Water chemistry, zooplankton and benthos in small lakes within the distribution area of the rare European pool frog *Pelophylax lessonae* (Camerano) in Norway

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Summary

In June 2018, water chemistry, benthos and zooplankton were investigated in 7 small lakes/ponds within the key area of the European pool frog *Pelophylax lessonae* (Camerano) in Norway. The geographic distribution of the Norwegian population is minor, limited to a very few small lakes in the county of Agder in southernmost Norway. Since the species is thermophilic, reproduction success only occurs in warm summers. Accordingly, the pool frog is one of the most rare vertebrates in Norwegian fauna, classified as critically endangered in the Norwegian red list. A breeding program is today established in order to rescue this species.The article deals with potential biotope challenges most relevant for the recruitment success of the pool frog in Norway, primarily related to physical, chemical and biological conditions in surface water. In addition, terrestrial biotope challenges and potential effects of low genetic diversity in the very small Norwegian population are discussed.

Sammendrag

Vannkjemi, zooplankton og bunndyr i små innsjøer innenfor leveområdet til en av Norges mest truete vertebrater, Europeisk damfrosk Pelophylax lessonae (Camerano). I juni 2018, ble vannkjemi, bunndyr og zooplankton undersøkt i 7 små tjern i kjerneområdet for den damfrosken *Pelophylax lessonae* (Camerano) i Norge. Utbredelsen av arten er svært begrenset, og populasjonen svært liten. Arten er relativt termofil, noe som sannsynligvis er hovedårsaken til den marginale utbredelsen. Arten er trolig den mest

sjeldne vertebrat i norsk fauna, klassifisert som kritisk truet på den norske rødlista. Derfor er det også etablert et avlsprogram for om mulig å kunne redde denne sjeldne vertebraten i norsk natur. I tillegg til artens klimatiske begrensninger i Norge, tar denne artikkelen opp ulike biotop- utfordringer, i første rekke i forhold til artens muligheter for reproduksjon i de akvatiske miljøene den er avhengig av. I tillegg, tas det opp enkelte terrestriske utfordringer for arten, samt effekter av lav genetisk diversitet i den svært marginale norske populasjonen.

Introduction

The Norwegian fauna hosts only six native amphibian species (DN, 2006; Dolmen, 2018). Laying of eggs and larvae development take place in freshwater, basically in small lakes and ponds, or in sheltered coves of larger lakes. Their main threats are drainage of wetlands, upfilling of small lakes and ponds, stocking of fish and pollution, including acid rain (Dolmen 1987), although the latter threat being significantly reduced (80-85%) in southernmost Norway since 1980 (Garmo and Skancke, 2017).

The European pool frog *Pelophylax lessonae* (Camerano, 1882) is widespread in much of mainland Europe, with isolated northerly populations in Norway and Sweden, generally accepted as native (Zeisset & Beebee, 2001; Gasc et al., 2004). The species was first discovered in Norway in 1986, but the locality was not further investigated until 1996 (Dolmen, 1996). The geographic distribution of the Norwegian population is very small, limited to a few small lakes in the county of Agder, southern Norway. The population is estimated to consist of less than 50 reproductive individuals, in some years far less (Dolmen 2012; Engemyr and Reinkind, 2019). As the species is thermophilic, reproduction success in Norway occurs only in warm summers, but also cold winters seem to negatively affect winter survival, primarily for young metamorphosed individuals (Dolmen, 2012). Besides these climatic demands, biotope quality and the presence of fish are probably the most limiting factors for the extensiveness of the pool frog in Norway. Accordingly, the pool frog is possibly the most rare vertebrates in the Norwegian fauna (DN, 2006) and classified as a critically endangered species on the Norwegian red list. Late 2017, the Norwegian Environmental Protection Agency (Miljødirektoratet) asked the county governor of Aust- and Vest-Agder (Agder from 01.01.2020) to carry out a breeding program in order to rescue this most threatened Norwegian vertebrate. The breeding program is being implemented under controlled conditions in Kristiansand Zoo, for restocking into natural biotopes.

During the most severe acid rain period in the western world, 1970-2000, surface water in southernmost Norway was severely impacted (Rosseland et al., 1986). The most affected county in Norway was Aust-Agder, the home of the pool frog. The county lost 2500 brown trout population and about 500 perch population during this period (NIVA, 2004). Accordingly, many scientists in the western world, also focused on potential water acidification effects on amphibians. Some focused on effects of low pH on adult amphibians (Beebee and Griffin, 1977; Pough and Wilson, 1977; Strijbosch, 1979; Clark, 1986; Gascon and Planas, 1986), others on effects of low pH on embryos (Gosner and Black, 1957, Beebee and Griffin, 1977; Nielsen et al., 1977; Saber and Dunson, 1978; Tome and Pough, 1982; Pierce et al., 1984; Dale et al., 1985; Pierce, 1985; Clark, 1986; Gascon and Planas, 1986).

Fewer studies were carried out on larval amphibians, and most of them focused on the ability of tadpoles to tolerate acute exposure to low pH (Gosner and Black, 1957; Beebee and Griffin, 1977; Saber and Dunson, 1978; McDonald et al., 1984; Skei and Dolmen, 2006). These studies showed that amphibians exhibited large variations in acid tolerance. McDonald et al. (1984) suggested larval amphibians to be more able to resist acid exposure than many acid-intolerant fish. However, in general, the density and diversity of amphibians were concluded to decrease as water pH decreased, and the adults seemed more resistant than earlier life stages.

Dolmen et al. (2008) carried out field investigations of amphibians' tolerance to acidity in southernmost Norway and concluded that amphibian reproduction and development in a few localities had taken place at a pH as low as 4.5 or 4.6, but that in general pH values above 5.0 seemed necessary. Higher pH also resulted in higher amphibian diversity.

Several studies also demonstrated toxicity of inorganic Al to amphibians (Clark and Hall, 1985; Clark and LaZerte, 1985; Cummins, 1986; Leuven et al., 1986; Freda and McDonald, 1990; Freda et al., 1990), whereas others found no correlation between Al and embryonic mortality or amphibian abundance (Gascon and Planas, 1986). Freda et al. (1990) observed an increased toxicity of Al when complexed to organic humic compounds, i.e. quite opposite to what observed in fish studies (Skogheim et al., 1986; Lydersen et al., 1990; Witters et al., 1990). However, Freda et al. (1990) concluded that purified humic and fulvic acids themselves might be toxic in water with high concentrations of dissolved organic carbon (DOC). In addition, they further concluded that labile aluminium (LAL, i.e. cationic inorganic Al) obtained by the ion exchange column, i.e. Amberlite IR-120 (Driscoll, 1984), was not a good predictor of pond water toxicity to amphibians at DOC concentrations higher than 5.7 mg L^{-1}, despite the fact that LAL has been documented being the most toxic Al-form to fish in numerous studies (e,g. Driscoll, 1984; Rosseland and Skogheim, 1984; Neville, 1985; Nevile and Campbell, 1988; Lydersen et al., 1990). Regarding toxicity of Al, it is probably also important to distinguish between amphibian larvae with external gills, and thus directly exposed to the pond water like in the smooth newt *Lissotriton vulgaris* and amphibian larvae with internal gills like the common toad *Bufo bufo*, exposed to their own internal environment (Skei and Dolmen, 2006). The latter gill system is much more protected towards the very surface-active inorganic, cationic Al (LAL) present in surface water, the primary toxic Al-species bound to fish gills, with subsequent hypoxia and death (Neville, 1985, Neville and Campbell, 1988). Also the pool frog has internal gills.

Our field investigation within the area of the pool frog population in Norway, includes water chemical characterization and investigations of zooplankton and benthos in 3 small lakes known to host pool frogs and 4 nearby lakes or ponds. The aim was to achieve more information about aquatic and terrestrial habitat criteria and population status of the thermophilic pool frog, at the climatic northern boundary of its existence.

Material and Method

In June 2018, water chemistry, zooplankton and benthos were investigated in 7 small lakes/ponds located in the county of Agder, the central distribution area for the Norwegian pool frog population (Figure 1). From each site, 1 L of sub-surface water was collected in clean prewashed polyethylene bottles for water chemical characterization. Zooplankton samples were collected by net hauling from approximately 1 m above the sediment to the surface by a Wisconsin seine net of 25 mm mesh. The samples were transferred to 100 mL glass containers before preserved with Lugol's iodine solution.

Figure 1. Map of the investigated pool frog area in Agder, southernmost Norway.

Benthos were collected with hand-hold dip nets, where two people sampled along the shoreline for a period of 15 min each, covering most of the shoreline of the small lakes/ponds. Sampled benthos were stored in glass bottles and conserved in 96% ethanol. When back from field, all samples were stored dark and cold (4°C) until analyzed.

Prior to sampling at each site, all sampling devices (net haule, dip nets, vaders etc.) were disinfected with Virkon S to avoid distribution of possible unwished contaminants between sites. Among many potential threats for the pool frogs, *Batrachochytrium dendrobatidis* is a fungus that causes the disease chytridiomycosis in amphibians (Jaeger et al., 2017). This disease was discovered in Norway for the first time in 2017 (Nielsen et al., 2019).

Water analyses were conducted in the laboratory at the University of South-Eastern Norway, Campus Bø, according to standard methods (Table 1). The acid neutralizing capacity (ANC-1) and ANC-2, incorporating strong organic anions, was estimated according to Lydersen et al. (2004).

Analyses of zooplankton were conducted at the Inland Norway University of Applied Sciences), while the benthos analyses were conducted at the University of Bergen.

Statistical analyses were conducted by Eirik Fjeld (Fjeld & Vann AS), former senior researcher at NIVA (Norwegian Institute for Water Research). As we had many physio-chemical environmental variables, often strongly intercorrelated, we tried to find a proper subset by removing superfluous variables that only had minor contributions to the description of the major environmental gradients. A PCA analysis showed that using a subset of 6 water-chemical variables (turbidity, pH, Ca, TOC, Tot-N and Tot-P), about 84% of the variations were described by the two first components, PC-1 and PC-2. Accordingly, we used these variables as candidates in the gradient analysis (CCA: Canonical Correspondence Analysis) on each of the two groups of invertebrates (zooplankton and benthos) separately. Here, we used the model-building approach outlined by Gardener (2014) to create a "minimum adequate" model for our CCA, starting with a blank model and using the full model to select the candidate variables. If any potentially significant terms were found, then we added the best to the model. This was repeated until there were no more significant terms. The statistical analyses were done by the computer programs JMP (SAS Institute, 2010) and R (R core team, 2019) with the package vegan (Oksanen et al., 2019).

For the sake of species and locality protection, we have not dealt in detail with respect to the position of the individual lakes and ponds.

Table 1. Water chemical parameters analyzed in the 7 small lakes/ponds in Agder, southernmost Norway, sampled June 19, 2018.

Parameter	**Unit**	**Reference**
pH	$-\log[H^+]$	NS 4720
Conductivity	$\mu S\ cm^{-1}$	NS-ISO 7888
Turbidity	NTU	NS-EN ISO 7027-1:2016
Color	$mg\ Pt\ L^{-1}$	NS-EN ISO 7887:2011 C
Alkalinity	$mmol\ L^{-1}$	NS 4754
Ca^{2+}, Mg^{2+}, Na^+, K^+, NH_4^+	$mg\ L^{-1}$	NS-EN ISO 14911
Cl^-, SO_4^{2-}, NO_3^-	$\mu g\ L^{-1}$	NS-EN ISO 10304-1:2009
Total-N	$\mu g\ N\ L^{-1}$	NS 4743
Total-P	$\mu g\ P\ L^{-1}$	NS-EN 1189
TOC	$mg\ C\ L^{-1}$	NS-EN 1484-1:1997

Figure 2. Pictures of the 7 investigated lakes and ponds in Agder, June 19 2018. Photo: Marijanne Holtan, USN.

Results

Background information

The seven investigated lakes and ponds are located in the county of Agder, about 5 km inland from the ocean. They are all relatively small, varying in surface area from about 100 m^2 (Pond F) to about 1500 m^2 (Pond E).

No vegetation mapping was implemented during our field trip, but we observed that all ponds were surrounded by *Sphagnum* moss dominated wetlands (Figure 2). In addition, Pond A was also surrounded by the helophytic macrophyte common reed (*Phragmites australis*). The near-pond terrestrial areas of the 6 other ponds, consist of much less microvegetation. Furthermore, Pond G was surrounded by far the largest open bog areas. As macro-vegetation contributes significantly to terrestrial hideouts for adult amphibians, the Pond G biotope is likely the most predator exposed biotope for adult amphibians.

In southernmost Norway, amphibians normally breed in April to early June. After one to three weeks (depending on water temperature), the eggs hatch to a larva/tadpole. The newly-hatched tadpole, like for fish larvae, have food reserves contained within their yolk sacs (left-over from the egg stage). After this, they start feeding on the bottom or in the free waters. The young tadpoles have featherlike external gills emerging from behind the head on either side. As the tadpoles mature, they develop legs, and the growth and use of their lungs is matched by a gradual shrinkage of the gills. After a total of 2-3 months they metamorphose and leave the water. During our investigation in June 19, we found tadpoles of the smooth newt, in Pond A (n=1), Pond B (n=2) and in Pond G (n=4), and one tadpole individual of pool frog in Pond A.

Table 2. Size and water chemistry in the 7 investigated lakes/ponds, sampled June 19 2018.

Parameter	**Unit**	**Pond A**	**Pond B**	**Pond C**	**Pond D**	**Pond E**	**Pond F**	**Pond G**
Max depth	m	3,5	6,3	4,9	4,7	5,8	1,1	2,9
Sight depth	m	1,7	1,8	0,9	1,9	1,5	>1,1	1,5
Water temp (1m)	°C	14,8	14,9	9,9	17,4	14,7	15,8	16,8
pH	$-\log[H^+]$	6,04	5,29	4,79	5,79	5,65	4,81	5,97
K_{25}	µS cm^{-1}	45,9	30,7	38,0	31,3	38,6	27,5	35,5
Turbidity	NTU	0,61	0,59	1,27	0,90	0,58	1,40	1,22
Ca^{2+}	mg L^{-1}	3,56	1,28	1,11	1,49	1,79	0,96	1,76
Mg^{2+}	mg L^{-1}	0,91	0,61	0,61	0,65	0,72	0,56	0,70
Na^+	mg L^{-1}	4,25	4,05	4,29	3,94	4,34	2,32	3,95
K^+	mg L^{-1}	0,38	0,31	0,37	0,41	0,65	0,15	0,35
NH_4^+-N	µg L^{-1}	0	0	0	0	0	0	53
SO_4^{2-}	mg L^{-1}	1,37	1,97	1,05	1,16	1,83	1,62	2,74
Cl^-	mg L^{-1}	5,82	6,24	6,94	6,02	6,44	4,24	5,69
NO_3^--N	µg L^{-1}	212	0	0	194	199	0	221
TOC	mg L^{-1}	9,55	8,68	15,19	5,99	10,15	10,75	5,75
TOT-N	µg L^{-1}	407	339	516	367	404	598	367
TOT-P	µg L^{-1}	2,6	3,8	7,2	3,1	3,5	4,3	4,8
Alkalinity	µekv L^{-1}	209	14	0	50	31	0	39
ANC-1	µekv L^{-1}	240	81	84	102	120	45	93
ANC-2	µekv L^{-1}	207	52	32	82	86	9	73

Based on life cycle knowledge, our investigation was implemented in mid June, when the new generation of amphibians (tadpoles) are permanently living in water and their destiny completely dependent on the physico-chemical conditions within the lake. Besides potential physico-chemical challenges/threats within the localities, also threats from predators such as fish, predatory water insects (mainly Coleoptera, Hemiptera and Odonata) and birds are real. As brown trout *Salmo trutta* has entered both Pond A and Pond E, gill net fishing has been implemented in both localities early 2018, and followed up in 2019. Thus, fish was almost extinct prior to our investigations in June 19 2018.

Prior to the field investigation in June 19 2018, we knew that the pool frog individuals live and reproduce (tadpoles) in Pond A, Pond D and Pond E, while adult individuals have occasionally also been observed in Pond B, Pond C and Pond F. In addition, the species (5 males and 3 females) has previously been introduced in Pond G where successful reproduction was documented the same year and adult individuals observed up till 2 years after the introduction.

We also knew that the smooth newt has been found in 5 of the lakes/ponds, only being absent in Pond C and Pond F. The European common frog *Rana temporaria* has earlier been reported breeding in all the investigated ponds, except for Pond C and Pond F.

Physical and chemical conditions in the ponds

The maximum depth of the lakes/ponds varied from 1.1 m (Pond F) to 6.3 m (Pond B). During the field investigation in June 19 2018, the water temperature at 1m depth, varied from 9.9°C (Pond C) to 17.4°C in Pond D (Table 2). Pond C was far the coldest pond (9.9°C at 1 m), i.e. 4.8°C lower than second coldest lake/pond.

As the sites are located relatively near the coast, the water chemistry in the ponds are significantly impacted by marine derived Na^+ and Cl^-, and thus these ions are the predominating cation and anion in all ponds. The marine vicinity also explains the relatively high concentration of Mg^{2+} in the ponds, compared with the much more terrestrial derived cations, Ca^{2+} and K^+.

Far the most acidic lakes/ponds were Pond C and Pond F, with pH of 4.79 and 4.81, respectively. Both localities also exhibited alkalinity values close to zero (Table 2). They also exhibited the highest TOC concentrations (15.2 mg L^{-1} and 10.8 mg L^{-1}), but the lowest Ca-concentrations and the lowest acid neutralizing capacity values (ANC-1 and ANC-2) among the ponds. Tadpoles of the three amphibian species registered, have so far never been found in these two ponds.

Far the highest Ca concentration (3.56 mg L^{-1}) was present in Pond A, which was the only lake/pond with pH above 6 (Table 2).

Regression analyses including all ponds, exhibited significantly positive relationships between pH and Ca and between pH and ANC-1 and/or ANC-2 (Figure 3).

Zooplankton distribution in the ponds

Altogether, 28 species/species groups were identified in the seven lakes/ponds, clustered into 6 different copepods, 8 cladocerans and 14 rotifers (Table 3). The number of species/species groups varied from only 6 species in Pond A up to between 11-16 in the other lakes/ponds.

The most common copepod was *Eudiaptomus gracilis* present in all ponds, except in Pond F, followed by *Mesocyclops leuckarti* found in 5 localities, down to the lowest occurrence, i.e. only one individual of *Macrocyclops albidus*, found in Pond B (Table 3). Among the cladocerans, the *Daphnia longispina* group and *Diaphanosoma brachyurum,* both showed up in 5 of the 7 investigated ponds, down to only one individual of *Moina* sp. found in Pond F.

Of the 14 rotifers species identified, *Polyarthra minor* was recorded in all ponds, except Pond A, closely followed by *Pompholyx sulcata*, *Lecane* spp. and *Trichocerca* sp., all with occurrence in 5 of 7 ponds. *Keratella serrulata*, *Keratella cochlearis* and *Filinia* sp. showed up in only

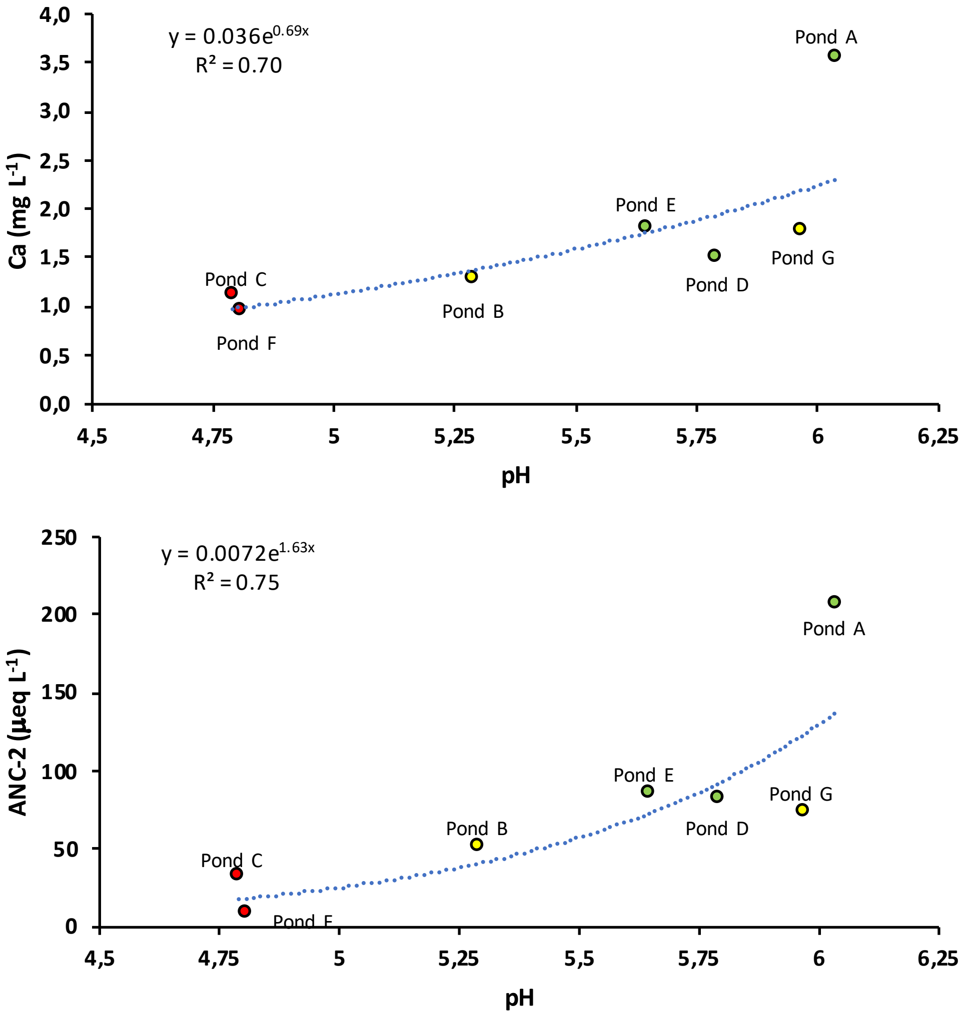

Figure 3. Relationships between pH and Ca, and pH and ANC-2 in the 7 lakes/ponds investigated, June 19, 2018. Green points: Pool frog: Small, but permanent population. Yellow points: Pool frog: Occasionally observed. Red points: Pool frog: Absent.

one site each, in Pond F, Pond G and Pond F, respectively (Table 3).

A Canonical Correspondence Analysis (CCA), ordinating zooplankton towards the most relevant chemical variables (turbidity, pH, Ca, TOC, Tot-N and Tot-P, based on PCA analysis), showed that Tot-P, Tot-N, pH and TOC, each can contribute significantly to explain the distribution of zooplankton between lakes/ponds. However, several alternative models have almost identical explanation power. A stepwise approach showed that the variables Tot-P and pH together, best explained the distribution of zooplankton between ponds, and CCA1 and CCA2 explained 27% and 22% of the variance in the dataset, respectively (Figure 4).

Benthos distribution in the ponds

All benthos species found are common in ponds and small lakes along the coast of southern Norway. Odonate larvae dominated in all the investigated ponds, with *Leucorrhinia dubia* as the most abundant dragonlfly (Anisoptera), and *Lestes sponsa* as the most abundant damselfly (Zygoptera). Thus, in absence of fish, odonates,

Table 3. List of all metazoan zooplankton species collected in the pelagic part of the 7 lakes and ponds, sampled 19 June 2018, by net hauling from approximately 1 m above the sediment to the surface, by a Wisconsin seine net of 25 mm mesh.

Species/Pond no.	**Pond A**	**Pond B**	**Pond C**	**Pond D**	**Pond E**	**Pond F**	**Pond G**
Copepoda:							
Heterocope saliens (Lilljeborg, 1863)					3		1
Eudiaptomus gracilis (G.O. Sars, 1863)	30	32	1	5	46		12
Cyclops scutifer G.O. Sars, 1863	5	1					
Mesocyclops leuckarti (Claus, 1857)		8	12	4	1		101
Macrocyclops albidus (Jurine, 1820)		1					
Diacyclops nanus (G.O. Sars, 1863)			1			1	
Cladocera:							
Daphnia longispina group G.O. Sars, 1862	59	14		3	31		2
Ceriodaphnia quadrangula (O.F. Müller, 1776)		1	3			2	
Diaphanosoma brachyurum (Liéven, 1848)	93	31	2	80			4
Bosmina longispina Leydig, 1860			1		4		11
Moina sp. Baird, 1850						1	
Polyphemus pediculus (Linnaeus, 1761)	4	3	44	1			
Acantholeberis curvirostris (O. F. Müller, 1776)			5		1	9	
Chydoridae spp. Stebbing, 1902		2	2		1	1	
Rotifera:							
Keratella serrulata (Ehrenberg, 1838)						4	
Keratella cochlearis (Gosse, 1851)							1
Keratella ticinensis (Callerrio, 1920)		1	13				
Pompholyx sulcata Hudson, 1855		1	2		1	7	9
Filinia sp. Bory de St. Vincent, 1824						1	
Polyarthra minor Voigt, 1904		4	3	2	2	3	2
Ascomorpha sp. Perty, 1850		1		12	1		
Conochilus unicornis Rousselet, 1892				29	3		10
Euchlanis spp. Ehrenberg, 1830					1	14	
Gastropus spp. Imhof, 1888		4	1			7	
Lecane spp. Nitzsch, 1827			2	1	2	24	2
Collotheca spp. Harring, 1913		1	4		1	2	
Trichocerca sp. Lamarck, 1801	1	1	1	8		5	
Asplanchna priodonta Gosse, 1850				19		1	

beetles (Dytiscidae) and water boatmen (Corixidae) are likely the primary aquatic top predators in these lakes/ponds, in addition to the amphibian species.

Small freshwater clams were only found in Pond A, the locality with the highest pH (> 6) and the highest Ca-concentration, i.e. 3.56 mg L^{-1} (Table 2).

Table 4. List of all benthos collected along the shoreline of the 7 lakes and ponds, sampled 19 June 2018, by use of hand-hold dip nets during 30 minutes of sampling.

Taxa/Pond no.	Pond A	Pond B	Pond C	Pond D	Pond E	Pond F	Pond G
Small freshwater clams							
Pisidium sp.	1						
Sphaerium sp.	1						
Odonata							
Zygoptera (damselflies)							
Lestes sponsa	5	2	4	9	16	20	12
Pyrrhosoma nymphula	1	2					1
Coenagrion sp.	2	1	1			2	
Enallagma cyathigerum	2	2	3	1			2
SZygoptera (damselflies)	**10**	**7**	**8**	**10**	**16**	**22**	**15**
Anisoptera (dragonflies)							
Aeshna grandis		1			3		
Aeshna sp.	1		2				
Leucorrhinia dubia	17	20	7	9	16	13	7
SAnisoptera (dragonflies)	**18**	**21**	**9**	**9**	**19**	**13**	**7**
Ephemeroptera (mayflies)							
Cloeon simile		1					
Hemiptera							
Corixidae (water boatmen)							
Corixidae indet. (juvenile)	1		2	5	3	2	11
Gerridae (water striders)							
Gerris sp.	2		3		3	2	
Coleoptera (beetles)							
Dytiscidae indet.				1		1	2
Gyrinidae indet.	2		1				
Diptera							
Chaoboridae (phantom midge)							
Chaoborus sp.		1		1			
Trichoptera (caddisflies)							
Agrypnia obsoleta							1
Aranea (spiders)							
Aranea indet.			3	1			
Amphibians							
Lissotriton vulgaris (larvae)	1	2					4

A CCA analysis ordinating benthos versus the most relevant water chemical variables, showed that none of the 6 *a priori* most promising water chemical variables contributed significantly to the model. The most promising variable was TOC, but its contribution was not

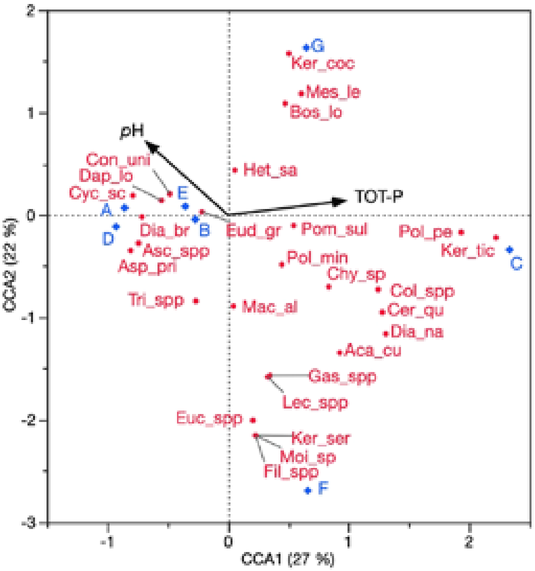

Figure 4. Canonical Correspondence Analysis of absolute occurrence of zooplankton species versus Tot-P and pH, expected à priori water chemical variables for biological occurrence. Lakes and ponds are marked blue letters. All zooplankton are marked red, crustacean species (Cladocera and Copepoda) named with 5 letters, Rotifera species with 6 letters.

significant. Thus, no water chemical variable measured could significantly explain the occurrence or distribution of benthos species in our 7 lakes/ponds.

Discussion

Historically, impacts of acid rain has likely been a bottle neck for the reproduction success of the pool frog in Norway, but this impact has been significantly reduced since the 1980's, i.e. up to 80-85% reduction in the input of strong acids in this area of southernmost Norway. Dolmen et al. (2018) also showed a recovery in the amphibian populations in southernmost Norway during the period 1988/89–2010. In 5 of the 7 investigated ponds, the at present water conditions seem to be acceptable for reproduction of the pool frog.

It is well documented that there is an ontogenetic variation in acid tolerance between amphibian, where toxic effects are most acute at fertilization, less toxic effects during embryo development, and even less toxic effects for tadpoles (Pierce, 1985). As far as we know, acid tolerance in adult amphibians has not been extensively tested.

As frog and toad tadpoles have internal gills, they are likely less sensitive to water acidification than newt larvae, which have external gills (Skei and Dolmen, 2006). The difference is predominantly due to inorganic, cation Al-species (LAL: labile Al) which has been shown to bind strongly to the surface of external gill surfaces of freshwater fish, a phenomenon well described, since first published, independently by Schofield (1977) and Dickson (1978). Thus, since larvae of the smooth newt have been found in as much as 5 of the investigated ponds, varying in pH from 5.29 to 6.04, it is reasonable to believe that the pH regimes of these ponds are acceptable for the reproduction success of the pool frog as well, as life stages of the smooth newt should be even more acid sensitive

Climate and the presence of (predatory) fish are very important limiting factors for the distribution of the pool frog in Norway (Dolmen, 2012). In addition, pH and TOC likely represent the primary limiting water chemical factors for its reproduction and further expansion. The lowest pH values (4.79 and 4.81) and highest TOC levels (15.2 and 10.2 mg C L^{-1}) measured in Pond C and Pond F, respectively, are probably too extreme for successful reproduction of many amphibian species (Freda et al., 1990, Skei and Dolmen, 2006). Consequently, these two locations are not proper reproduction and expansion sites for the pool frog in the area.

However, we should not underestimate the fact that non-lethal effects, including depression of growth rates and increases in developmental abnormalities, can occur at higher pH values than what have been documented being lethal (Pierce, 1985). This is likely also relevant for the pool frog in our ponds with pH-values from 5.29 – 6.04.

Toxic effects of high TOC concentrations *per se* on embryos and tadpoles of the pool frog in these 5 lakes/ponds, is unlikely as the TOC concentrations are not very high, and the pH values higher than that reported being lethal to embryos and tadpoles of several amphibian species studied (Freda et al., 1990).

The fact that the CCA demonstrated a significant relationship between the occurrence of zooplankton species and water chemical variables (pH and Tot-P), whereas no such similar significant relationship was found for benthos, might be spurious. However, a significant relationship between zooplankton and water chemistry is more likely as all these species are permanent water organisms, probably with more specific requirements with regard to water chemistry and their localities' trophic status. Most benthos species are however temporal water organisms, except for the small freshwater clams in Pond A. As for macro-invertebrates on the bottom etc., dragonfly larvae in general have been shown to be very tolerant to acidic water throughout their life stages (Dolmen and Pedersen, 2018). Also many Dytiscidae larvae in bog pools are known to be tolerant.

Pool frog tadpoles graze on "Aufwuchs" on the vegetation, on stones etc., besides organic remains in the mud, but they also filter-feed micro-organisms in free waters (e.g. Rist et al., 1997 and Wells, 2007). The presence of some zooplankton species in certain life stages, alive or dead, may therefore possibly be important as food for tadpoles. In general, also a high number of invertebrates in a locality results in a richer organic percentage in the bottom mud, i.e. more nourishment for the tadpoles. At least in part, this may explain why pool frogs choose some lakes and others.

Conclusion

The European pool frog is generally accepted as native in this area of Norway.

As the pool frog is thermophilic, the geographic distribution of the Norwegian population is minor, limited to only a very small area in the county of Agder, southernmost Norway. Thus, climate change, i.e. warmer and wetter weather in this area, and the significant reduction in acid rain, will likely favor an expansion of this species in the years to come.

One critical water chemical parameter that still remains to be better evaluated, is the concentration of total organic carbon (TOC). TOC has likely increased in the ponds within this area during the latest years. One reason is the reduction in acid rain, causing a pH increase with subsequent increased mobilization of organic carbon from the catchment (Monteith et al, 2007). Another reason is warmer and wetter climate, also causing an increased TOC mobilization from terrestrial into aquatic systems. High TOC *per se* might be toxic for amphibians and thus a potential limiting reproduction factor. However, regarding both embryos and tadpoles, no such TOC effect has so far been documented at pH above 5 (Freda et al. 1990). The recovery of e.g. the *Daphnia longispina* group in this area, as a consequence of reduced acid stress (Nilssen and Wærvågen 2002) may also provide an important food resource for the pool frog tadpoles, alive or dead. In addition, a high number of invertebrates in a locality *per se* results in a richer organic percentage in the bottom mud, i.e. more nourishment for the tadpoles.

Other threats for the Norwegian pool frog population is the presence of predators. Tadpoles are eaten by fish, beetles, dragonfly larvae and birds, while adult frogs have predators as grey heron, crows, gulls, ducks, badger, otter and snakes, in this area primarily the grass snake. As brown trout has entered (released?) some of the small lakes, gill net fishing has been implemented to eliminate the only "invasive" species in this area.

Another challenge for the pool frog is the limited number of individuals present in the Norwegian population, i.e. < 50 individuals. Small populations normally lead to loss of heterozygosity and reduced genetic diversity and loss or fixation of alleles and shifts in allele frequencies (e.g. Frankham et al., 2002). Thus, small populations are then more susceptible to demographic and genetic stochastic events, which can impact the long-term survival of the population. Therefore, small populations are often considered at risk of endangerment or extinction. A breeding program funded by the Norwegian Environmental Protection Agency, is therefore established in order to reduce this problem and rescue the European pool frog in the Norwegian fauna.

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