	A, Ø,	10	40	(7 x 2 x 2)
	Hæra watercourse	Ø	7	28	(7 x 2 x 2)
	Total		44	176	

[1]A = Akershus, Ø = Østfold, I = Innlandet.

Table S2. 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]					
						June			August		
	S[1]	Latitude	Longitude	#	L	CP	SC	NC	CP	SC	NC
Lierelva, Bjørkelagen	R	59.885778	11.575056	4	9	0	0	2	0	0	2
Fosserdam	R	59.821278	11.495306	4	16	0	0	2	0	0	2
Fossersjøen	C	59.816389	11.492333	4	12	0	0	2	0	0	2
Lundsfoss	R	59.701806	11.537611	4	16	0	0	2	0	0	2
Hemnessjøen pier	R	59.696472	11.418806	4	15	0	0	0	0	0	0
Hemnessjøen outlet	R	59.725444	11.419861	4	12	0	0	2	0	0	1
Daltorpsfoss	R	59.720694	11.482250	4	8	0	0	2	0	0	0
Hølandselva north	C	59.768722	11.485444	4	14	0	0	2	0	0	2
Hølandselva middle	C	59.720333	11.492167	4	15	1	0	2	0	0	2
Hølandselva outlet	C	59.675139	11.530778	4	14	0	0	1	0	0	2
Rødenessjøen Kroksund	C	59.618583	11.584790	4	15	0	0	0	0	0	0
Rødenessjøen Ysterud	C	59.488306	11.639750	4	20	1	1	0	2	2	0
Total				48	165	2	1	17	2	2	15

[1] C = Crayfish plague restriction zone, R = risk area

[2] # = Total number of water samples (June & August summarized), L = total water volume summarized for all samples

[3] Number of samples in June and August with positive detection of eDNA from crayfish plague (CP), noble crayfish (NC), and signal crayfish (SC).

Table S3. 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]					
						June			August		
	S[1]	Latitude	Longitude	#	L	CP	SC	NC	CP	SC	NC
Bindingsvann, outlet	C	59.789510	10.954824	4	13	0	0	2	0	0	0
Tangentjern, inlet, bridge on brusagav.	C	59.788393	10.900794	4	16	0	0	2	0	0	2
Sværsvann	C	59.817607	10.890148	4	14	0	0	0	0	0	0
Tangentjern, inlet, bridge on Hareveien	C	59.790558	10.890969	4	19	0	0	0	0	0	0
Langen, inlet, bridge on Bru-fjellv.	C	59.779107	10.910734	4	13	0	0	0	0	0	0
Langen, bridge on Skiveien	C	59.726024	11.003307	4	14	0	0	0	0	0	0
Våg	C	59.736235	11.020769	4	12	0	0	0	0	0	0
Tangenelva, bridge on Tomterveien	C	59.722254	11.055222	4	14	0	0	0	0	0	0
Hobølelva, Sagbruk	C	59.671376	11.019753	4	15	0	0	0	0	0	0
Hobølelva, Elvestad	C	59.624056	10.952501	4	16	0	0	2	0	0	0
Total				40	146	0	0	6	0	0	2

[1] C = Crayfish plague restriction zone, R = risk area

[2] # = Total number of water samples (June & August summarized), L = total water volume summarized for all samples

[3] Number of samples in June 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 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]					
						June			September		
	S[1]	Latitude	Longitude	#	L	CP	SC	NC	CP	SC	NC
Gjesa, Outlet Østersjøen	C	61.127340	11.455511	4	20	0	0	0	0	0	0
Gjesa, Sameiebuveien	C	61.156383	11.452283	4	20	0	0	0	0	0	0
Jussula, Sameiebuveien	C	61.159680	11.445496	4	20	0	0	0	0	0	0
Glomma, Flisa	C	60.589739	12.019428	4	15	0	0	1	0	0	0
Glomma, Grue	C	60.427744	12.039228	4	13	0	0	0	0	0	0
Glomma, Hvebergåa	C	60.353157	12.051679	4	20	0	0	0	0	0	0
Glomma, Nugguren		60.340868	12.047194	4	20	0	0	0	0	0	0
Total				28	128	0	0	1	0	0	0

[1] C = Crayfish plague restriction 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 S5. 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		
	S[1]	Latitude	Longitude	#	L	CP	SC	NC	CP	SC	NC
Vrangselva, Åbogen	C	60.112260	12.116651	4	20	0	0	2	0	0	2
Søndre Åklangen, Badeplass	C	60.053552	12.139129	4	16	0	0	1	0	0	0
Vrangselva, Skotterud	C	59.982010	12.121258	4	15	0	0	0	0	0	0
Vrangselva, Magnor bad	C	59.950789	12.199625	4	17	0	0	0	0	0	0
Finnsrudelva, Finnsrudvegen	C	59.997405	12.318277	4	20	0	0	2	0	0	2
Finnsrudelva, Billavegen	C	59.979207	12.337286	4	20	0	0	2	0	0	2
Buåa, Eidskog	C	59.925330	11.993562	4	11	0	0	0	0	0	0
Buåa, Riksgrense	C	59.899040	11.986509	4	9	0	0	0	0	0	0
Total				32	128	0	0	7	0	0	6

[1] C = Crayfish plague restriction 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 S6. Locations for water sampling in the River Hæra 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			August		
	S[1]	Latitude	Longitude	#	L	CP	SC	NC	CP	SC	NC
Dalselva, Dalveien	R	59.587356	11.446157	2	8	-	-	-	0	0	2
Dalselva, Åmotveien	R	59.602203	11.421013	4	17	0	0	2	2	0	2
Hæra, Tangen	R	59.604283	11.409403	4	6	0	0	2	2	0	2
Hæra, Krogstad Bruk	R	59.590706	11.398609	4	13	2	0	2	2	0	2
Hæra, Krogstad Bruk Downstream	R	59.589149	11.398139	2	10	2	0	2	-	-	-
Hæra, Kapellveien	R	59.549690	11.323278	4	10	0	0	0	2	0	2
Dugla, Sloraveien	R	59.539237	11.419605	2	10	-	-	-	0	0	0
Dugla, Kongeveien	R	59.549418	11.411439	2	10	0	0	0	-	-	-
Dugla, E18	R	59.559744	11.384363	4	17	0	0	2	0	0	2
Total				28	100	4	0	10	8	0	12

[1] C = Crayfish plague restriction zone

[2] # = Total number of water samples (June & August), L = total water volume summarized for all samples

[3] Number of samples in June and August with positive detection of eDNA from crayfish plague (CP), noble crayfish (NC), and signal crayfish (SC).

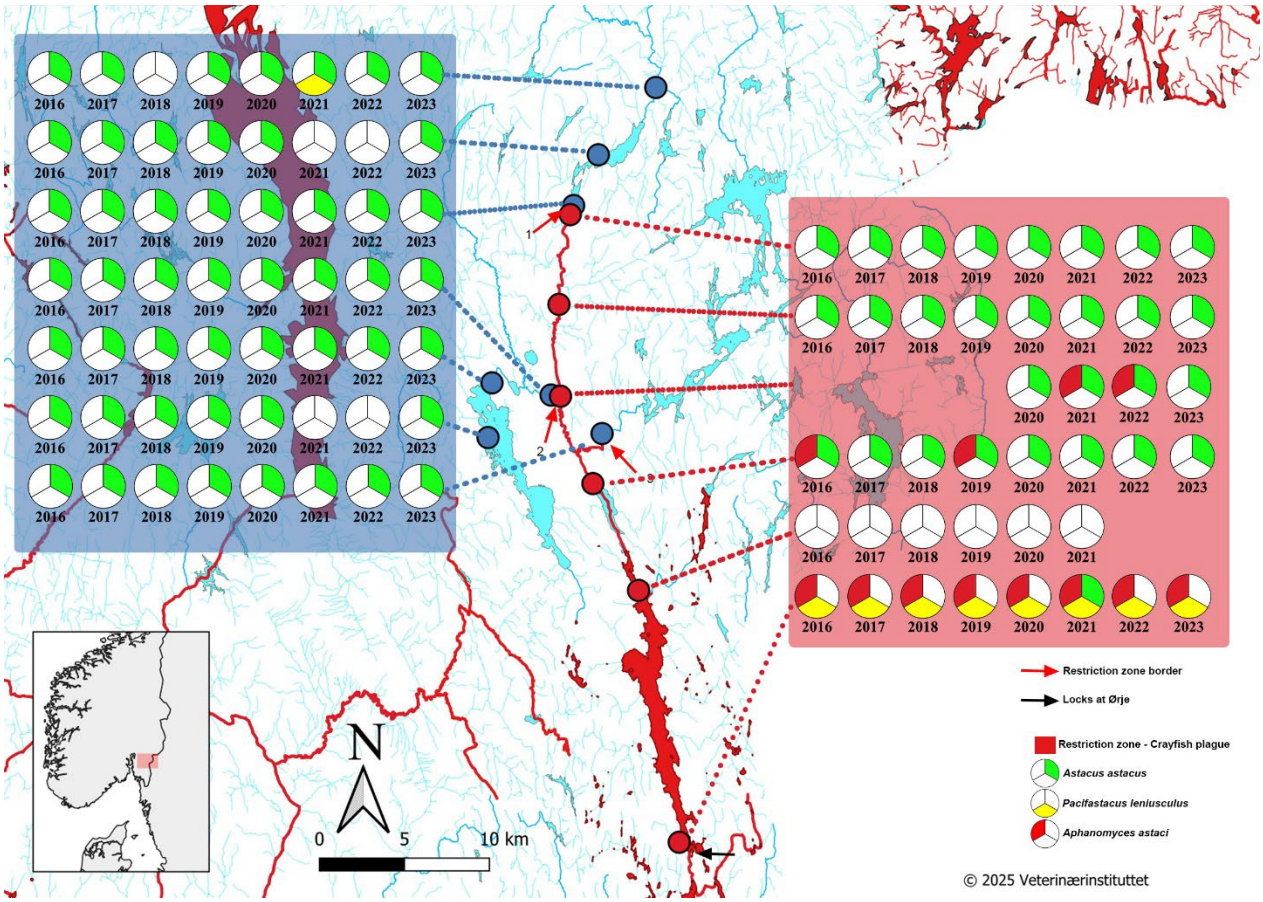

Figure S1. . Environmental DNA results from 2016 – 2023 in Halden watercourse, showing the stable detection of noble crayfish eDNA within the crayfish plague restriction zone (red sample sites) from the middle part of River Hølandselva up to the boarder of the restriction zone at Fosserdam. Water samples from the risk zone (blue sample sites) also show overall stable detection of noble crayfish eDNA.

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