Impact of nearby agricultural fertilizer application on nutrient content of freshwater ponds at Naphill Common, Buckinghamshire.

Report of an individual study carried out as part of the requirements for the Degree of Bachelor of Science of Canterbury, Christ Church University, Canterbury.

The impact of nearby agricultural fertilizer application on nutrient content of freshwater ponds at Naphill Common, Buckinghamshire.

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Abstract: Agricultural land dominates much of Western Europe and Britain and is necessary to provide food for an increasing human population. However, inorganic fertilizers which promote higher yields and profit for farmers are being applied in large volumes resulting in increased leaching and run-off rates depending on soil type. In this study, Naphill Common within the Chilterns AONB is the proposed site at risk of nutrient enrichment, with adjacent Bradenham farm as the hypothesised non-point source of pollution. The freshwater concentrations of phosphate (P), ammonia (A) and oxidised nitrogen (TON) were determined through colorimetric analysis of samples collected at five ponds, then correlated to each ponds' distance from the farm. Field measurements of pH revealed no immediate threat, while conductivity highlighted Pickups' pond (206 µS) as a likely site of ammonia enrichment. A relationship was observed between TON and farm distance (p=0.033), and nutrient analysis revealed 80% of ponds to have P concentrations exceeding 100 ug/l, while 40% exceeded 1000ug/l. Eutrophic conditions such as this reduce the suitability of habitats available for the great crested newt, *Tritarus cristatus* populations which inhabit ponds at Naphill Common and pose a threat to biodiversity legislation aimed at increasing numbers and range of the animal. Total expenditure by the UK to mitigate eutrophication of freshwaters is aimed mostly at lakes and streams, therefore ponds remain criminally underrated in terms of biodiversity and ecosystem services. This project aimed to describe the degree of nutrient enrichment impacting ponds within a broadleaf woodland stand of the Chilterns and discusses how variables not considered here could be analysed in future projects.

Key words: freshwater, pollution, phosphorous, nitrogen, ammonia, agriculture, woodland, eutrophication, spectrophotometry, amphibian

INTRODUCTION

In the UK, an estimated 400,000 ponds cover 14% of the country (Bailey-Watts *et al.,* 2000), and despite their perceived low value, many are home to a greater diversity of aquatic invertebrate species than any other freshwater body type relative to area (Williams *et al.,* 1998). However, the high diversity of associated species relies on dense distribution of ponds, and so it is important that we manage these sustainably to prevent hydrosere succession into terrestrial land (Williams *et al.,* 2010), risking the loss of these species. An example is the Great-crested newt, *Triturus cristatus* L. (Caudata Salamandridae), an amphibious species which is known to be highly sensitive to water quality changes (Arntzen *et al.,* 2009), and whose decline is directly linked to habitat loss (Perring and Franklyn, 1976). For *T. cristatus* and other protected species, population decline because of habitat destruction and reduced water quality from anthropogenic activities is the largest threat of which are experiencing population decline due to habitat destruction and reduced water quality (Gustafson *et al.,* 2009).

Pond systems are under threat from urban development and agricultural practices which reduce pond density and therefore connectivity of this unique habitat. A Government-led survey of ponds in Britain linked the decline in quality and number of countryside ponds to intensive arable farming (Williams 1996).

Spray drift from application of nitrogen to arable fields also disrupts distribution patterns and reduces biodiversity (Marshall 1988). Intensive farming and modern mechanised practices have improved the average UK cereal yield from 5 t/ha^{-1} to 7 t/ha^{-1} since 1987 (Barr *et al.*, 1993), and in that period Nitrogen application on grass crop fields increased by 40 kg/ha^{-1} (Benford, 2017).

As human populations increase, it is no surprise agriculture dominates land use throughout Western Europe (Robinson and Sutherland, 2002), food is needed for a predicted 13% population increase by 2050 (Nash, 2017). However, fertilizer application can lead to significant run-off that results in pollution of freshwater systems that are poorly buffered. Nutrients including nitrogen and phosphorous become pollutants of particularly freshwater bodies when their concentration is high enough to stimulate growth of plants or algae beyond a sustainable level (VanLoon and Duffy, 2011). As photosynthesis occurs and nutrient availability no longer becomes a limiting factor, growth rates increase exponentially (Schindler, 2006) causing blooms until dissolved oxygen is depleted and the water is considered hypoxic. Algal blooms are more common in ponds as waters are usually calm (Jöhnk *et al.,* 2008), but as resources are depleted, death and decomposition of algae further depletes oxygen levels and even tolerant taxa will begin to die off. Hypereutrophic phosphate levels at Naphill could result in large algal blooms during the Summer months as longer daytime hours provide sunlight needed for photosynthesis of cyanobacteria. Recent figures suggest impact abatement and mitigation strategies in the UK alone cost £114 000 000 annually (Pretty *et al.,* 2003).

The aim of this project was to determine the nutrient levels of five natural ponds and compare literature to find the greatest risks to organisms which inhabit these ponds. This was done through nutrient analysis of collected water samples. The non-point source polluter being studied here is Bradenham farm, a cereal-crop farm occupying land directly adjacent to Naphill Common. Grid reference data were also collected, and analysis run to determine correlation between concentration and distance from the farm. This paper seeks to characterise the current state of nutrient enrichment of ponds at Naphill Common and determine the level of risk to populations of the great crested newt, *Triturus cristatus.*

1. Null: There is no significant relationship between any pond's distance to Bradenham farm and its measured concentration of phosphate, ammonia or total oxidised nitrogen.
2. Null: Nutrient concentration does not exceed published water quality guidelines.
3. Null: Water quality of pond poses no threat to associated species.

METHODS

Study Area

Naphill common comprises 61 hectares of the Chilterns AONB in Buckinghamshire. Five ponds were chosen, Pickups; Daisy; Lady Horses; Willow and Shipwash, each at varying distances from nearby arable land suspected to be the cause of nutrient enrichment.

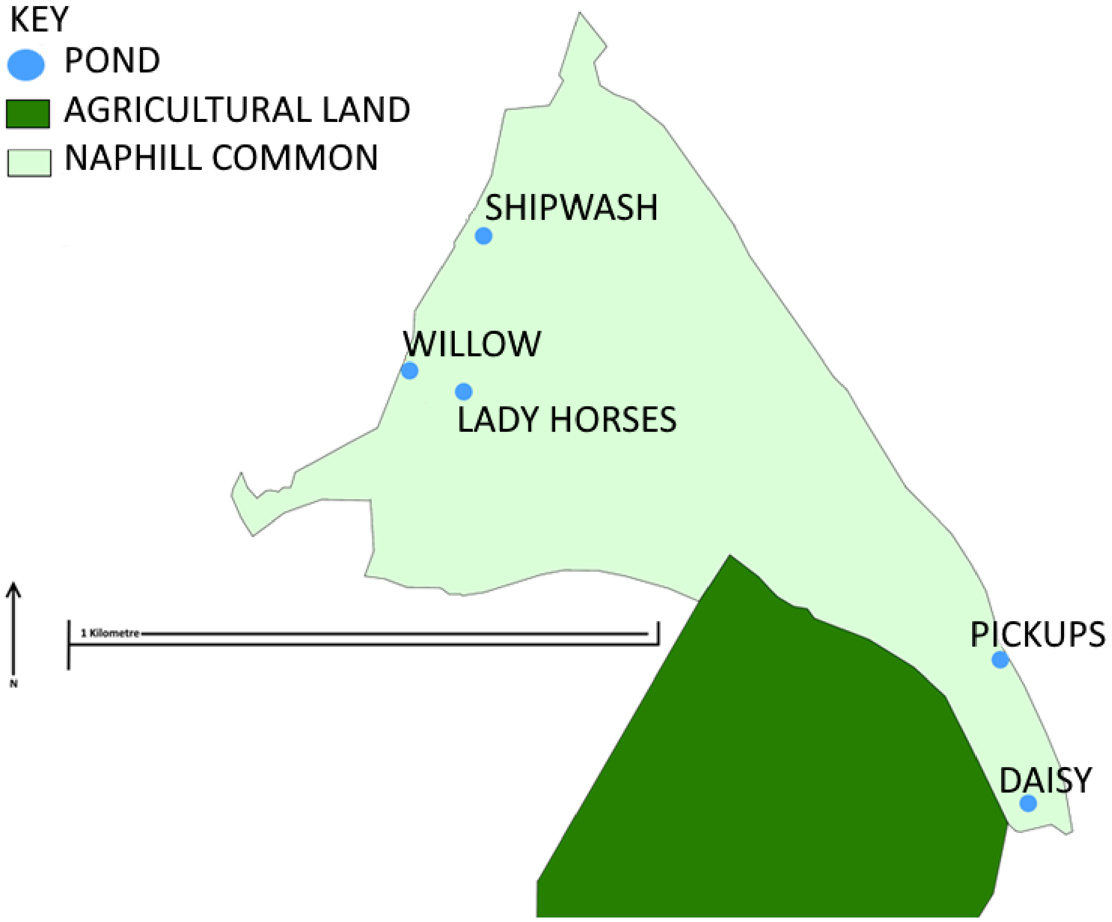

Figure 1. Map of pond site locations within Naphill Common. The site is roughly 61ha, and Bradenham farm is visible at the lower boundary to Naphill. Agricultural land here is used mostly to farm wheat and cereals.

Sample collection

Water samples were collected from five ponds at Naphill Common on the 14th February 2018, in 50ml capped plastic bottles. Weather was overcast but dry, air temperature approx. 5°C. One sample and one replicate were taken at the central point of each pond for a total of ten samples. Grid references recorded using GPS status application on a Samsung Galaxy S7 (MobiWIA Ltd, Miskolc, Hungary). Grid reference also taken at several points along common-farm boundary to calculate distances using mobile application.

Conductivity measured in situ using DiST 3 EC tester and pH using pHep®4 tester (HANNA® Woonsocket, USA); each held in place until stable value was given. These were rinsed between sites using distilled water to prevent cross-contamination.

Standards and Reagents

Phosphate	***Ammonia***	***Total oxidised nitrogen (TON)***
PHOS R1 (984366)	AMM R1 (984362)	TON R1 (984369)
PHOS R2 (984366)	AMM R2 (984363)	TON R2 (984370)

		TON R3 (984371)

Four standards were prepared to create colorimetric calibration curves for PO_4, TON and Ammonia (Appendix A1-3). Only the highest concentration standard is required as the instrument automatically dilutes solutions to produce a standard calibration curve. Phosphorous standard prepared by pipetting 1ml of 9.49 x 10^{-1}M PO4 stock into a sterile volumetric flask and diluted to 100ml with deionised water to produce a working standard of 10mg/l. This was capped and shaken thoroughly for 1 minute prior to use. TON standard: 20mg/l TON; and Ammonia standards: 2mg/l NO_2 and 1mg/l NH_4 prepared by lab technicians and stored in a dark refrigerator prior to analysis.

Table 1. List of reagents used to determine concentration of three analytes in nutrient analysis.

ThermoFisher© brand reagents (ThermoFisher Scientific, Waltham USA) were used in discrete analysis, therefore exact composition of each reagent is unavailable. Table 1 shows the reagents used for each test parameter along with its ThermoFisher© product ID.

Bottled reagents had caps removed and were placed in Gallery in two reagent racks, ensuring TON R1 and ammonia R2 kept separately as interference occurs during calibration. Standards used to fill 2ml cuvettes and placed into sample rack of Gallery. Calibration run from Gallery software following insertion of standards and reagents.

Chemical analysis

Ten water samples had their concentrations of PO_4, Total organic nitrogen and Ammonia determined by the colorimetric method using a ThermoFisher Scientific Gallery Automated Analyzer© (ThermoFisher Scientific, Waltham USA). Samples were gravity-filtered prior to analysis using a stemmed funnel and folded paper and frozen until needed.

Following calibration, water samples plus one control of distilled water were poured into individual 2ml cuvettes and placed in the Gallery sample rack. Using predetermined settings, tests for Ammonia, PO_4, and NH_4 as N were selected from the software interface and run for all samples. UV-Vis spectrophotometry and Beer-Lambert law calculations determine concentration of analyte proportionally to light absorbance of sample. Light absorbance measured at 880nm for PO_4, 540nm for TON and 660nm for Ammonia.

Statistical analysis

Analysis of data completed in MiniTab 17® (MiniTab Ltd, Pennsylvania USA). Distribution of data tested using Ryan-Joiner normality. P-value exceeding alpha of 0.05 is normally distributed (Ryan and Joiner 1976).

Pearson correlation applied to determine whether distance from Bradenham farm has a significant negative relationship to nutrient concentrations. Significant result inferred at the 99.5% confidence interval by a p-value lower than 0.05.

Results

Phosphate concentrations ranged from 60 µg /L at Pickups pond to 1115 µg /L at Daisy pond. Shipwash contained 1025 µg /L, while the remaining Lady Horses and Willow contained 245 and 100 µg /L respectively (Figure 2). There was no significant relationship between distance from agricultural land and PO_4 concentration (Pearson correlation= -0.015, p=0.968).

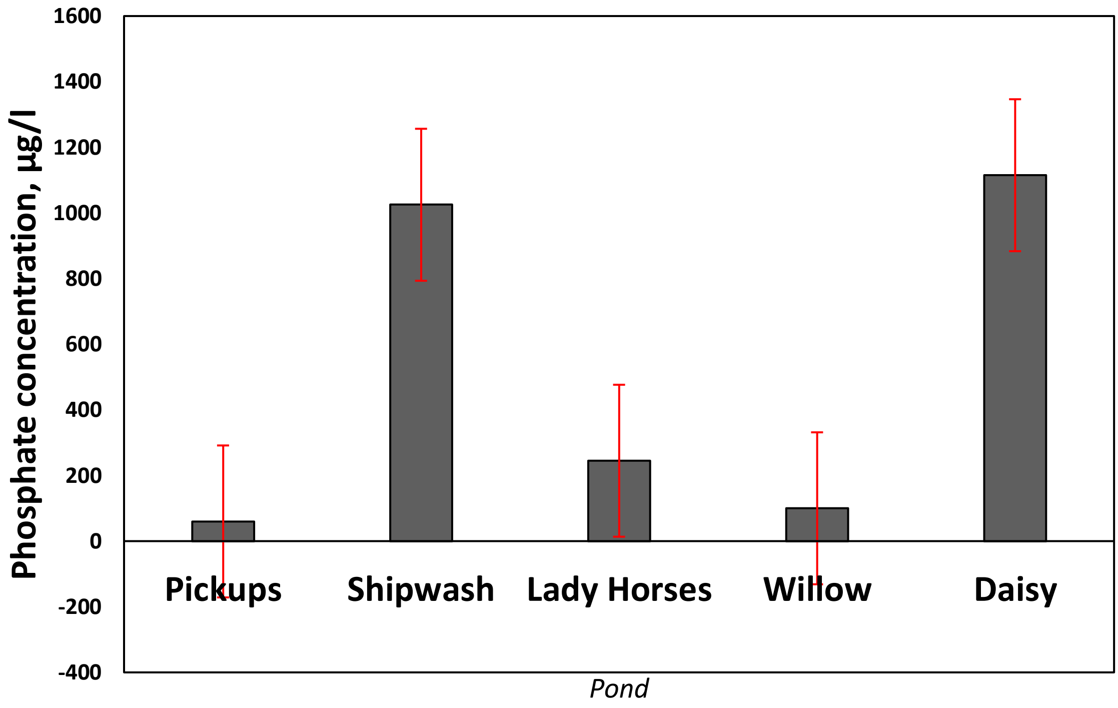

Figure 2. Phosphate concentrations determined in water samples collected from five ponds at Naphill. Standard error bars shown in red.

Ammonia and TON analysis returned negative concentration values for all sites (Figures 3; 4). TON of water samples ranged between -200 µg /L at Pickups to -135 µg /L at Shipwash (Figure 3). However, TON variation between ponds correlated significantly with distance from agricultural land (Pearson correlation = 0.674, *p*=0.033).

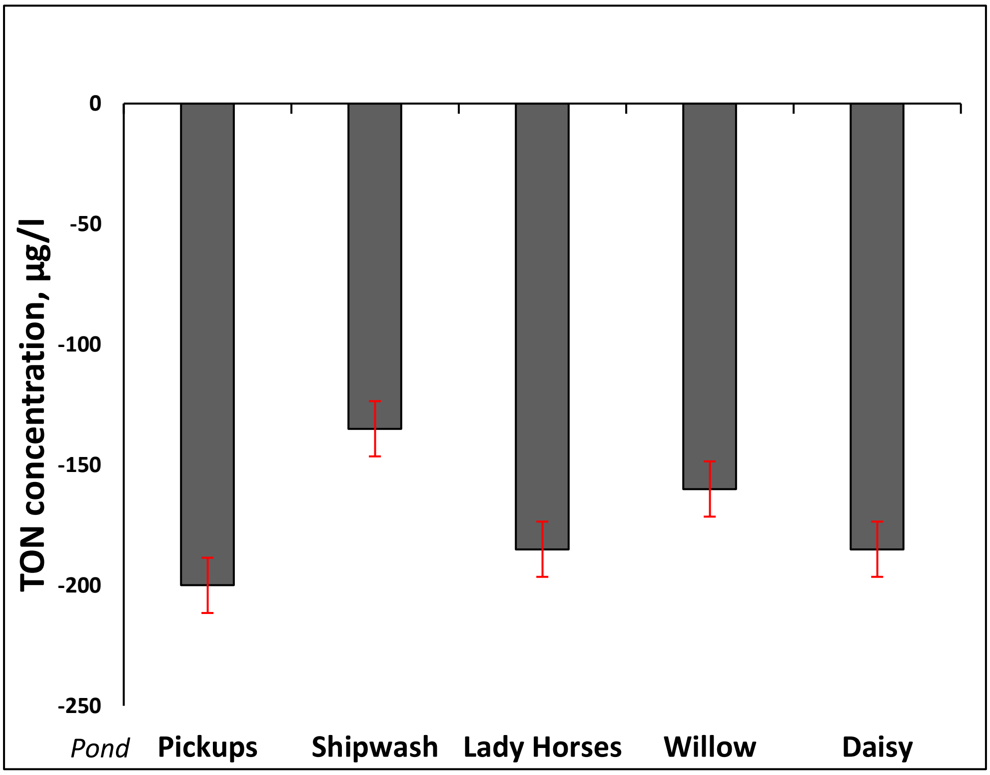

Figure 3. Total oxidised nitrogen (TON) concentrations in water samples taken from ponds at Naphill. Standard error bars shown in red.

The range of ammonia concentrations between ponds, 1845 µg /L in total (Figure 4). The lowest value -2050 µg /L at Daisy and the greatest being -205 µg /L at Shipwash. No significant relationship linking ammonia concentration with distance from agricultural land (Pearson correlation = 0.036, *p*=0.922).

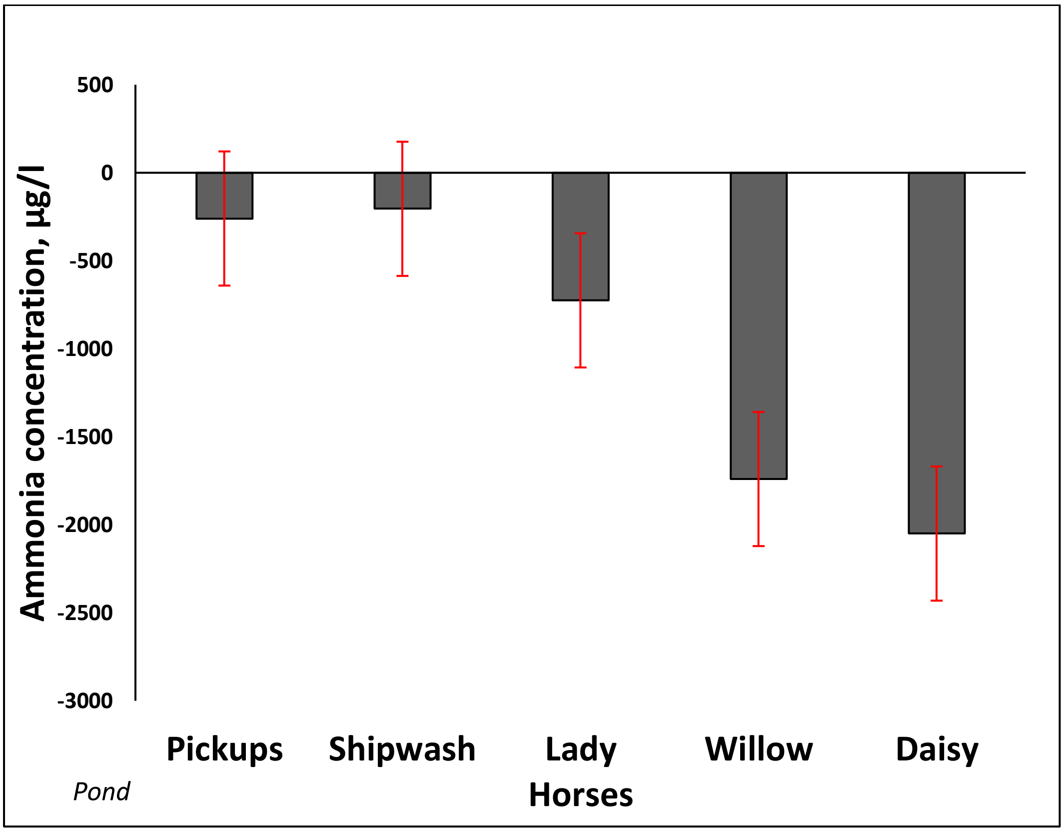

Figure 4. Concentration of ammonia ions present in water sampled from ponds at Naphill. Standard error bars shown in red.

Table 2. Pond's distance from Bradenham farm.

Pond	**Distance from farm** **m**
Pickups	120
Daisy	190
Lady horses	520
Willow	550
Shipwash	630

Ponds were sampled in the range of 0.12 and 0.63 km from Bradenham farm (Table 2). Lady Horses is 30m closer to farm than Willow, and Pickups-daisy distance vary by only 70 metres.

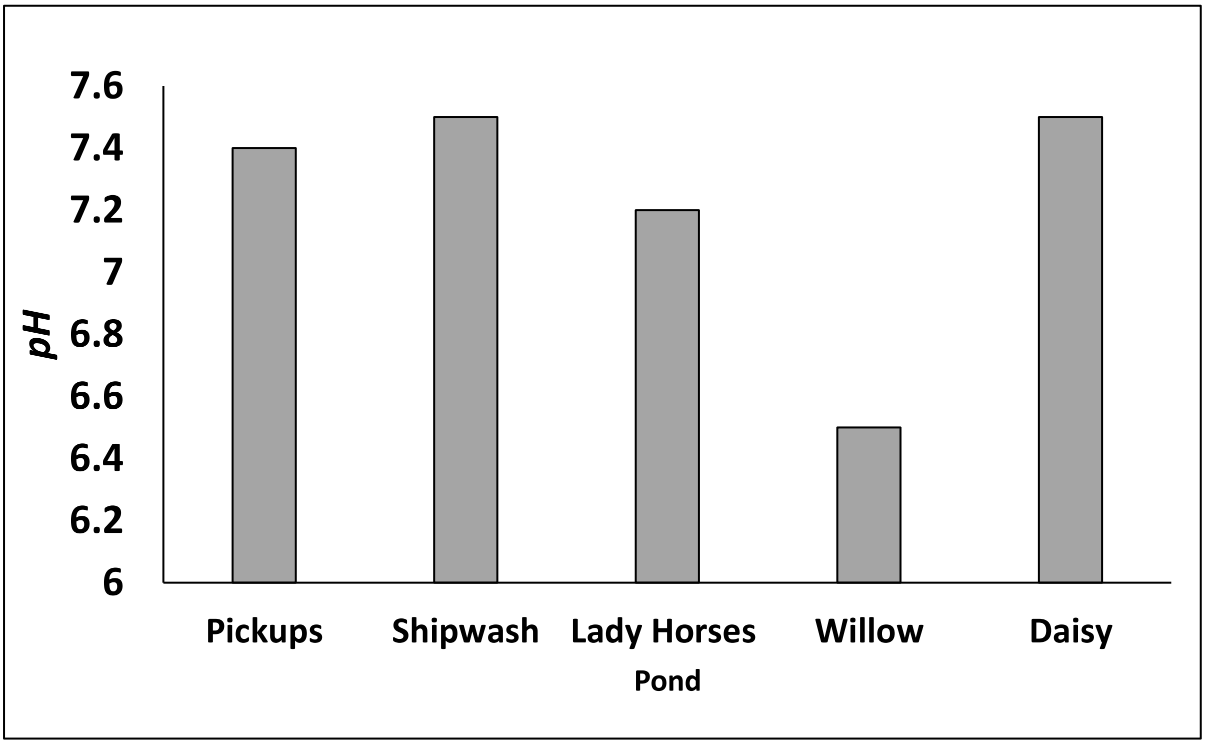

Figure 5. Single-measurement pH values of ponds at Naphill. Determined in-situ using HANNA© pHe 4.

Results of pH measurement show little variation, all values obtained within 6.5 and 7.5 (Figure 5). Willow had the lowest pH, Daisy and Shipwash the greatest.

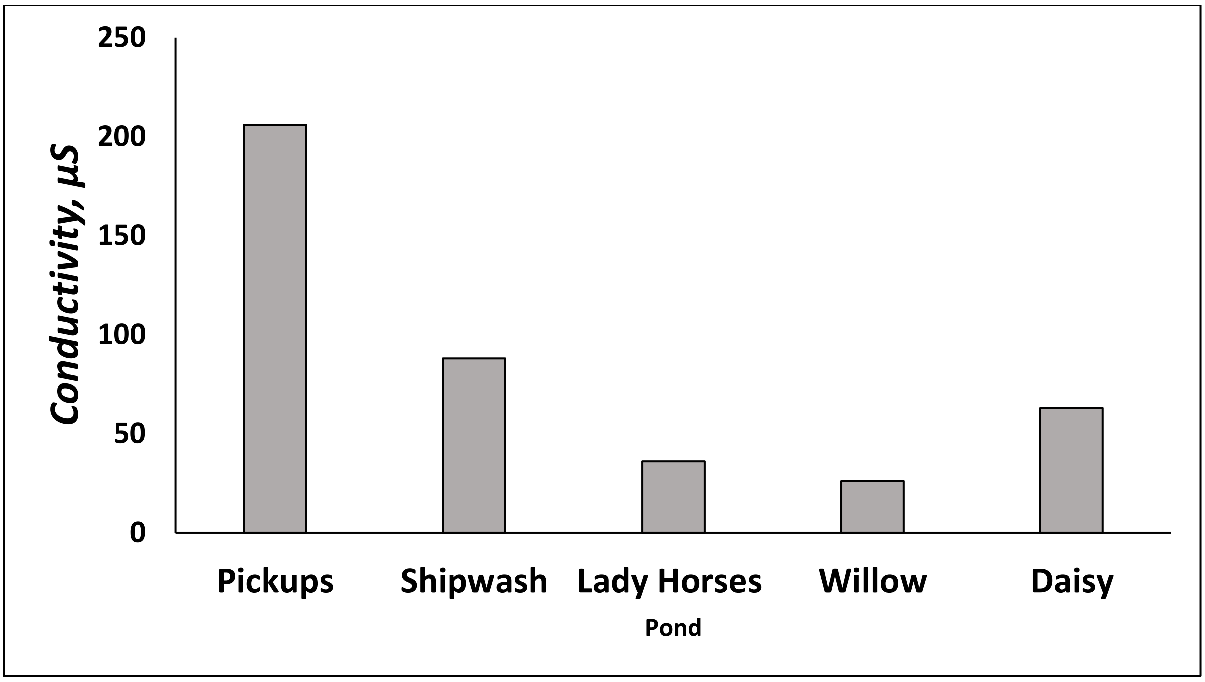

Figure 6. Single-measurement conductivity measurements of ponds at Naphill Common. Taken in-situ using HANNA EPT 3.

Greatest conductivity of 206 measured in Pickups, and lowest (26) at Willow (Figure 6) comprise a total range of values 180*µS.* All ponds but Pickups had conductivity below 100 *µS*.

DISCUSSION

The data obtained from nutrient analysis show phosphate concentrations to be elevated in 4 out of 5 ponds at Naphill (Figure 3). This is in comparison to known unpolluted ponds, which can exhibit anywhere from 0-150µg /L total phosphate (TP), however, phosphate concentrations above 40µg /L are considered eutrophic, and above 100 µg /L conditions are hypereutrophic with suspected anthropogenic input (Williams *et al.,* 2010). Pickup's pond contained 60 µg /L and is considered eutrophic, while the remaining four ponds exceed hypereutrophic conditions and are considered highly polluted. Daisy and Shipwash ponds contained TP concentrations an order of magnitude above the hypereutrophic boundary set by the freshwater habitats trust, therefore endangering the populations of associated species such as *T. cristatus*, which have a measurable preference for ponds containing low phosphate and nitrogen levels (Gustafson *et al.,* 2009).

In 4/5 ponds studied, phosphate concentrations appear to be elevated to a point where eutrophication is likely to occur (Schindler 2006). Alongside phosphate limits, water quality guidelines state the maximum acceptable level of ammonium or total nitrogen to be 500µg /L in freshwater (Williams *et al.,* 2010; VanLoon and Duffy 2011). It can not be stated whether ponds at Naphill are above this due to a technical fault with the Gallery providing negative values for TON and ammonia nutrient analyses (Figures 3; 4). Issues with calibration will be discussed further on.

Despite negative TON values (Figure 4), relative variation within the data obtained correlated with the distance between ponds and Bradenham farm to a significant degree ($p = 0.033$). This allows me to reject the second null hypothesis for TON and conclude that agricultural land is impacting TON concentrations at Naphill. Recorded TON concentrations also exhibited the lowest standard error range compared to other nutrient analysis, with low variation between values of different ponds.

Populations of *T. cristatus* known to inhabit ponds at Naphill Common (Marshall and Bennett 2013) may be at risk of losing their breeding sites if nutrient concentrations at are not significantly reduced. If species such as filamentous algae outcompete submerged and marginal vegetation by rapidly consuming P and N, blooms greatly threaten quality of water (Schindler 2006). Plants capable of rapidly converting nutrients into biomass pose a serious threat to ponds. Reduced water quality currently contributes to *T. cristatus* decline in

Europe (Gustafson *et al.,* 2009), but accelerated habitat loss may also occur as floating sweet grass; *Glyceria fluitans*, the favoured site for newt egg-laying is outcompeted by rapid-growth algae (Williams *et al.,* 2010). Furthermore, invasive species including the Australian swamp stonecrop, *Crassula helmsii* form dense surface cover and reduce the available space for male mating displays (Green 2010). The plant is often imported for use in garden ponds, however evidence suggests *C. helmsii* leaves as unsuitable sites for egg-laying amphibians due to small individual size and rigidity (Watson 1999). High nutrient availability will only serve to accelerate the decline of *T. cristatus.* Under the EU Habitats directive, Annex II includes management according to ecological requirements of *T. cristatus* (European Commision 2003b). Evidence collected here suggest concentrations of biologically available nutrients pose direct threats to the habitat requirements, and therefore violate legislation put in place to protect the species.

No survey was carried out to assess the microbiology of ponds, although this may profoundly impact the concentration of nutrients measured. Fixation of nitrogen is mediated by those including *Rhizobium spp.* a bacterial symbiote which converts inorganic nitrogen into usable organic forms within nodules attached to roots, facilitating nutrient uptake. In aqueous environments, *Nitrosomonas europaea* oxidizes nitrate to nitric oxide, a colourless gas which is then released into the atmosphere (Caranto *et al.,* 2017), and is then oxidised further by oxygen to form nitrite. The optimal pH for these reactions to occur is 6.5-8, and this range being met by all five ponds at Naphill (Figure 5) suggests nitrification may be impacting the ammonium ion concentrations of these ponds (VanLoon and Duffy 2011). Significant oxidation of ammonia in the ponds surveyed may be reflected in a linear relationship to TON concentration in which TON is consistently greater. However, TON concentrations at Naphill were found to be consistently higher than ammonia with no significant correlation (p=0.904), and nitrogen cycle fluctuations can not be characterised. Instrument calibration for the ammonia test was less accurate than P or TON tests (Appendix 3), so it is possible that data is not truly representative of ammonia concentration present in ponds.

Good quality freshwater can contain anywhere from 50-1500 μS of ions, but for a biologically diverse community to thrive a range of 150-500 μS is preferred (Williams *et al.,* 2010). No ponds exceed these values, although Pickup's (Figure 6) high conductivity may be indicative of high nutrient input or significant reduction of nitrogen compounds into ammonia ions (VanLoon and Duffy 2011).

The data suggest that combined fertilizer run-off is contributing partially to the volume of oxidised nitrogen in ponds but repeat analyses must be performed on a greater sample size which includes known unpolluted ponds to serve as controls. The hypothesised non-point source, Bradenham farm, could mitigate nutrient run-off and reduce waste by installing bio-beds adjacent to spray stations. These biological filters contain microbial communities which decompose fertilizers and pesticides into organic compounds which can then be used as manure to improve crop growth. Campbell *et al.,* (1991) first proposed the increased use of sprays as a cause for declining biodiversity near agricultural land, and although emitting phosphorous from sprays has shown reduced freshwater pollution, Haygarth *et al.,* (2016) predict that current damage to natural systems will take a lot more effort to remediate. As stated earlier, current costs to mitigate fertilizer-related damages exceed £100M y^{-1} and despite growth of agro-industries, the value of UK cropping farms increased to in 2016 and 2017 despite a 2% decrease in yield. This was offset by 13 percent increase in basic payment (National statistic 2017), a government incentive to increase the number of farmers and improve the nations self-sustainability. However, reported and actual application rates of fertilizer often vary (Leslie *et al.,* 2017), therefore reducing the reliability of environmental initiative incentives for farmers to reduce fertilizer waste.

Automated discrete analysers have improved the rate at which nutrient analyses can be completed on a sample or batch, and greatly reduce the risk involved in manual handling of reagents such as Molybdenum phosphosulfide (MoPS), used to determine phosphorous in colorimetric analyses (Holman 1943; He and Honeycutt 2005). Unfortunately, poor calibration and time constraints to fully troubleshoot this meant that the nutrient concentration data collected here is not as reliable as needed for this type of project. Calibration curves generated from standards (Appendices 1-3) were within 0.001 unit of 1 for P and TON but ammonias accuracy was noticeably lower (0.895) further reducing reliability of data. Inaccurate reading of standards could be due to human error during preparation of the working standard, although issues with the gallery were known prior.

A future study should include a detailed soil analysis of Naphill and characterisation of the variation in pH, texture and organic matter content. As soils are not well-suited to agricultural use, it has remained common land long enough for succession from heath scrub to dense broadleaf woodland (Eyers 2012). The common is underlain centrally by Loess deposits which give rise to sandy loam type soil, while the area around this contains clay type soils where reading formation clay deposits sit (Eyers 2007). Therefore, nutrient run-off dynamics will vary greatly across the common, where areas rich in clay have the potential to retain up to 83% more nitrogen and 200% phosphorous leachate (Tahir and Marschner 2017). Retention of nutrients by soils is controlled highly by cation exchange capacity (CEC), the ability of soil components to adsorb positive ions to their surface (VanLoon and Duffy

2011). In the context of this study, nitrate anions are particularly susceptible to leaching because they do not become adsorbed to negatively charged surfaces present in the soil matrix (Lehmann and Schroth 2003). This may explain to some degree why a distance-based relationship was observed for TON but not phosphate, as the latter will only be leached when present in mobile forms such as organic phosphorous (Havlin *et al.,* 1999).

The continuous presence of trees at Naphill also provide opportunities for nutrients to be retained and transformed into biomass, with multiple studies confirming lower nutrient concentrations in agroforest systems when compared to conventional agricultural land (Seyfried and Rao 1991). Responsible for this is likely the increased organic matter volume that is associated with decomposing leaf litter, which increases the CEC of soil components (Lehmann and Schroth 2003). Therefore, despite data suggesting 80% of ponds at Naphill to be in a state of hypereutrophication, increased organic matter in sandy-soil areas of the site may reduce mobility of leached nutrients and subsequently the amount of biologically available N and P that facilitates algal blooms. Additionally, sediments with high volumes of retained N and P could be manually removed from ponds to reduce the degree of nutrient enrichment, however this strategy would not be applicable at Naphill as to the protection status of *T. cristatus* restricts those without a license from disturbing known population habitats (European Commision 2003b).

CONCLUSION

Based on the data presented here, current P levels pose a threat to Great crested newt populations at Naphill, and physical removal of affected sediments is not possible due to protection under the EU habitats directive. Oxidised nitrogen concentrations in ponds correlated with distance from Bradenham farm, but detailed information regarding the geology, hydrology and soil retention characteristics must be gathered before this is determined as the major source of nutrient input at Naphill. Additionally, the impacts of biotic cycling of nutrients were not quantified here although they may have profoundly impacted the data collected. Many standard methods are available through the freshwater habitats trust to measure these factors and should be carried out in a future iteration of this project to improve power of conclusions. Improper calibration of the Gallery automated analyser greatly impacted these results to reduce the overall reliability, although this could be repaired and samples re-analysed with relative ease.

ACKNOWLEDGMENTS

I am grateful to Dr. Christopher Harvey for supervising this project and providing constructive feedback on an earlier manuscript. Thanks is given to the Buckinghamshire and Oxfordshire team at Natural England for granting permission to sample at Naphill Common, as well as Chris Miller of the Friends of Naphill Common who provided a walking tour of the site and its features in the summer of 2017. Thanks also to Dr. Phil Buckley and Rosemary Talbert at Canterbury Christ Church University for aiding in chemical analyses of water samples; along with Chris Hobbs who provided a methodology for the gallery automated analyser. I am also grateful to Liam Quigley for helping transport water samples and equipment from the study site.

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APPENDICES

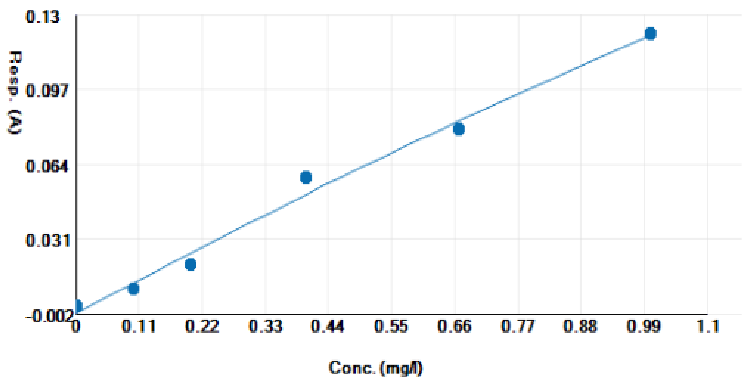
Calibration results

PO4 H Version number 1.2

Date	3/29/2018	**User**	Main user
Time	1:28:48PM	**Software version: 6.0.1**	
Analyzer name	Analyzer 1		

Test	PO4 H	**Coeff. of deter.**	0.990438
Status	Accepted	**Total factor**	8.129
Accepted	3/29/2018 11:43 AM		
Checked	3/29/2018 11:43 AM	Zero-order factor	-0.002
User name	Main user	1st order factor	0.136
Comment		2nd order factor	-0.013
Errors	Coeff. of det. limit		

Cal/Ctrl	Response	Calc. conc.	Given conc.	Lot	Errors
Water Blank	0.001	0.022	0.000	Default	
PO4-High	0.009	0.080	0.100	Default	
PO4-High	0.020	0.163	0.200	Default	
PO4-High	0.058	0.459	0.400	Default	
PO4-High	0.079	0.639	0.667	Default	
PO4-High	0.121	1.005	1.000	Default	



Appendix 1. Calibration curve generated from light absorbance readings on solutions containing phosphate in concentrations ranging 0 to 1 mg/l. Closeness of coefficient of determination value to 1.0 represents accuracy of calibration; calculated from expected and actual concentration responses.

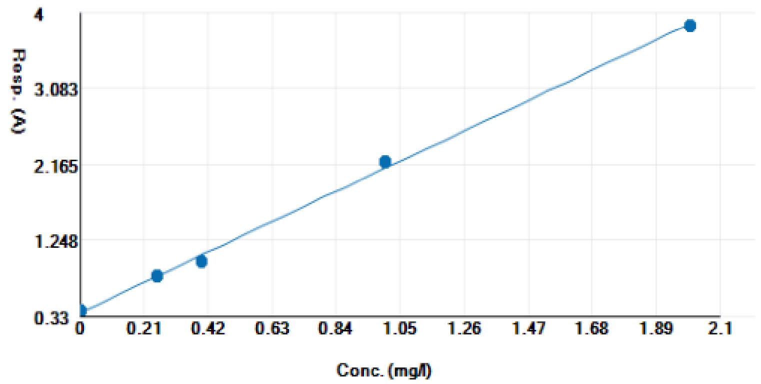
Calibration results

TON as N   Version number 1.2

Date	3/29/2018	**User**	Main user
Time	1:29:08PM	**Software version: 6.0.1**	
Analyzer name	Analyzer 1		

Test	TON as N	**Coeff. of deter.**	0.998255
Status	Accepted	**Total factor**	0.574
Accepted	3/29/2018 11:43 AM		
Checked	3/29/2018 11:43 AM	Zero-order factor	0.369
User name	Main user	1st order factor	1.743
Comment		2nd order factor	0.000
Errors			

Cal/Ctrl	Response	Calc. conc.	Given conc.	Lot	Errors
Water Blank	0.388	0.010	0.000	Default	
TON-High	0.807	0.251	0.250	Default	
TON-High	0.990	0.356	0.400	Default	
TON-High	2.195	1.047	1.000	Default	
TON-High	3.830	1.985	2.000	Default	Abs. high, Init abs. high



Appendix 2. Calibration curve generated for colorimetric determination of TON concentration in standards in concentrations ranging 0-2.0 mg/l. Coefficient of determination within 0.012 units of optimal calibration.

Calibration results

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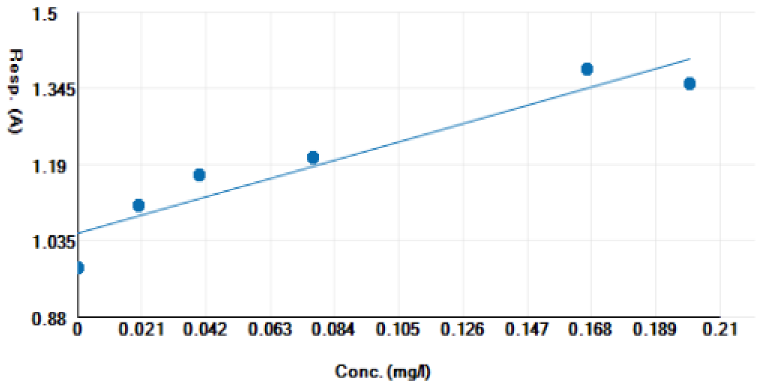
NH4 as N L Version number 1.2

Date	3/29/2018	**User**	Main user
Time	1:27:38PM	**Software version: 6.0.1**	
Analyzer name	Analyzer 1		

Test	NH4 as N L	**Coeff. of deter.**	0.895054
Status	Accepted	**Total factor**	0.564
Accepted	3/29/2018 11:42 AM		
Checked	3/29/2018 11:42 AM		
User name	Main user		
Comment			
Errors			
Factor	0.564		
Bias	1.050		

Cal/Ctrl	Response	Calc. conc.	Given conc.	Lot	Errors
Water Blank	0.978	-0.041	0.000	Default	
NH4-Low	1.105	0.031	0.020	Default	
NH4-Low	1.169	0.067	0.040	Default	
NH4-Low	1.204	0.087	0.077	Default	
NH4-Low	1.385	0.189	0.167	Default	
NH4-Low	1.354	0.171	0.200	Default	



Appendix 3. Calibration curve generated for colorimetric determination of ammonia concentration in standards ranging from 0-0.2mg/l. Poorer calibration than P, TON. Farther than 0.1 units from optimal calibration.

Male Great Crested Newt (*Triturus cristatus*)
Aquatic Stage

Male Common Newt (*Triturus vulgaris*)
Aquatic Stage

<u>DISCLAIMER: The above diagrams are for illustrative purposes only and serve as supplementary material. Source: Michael Klimpke</u>