DNA metoder til sporing af beskyttede arter i ferskvand

STATENS NATURHISTORISKE MUSEUM
KØBENHAVNS UNIVERSITET

Amphi Consult

eurofins

DNA metoder til sporing af beskyttede arter i ferskvand

-Forskningsprojekt i samarbejde med Amphi Consult, Eurofins og Københavns Universitet (2012-2015)

-Valideringer af eDNA detektion til overvågning af vandhulsarter (Naturstyrelsen 2012-2014)

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Baggrund

- 2011: Dansk forskning peger på potentiale for eDNA brug i naturovervågningen

MOLECULAR ECOLOGY

Molecular Ecology (2011) doi: 10.1111/j.1365-294X.2011.05418.x

FROM THE COVER

Monitoring endangered freshwater biodiversity using environmental DNA

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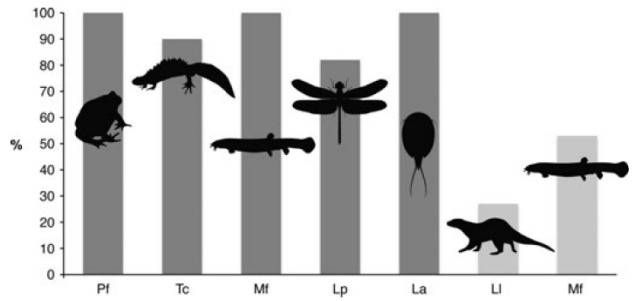
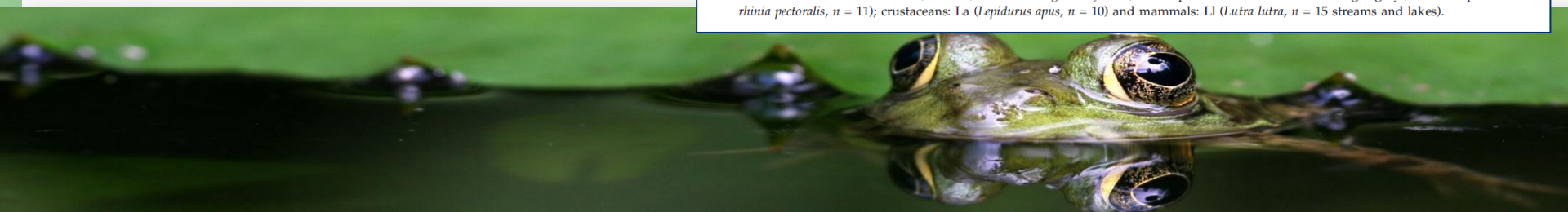
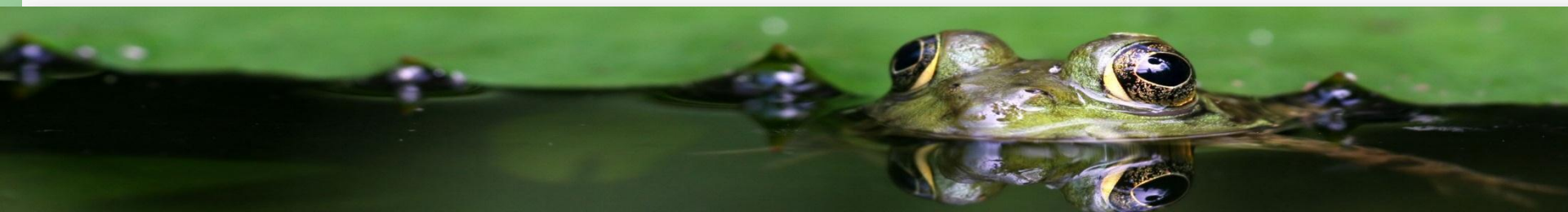


Fig. 2 Environmental DNA detection rates by qPCR in natural freshwater ponds with 100% occurrence of the species confirmed in the field (dark grey) or larger freshwater systems with known occurrence in the area (light grey). Detection rates are given in percentage positive localities out of the total number of localities surveyed for each species. Data covers amphibians: Pf (*Pelobates fuscus*, $n = 9$) and Tc (*Triturus cristatus*, $n = 11$); fish: Mf (*Misgurnus fossilis*, $n = 11$ ponds and $n = 15$ streams—light grey); insects: Lp (*Leucorrhinia pectoralis*, $n = 11$); crustaceans: La (*Lepidurus apus*, $n = 10$) and mammals: Ll (*Lutra lutra*, $n = 15$ streams and lakes).



Potentielle fordele – eDNA

- Standardiseret dataindsamling
- Uafhængighed af felteksperter
- Uafhængighed af umiddelbare vejrforhold
- Non-invasiv metode
- Redskabs- og habitatuafhængig
- Resource besparende?
- Højere sensitivitet ?



Hvordan?

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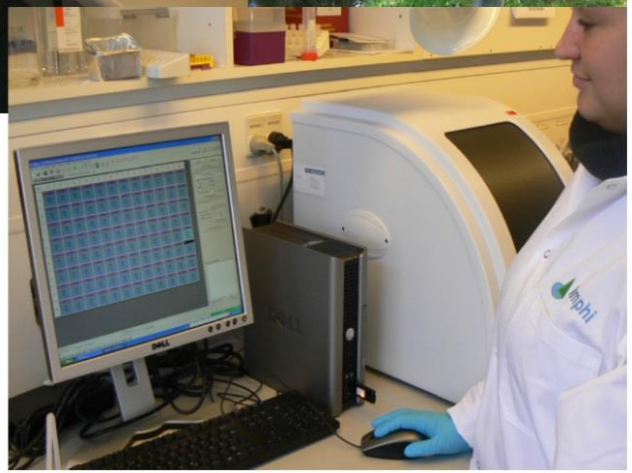


Udvikling af artsspecifikke DNA detektionssystemer (baseret på qPCR)

- *In Silico*
  - Udvikling af artsspecifikke detektionssystemer ud fra DNA-database og sekvensoplysninger (på computer)
- *In Vitro test*
  - Indsamling af væv fra target og non-target arter til DNA bank. Test og optimering.
- *In Vivo test*
  - Indsamling af vandprøver fra kendte forekomster og test i lab.
- *Validering*
  - stor skala afprøvning på mange prøver med kendte positive/negative forekomster

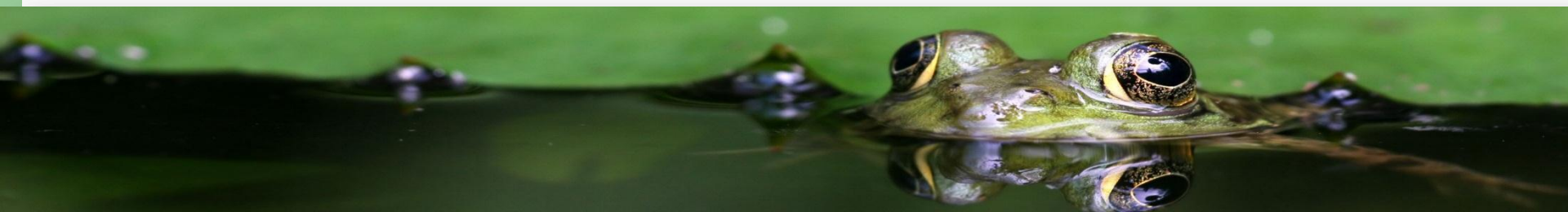


Table 2. Comparison in percentage between the target-sequence in the target-species and the non-target-species. 'N' and 'P' indicates a positive or a negative result from the in vitro test against a DNA extraction from the various target- and non-target-species. A lower percentage in mismatch indicates that there is a slight risk that the assay might also be specific against these other closely related species.

Neg / Pos in Assay test against DNA extracted from tissue from non-target-species (in vitro test)

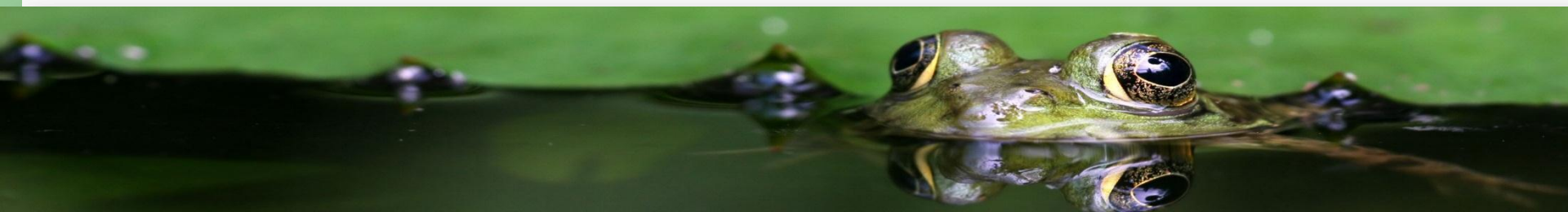
In silico mismatch (%) in target-sequence among non-target species

IN VITRO: IN SILICO:

mtDNA fragment target length (bp)									
	Assay	[CobtaeCB]	[MisangCB]	[MisFosCB]	98	93	115		
Common name (DK)	**Target species**	*Cobitis taenia*	*Misgurnus anguillicaudatus*	*Misgurnus fossilis*	*Cobitis taenia*	*Misgurnus anguillicaudatus*	*Misgurnus fossilis*	**Occurence**
Pigsmerling	*Cobitis taenia*	P	N	N	0	27	23	Europe, Asia, Baltic
Orientalsk dyndsmerling	*Misgurnus anguillicaudatus*	N	P	N	19	0	17	Asia, introduced in Europe
Dyndsmerling	*Misgurnus fossilis*	N	N	P	19	16	0	Europe, Asia, Scandinavia
Brasen	*Abramis brama*	N	N	N	19	26	26	Europe, Asia, Scandinavia
Løje	*Alburnus alburnus*	N	N	N	21	23	25	Europe, Asia, Scandinavia
Flire	*Blicca bjoerkna*	N	N	N	19	24	25	Europe, Asia, Baltic
Sølvkarusse	*Carassius auratus*	N	N	N	21	30	23	Asia, introduced in all the world
Karusse	*Carassius carassius*	N	N	N	23	30	24	Europe, Asia, Baltic
Græskarpe	*Ctenopharyngodon idella*	N	N	N	17	26	25	Asia, introduced in Europe
Karpe	*Cyprinus carpio*	N	N	N	23	29	23	Europe, Asia, Baltic
Grundling	*Gobio gobio*	N	N	N	21	27	23	Europe, Baltic
Sølv karpe	*Hypophthalmichthys molitrix*	N	N	N	18	30	23	Europe, Asia
Regnløje	*Leucaspius delineatus*		N	N	23	25	26	Europe, Asia, Baltic
Rimte	*Leuciscus idus*	N	N	N	21	25	25	Europe, Asia, Baltic
Strømskalle	*Leuciscus leuciscus*	N	N	N	21	24	26	Europe, Asia, Baltic
Elritse	*Phoxinus phoxinus*	N	N	N	21	28	27	Europe, Asia, Baltic
Bånd grundling	*Pseudorasbora parva*	N	N	N	20	27	24	Asia, introduced in Europe
Europæisk bitterling	*Rhodeus amarus*	N	N	N	22	29	31	Europe, Baltic
Skalle	*Rutilus rutilus*	N	N	N	24	31	25	Europe, Asia
Rudskalle	*Scardinius erythrophthalmus*	N	N	N	20	30	26	Europe, Asia
Suder	*Tinca tinca*	N	N	N	18	29	23	Europe, Asia, Scandinavia
Smerling	*Barbatula barbatula*	N	N	N	24	27	28	Europe, Scandinavia

List prepared by consulting the Danish checklist of freshwater fishes:

Konventionel overvågning



DNA sampling og analyse

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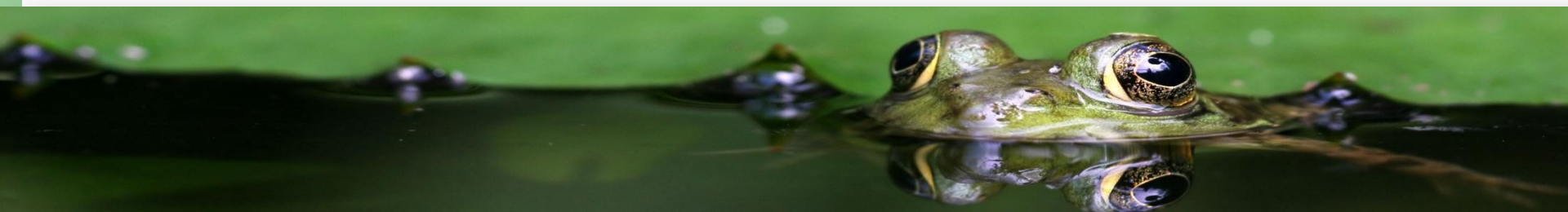
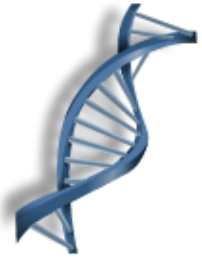
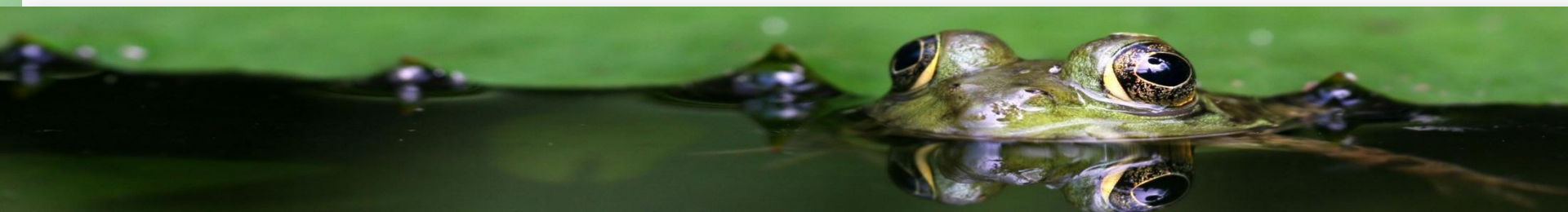


Validering:

VS.

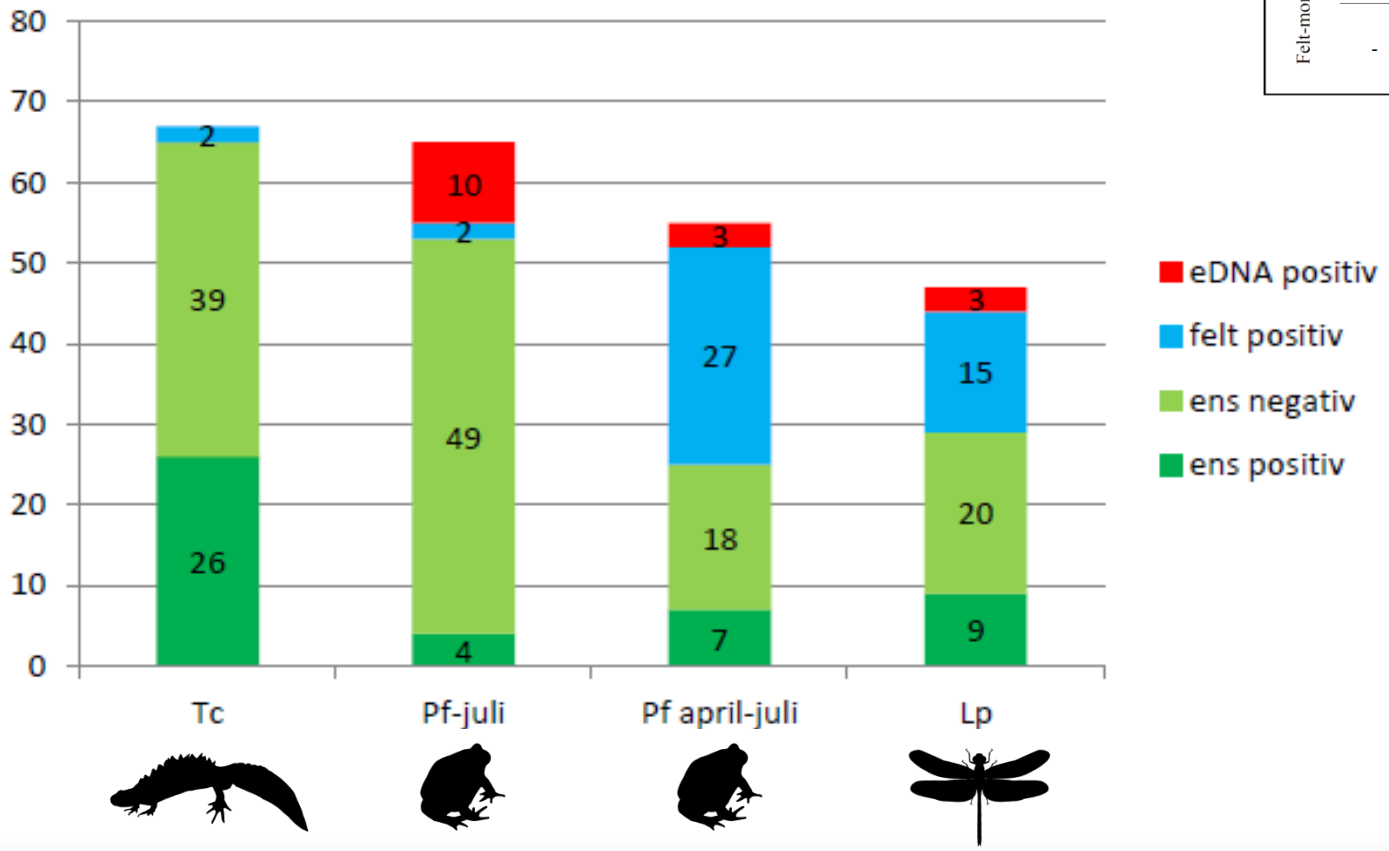


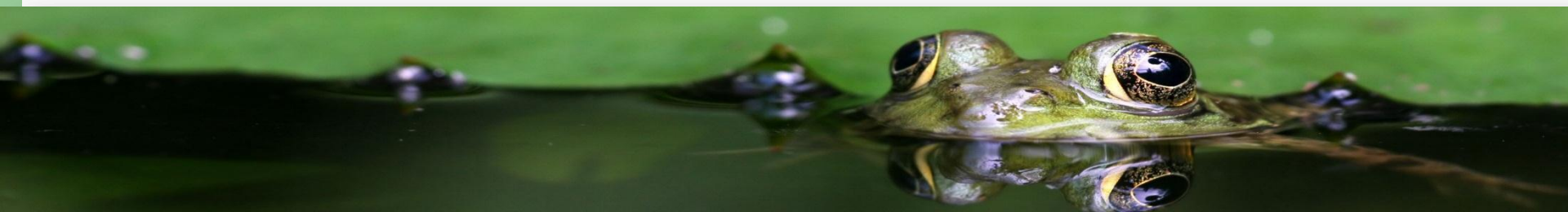
		eDNA-monitering	
		+	-
Felt-monitering	+	●	●
	-	●	●



Resultater - *Validering*

		eDNA-monitering +	eDNA-monitering -
Felt-monitering	+	●	●
	-	●	●

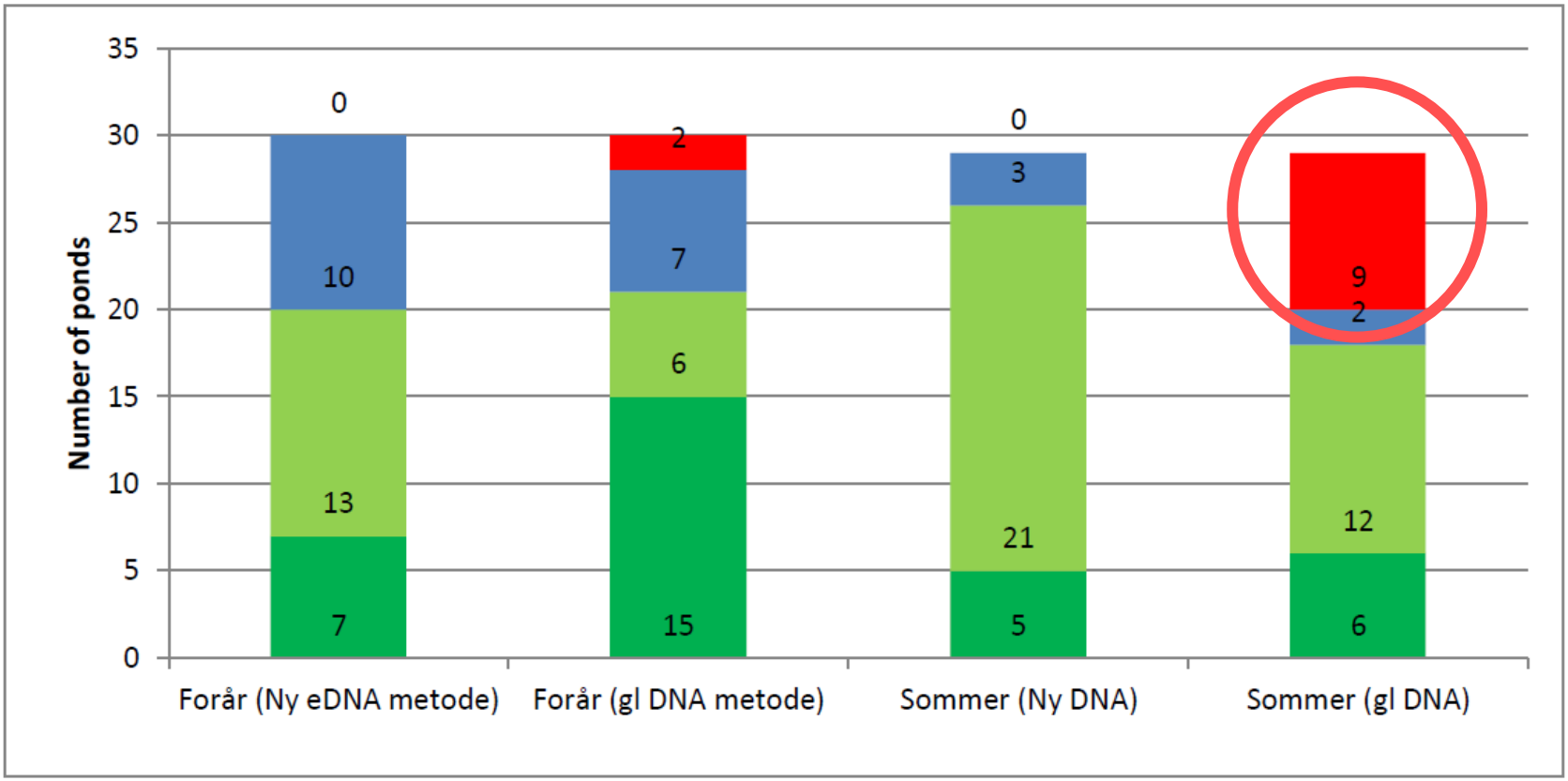


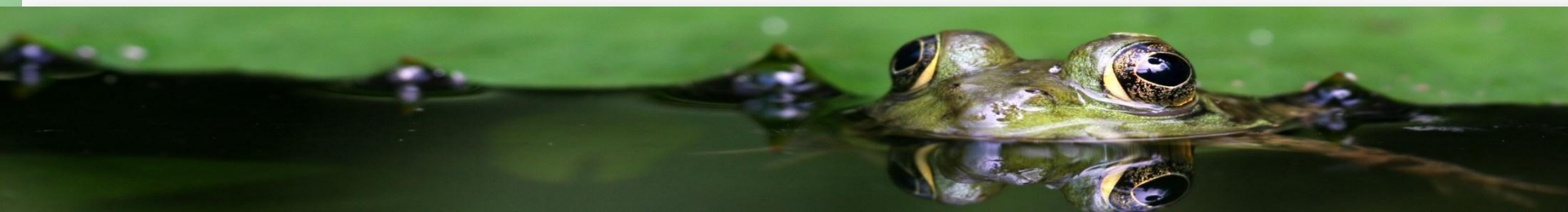


Resultater –

Validering af to forskellige artsspecifikke eDNA detektionssystemer til løgfrø (samme prøver)

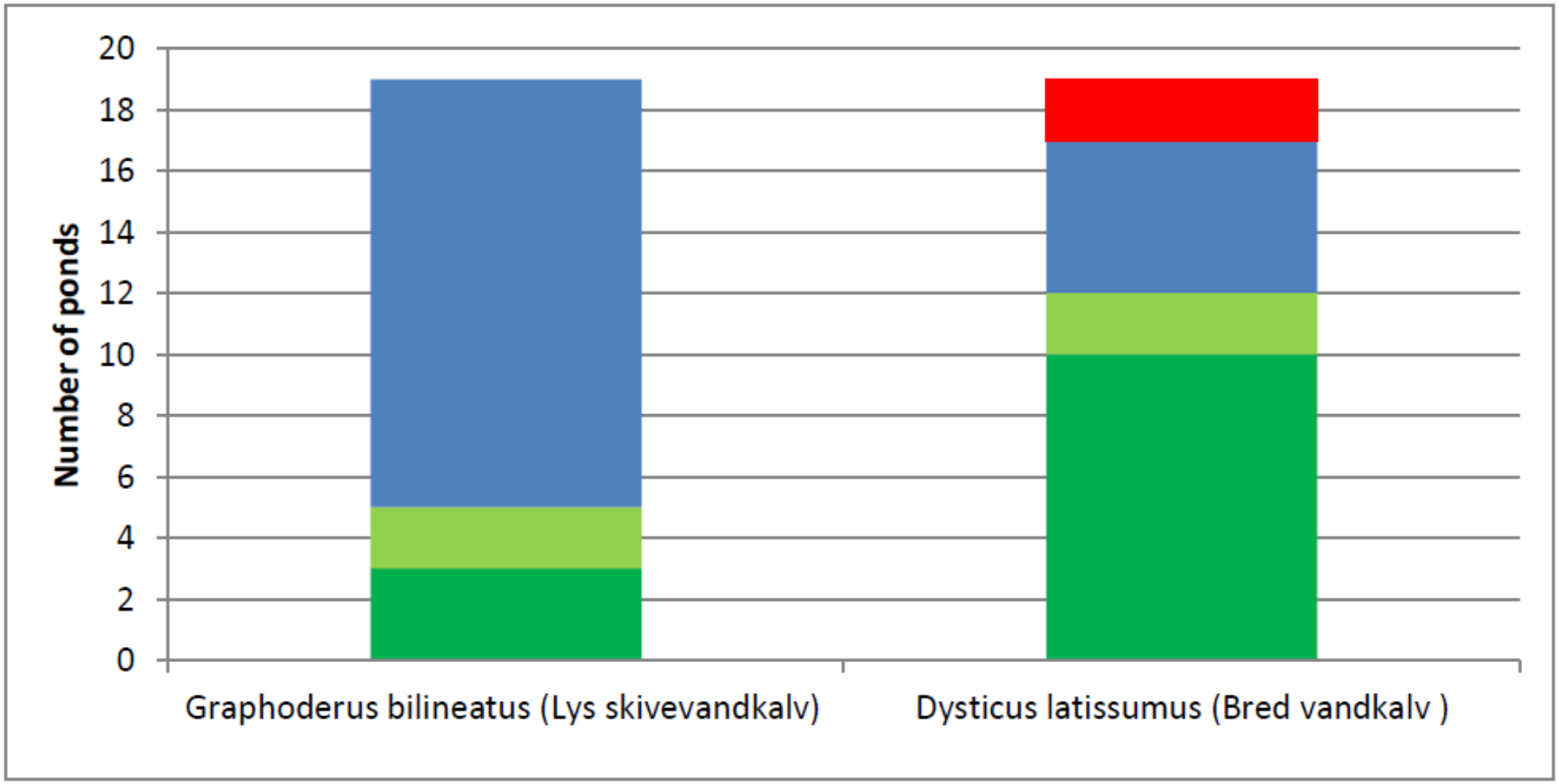
		eDNA-monitering +	eDNA-monitering -
Felt-monitering	+	●	●
Felt-monitering	-	●	●

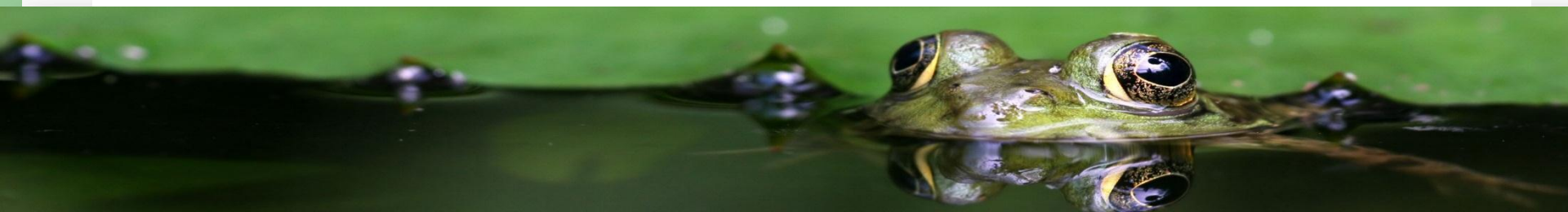




Resultater - *Validering*

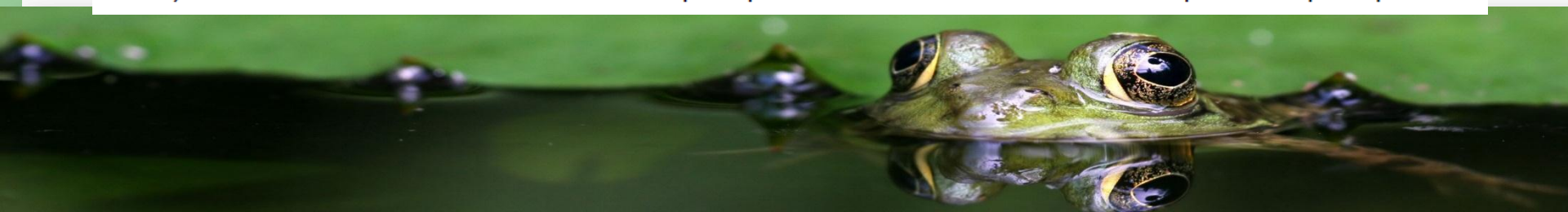
		eDNA-monitering +	eDNA-monitering -
Felt-monitering	+	●	●
Felt-monitering	-	●	●





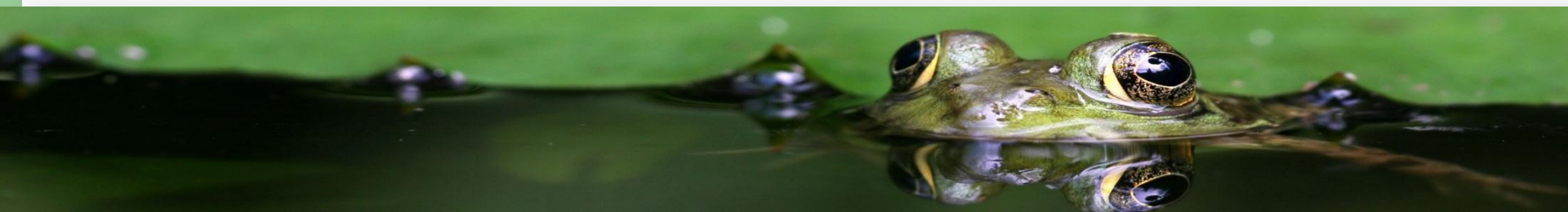
Artsspecifikke eDNA systemer udviklet og testet:

Art	UK	DK
Triturus cristatus	Great crestered newt	Stor vandsalamander
Rana avarlis	Moor frog	Spidssnudet frø
Rana dalmatina	Agile frog	Springfrø
Hyla aborea	European treefrog	Løvfrø
Pelobates fuscus	European common spadefoot	Løgfrø
Bombina bombina	Fire-bellied toad	Klokkefrø
Bufo calamita	Natterjack toad	Strandtudse
Bufo viridis	European green toad	Grønbroget tudse
Dytiscus latissimus	N/A	Bred vandkalv
Graphoderus bilineatus	N/A	Lys skivevandkalv
Misgurnus fossilis	Weather loach	Dyndsmerling
Cobitis taenia	Spined loach	Pigsmerling
Misgurnus anguillicaudatus	Oriental weather loach	Orientalsk dyndsmerling
Emys orbicularis	European pond turtle	Europæisk sumpskildpadde

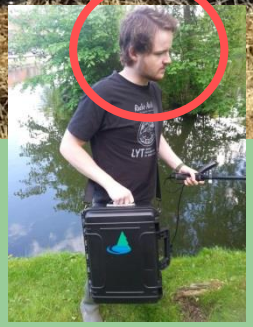


Konklusion

- Artsspecifikke eDNA systemer er nu til rådighed for de fleste ferskvands Bilag IV arter i Danmark.
- For stor vandsalamander ses 97% sammenfald mellem eDNA metoden og feltobservationer i storskala validering.
- I lokaliteter med >10 løgfrøer er der i alle tilfælde positivt eDNA signal. Ved små bestande af løgfrø er metoden mindre følsom end dygtig feltbiolog (- forventes forbedret med forbedret sampling teknik-).
- For invertebrater er eDNA metoden pt væsentligt mindre sensitiv end dygtig feltbiolog (- forventes forbedret med forbedret sampling teknik-).
- eDNA metoder kan give falske positive resultater. For at undgå dette, skal detektionssystemer være tilstrækkeligt testet og valideret.



DNA sporing af beskyttede arter:



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Læs mere om vores arbejde på:

www.amphi-consult.dk