Clean Water for Wildlife Technical Manual

Evaluating PackTest nitrate and phosphate test kits to find clean water and assess the extent of nutrient pollution

heritage lottery fund

LOTTERY FUNDED

June 2017

For further information please contact:
Freshwater Habitats Trust
Bury Knowle House
North Place
Headington
Oxford, OX3 9HY

This report should be cited as:

Biggs, J., McGoff, E., Ewald, N., Williams, P., Dunn, F. and Nicolet, P. 2016. Clean Water for Wildlife technical manual. Evaluating PackTest nitrate and phosphate test kits to find clean water and assess the extent of pollution. Freshwater Habitats Trust, Oxford.

Acknowledgements
We are enormously grateful to: the *Heritage Lottery Fund* who fund Clean Water for Wildlife as part of the 'People, Ponds and Water' project and to *Earthwatch* who funded the collection and analysis of samples in Woking and the New Forest.

Very many thanks to Anita Casey who collected water samples from Loddington and undertook most all of the laboratory analysis, and to all those who have provided advice during the development of this manual, particularly Geoff Phillips and Tristan Hatton-Ellis. We would also like to thank the New Forest National Park Authority and the New Forest Catchment Partnership for funding laboratory analysis of water samples.

We would especially like to thank Ian Barker, Stewart Clarke, Ruth Hall, Stephen Loiselle and Ian Thornhill for reviewing the report. Their comments helped us to substantially improve the document.

Contents

Summary

Quick nutrient testing kits are increasingly becoming more sensitive, cheaper and easier to use. This provides scope for a wide range of uses: both creating opportunities to discover much more about the levels of pollution in the landscape and democratising water quality testing by making it available to a wide audience.

However, all quick kits have their limitations, and none that we have tested so far approach the accuracy of laboratory analysis. It is therefore important to understand the strengths and limitations of these kits: to ensure that their results are validly used and interpreted.


PackTest nitrate kit in use

This guide provides the technical background for using and interpreting one type of quick kit: the Kyoritsu PackTest low range phosphate and nitrate test kits that we use in the Clean Water for Wildlife survey.

The guide outlines the results of trials to look at how nutrient concentrations measured with the PackTest kits compare to laboratory analysed water samples. It also provides contextual information: defining what is meant by the term clean water, briefly reviewing the main detrimental effects of nutrients on freshwaters, and summarising the nutrient concentrations which are synonymous with clean water in ponds, lakes, streams, rivers and ditches.

About the quick test kits

The Kyoritsu PackTest kits measure phosphate-phosphorus with a minimum detection limit of 0.02 mg L^{-1} and nitrate-nitrogen with a minimum detection limit of 0.5 mg L^{-1}. Tests are based on colourimetry and judged by eye against a colour chart. Each test takes either 5 minutes (phosphate) or 3 minutes (nitrate). In the Clean Water for Wildlife survey we use the PackTest phosphate and nitrate kits to assign a waterbody to one of three nutrient pollution categories:

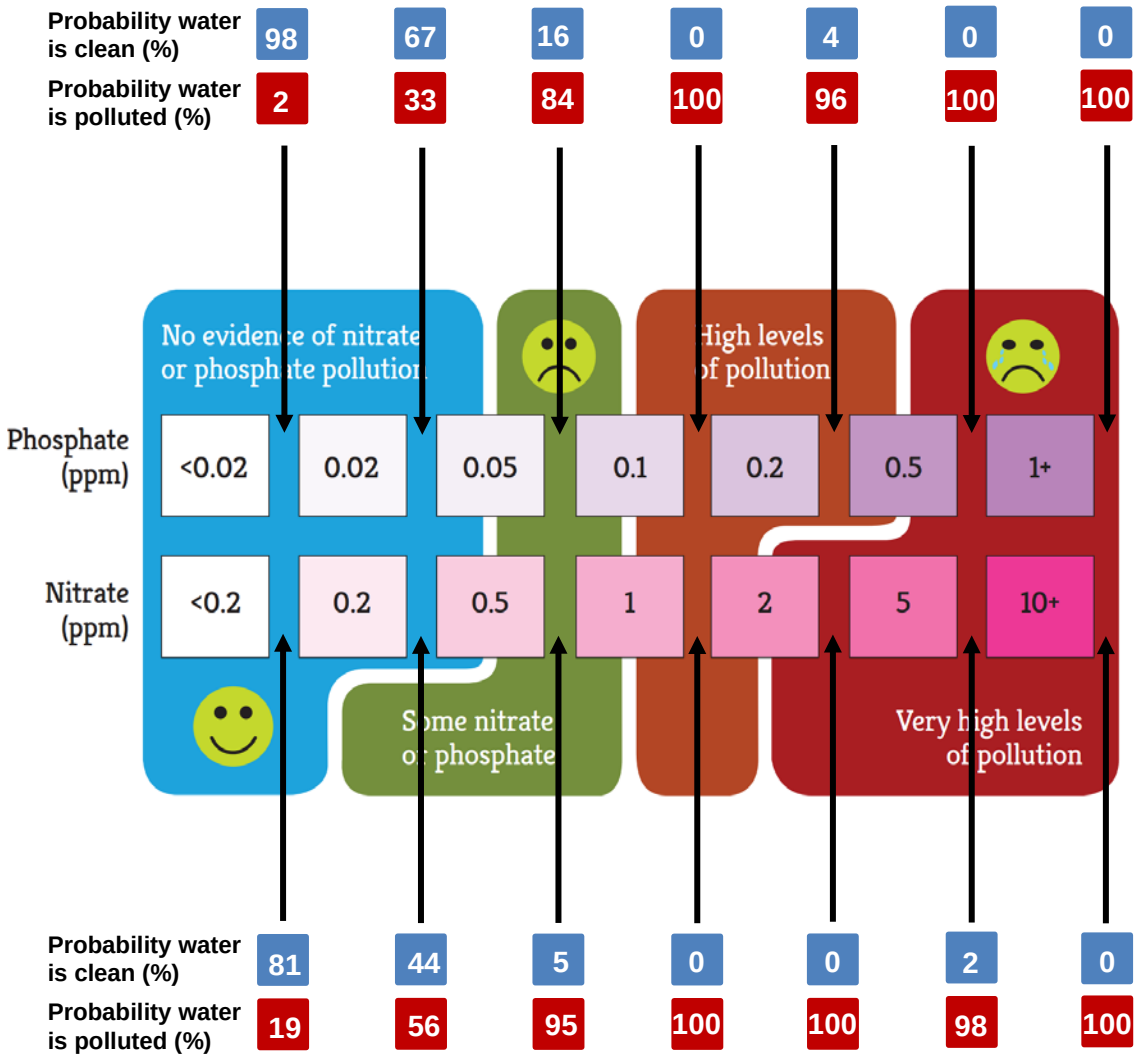
	Phosphate (mg L^{-1} PO_4-P)	Nitrate (mg L^{-1} NO_3-N)
Clean water	<0.05	<0.5
Some evidence of pollution	0.05-0.1	0.5-1
High or very high levels of pollution	>0.1	>1

The results of the trials comparing PackTest kits with laboratory analysed samples showed that, when analysing standard solutions (nutrient solutions made up with pure water in the laboratory), the kits performed well, broadly matching the results of laboratory analysed samples. Note that it is possible that the ‘clean water’ levels identified by the PackTest kits could sometimes we exceeded in winter when, with less denitrification occurring naturally, concentrations may exceed these values.

‘Natural’ water samples from ponds, lakes, ditches, streams and rivers provide a harder test because these waterbodies can contain a wide range of chemicals and sediment that may potentially interfere with water test results. At present we have only limited understanding of these effects. Comparison of the PackTest kits with laboratory analysis of natural waters showed that, overall, the kits can separate clean and polluted sites with sufficient reliability. Sites where the kits show no colour change are highly likely to be clean waterbodies with low nutrient levels (98% probability for phosphorus, 81% for nitrate). Sites with a moderate or

strong colour change are highly likely to be polluted (95% probability for phosphate, 84% for nitrate). However, about a third to a half of the sites bordering the clean water boundary (phosphate: 0.02-0.05 mg L^{-1}, nitrate 0.2-0.5 mg L^{-1}) may be mildly polluted, rather than clean. This means that at landscape scale, the kits will slightly over-estimate the amount of clean water present, but they are highly unlikely to over-estimate the level of either phosphate or nitrate pollution in waterbodies. At sites which should naturally have very low nutrient levels - especially acid lakes, both lowland and upland - they should be used with caution.

Overall, although it is important to recognise the limitations of the PackTest kits, our results suggest that they are a simple, rapid and cost effective way to identify nutrient pollution, especially in large landscape-wide surveys where the costs of laboratory analysis are likely to be prohibitive.



Probability that sites allocated to a particular PackTest phosphate or nitrate class are correctly classified as either clean or polluted

Introduction

1.1. Context

This guide is one of the outputs from the *Clean Water for Wildlife* project: a citizen science survey aiming to raise awareness of the true extent of nutrient pollution in the UK, and to identify clean water habitats, with the ultimate aim of helping to protect freshwater biodiversity.

Information about Clean Water for Wildlife can be found at: http://freshwaterhabitats.org.uk/. The survey results, including the raw data are available on Freshwater Habitats Trust's WaterNet database. All information shown on the database is in the public domain. However users also have the option to keep their results private, in which case they can only be used for analysis by the Freshwater Habitats Trust.

1.2. Aim of the guide

The aim of this technical guide is to summarise the results of reliability testing for the PackTest rapid phosphate and nitrate test kits which are used in the *Clean Water for Wildlife* survey.

The guide also defines what is meant by the term clean water, reviews the main detrimental effects of nutrients on freshwaters habitats and discusses how to interpret the results from the nutrient kits.

1.3. The problem with nutrients

In the absence of people, most freshwaters would naturally have low nutrient concentrations, and since freshwater plants and animals have evolved over millions of years in these conditions, the majority of species require these naturally low nutrient environments to flourish. Although excess nutrients are not usually directly toxic to freshwater plants and animals, freshwater ecosystems are often radically changed (nearly always for the worse) when nutrient enrichment favours one or a few species, usually plants and algae, which can exploit the excess nutrients. This can lead to less nutrient tolerant plants, normally the majority, being outcompeted and cause knock-on effects by eliminating the habitats of animals and changing the physical and chemical environment of the water.

Today, nutrients, particularly phosphate and nitrate, are amongst the most pervasive pollutants of freshwater across the globe. High levels of nutrients in the water results in excessive growth of aquatic plants, including algae, which suppresses less tolerant species. This, in turn, causes a raft of biological, health and economic impacts, including loss of plant, invertebrate and fish diversity, declines in the visual appeal and amenity value of waterbodies, and in some cases the development of toxic blue-green algae blooms that are harmful if ingested by humans and animals. As a result of their wide ranging effects, levels of phosphate and nitrate are widely used measures for assessing waterbody quality in international monitoring programmes such as the Water Framework Directive (Liu *et al.* 2012; Brahney *et al*. 2015; Mekonnen and Hoekstra 2015). A short technical summary of the effect of these nutrients is given in Section 1.9 below.

1.4. The Clean Water for Wildlife PackTest kits

Although nutrient pollution (often termed eutrophication) is widespread and has a deeply degrading effect on freshwater environments and wildlife, it has always been difficult to discover the level of nutrients in any individual waterbody without expensive laboratory analysis. Newly available nutrient test kits now offer a potential solution to this problem because, although they do not provide the detail of laboratory analysis, they are cheap and easy to use and can potentially provide an indication of nutrient levels in just a few minutes. The availability of quick nutrient kits opens many new opportunities for freshwater monitoring, particularly for assessing the true extent of pollution in large numbers of waterbodies across whole sites or landscapes; work which would normally costs tens or hundreds of thousands of pounds. This makes test kits especially useful for exploring new areas where no existing data are available, for tracing water pollution sources in a landscape, and for finding waterbodies with clean and unpolluted water which, these days, are increasingly rare.

The kits offer a particularly important opportunity to assess the quality of *small* waterbodies like ponds, ditches, and smaller lakes and streams. Larger waterbodies like rivers or major streams and very large lakes often already have data collected by the Environment Agency, Natural Resources Wales, SEPA or the Department of the Environment in Northern Ireland. Increasingly the results are also freely available online. However the quality of the huge network of small waterbodies, including thousands of kilometres of streams and ditches, and almost half a million ponds and most lakes is almost completely unknown.

To some extent, evidence of nutrient pollution is also a marker for other types of pollution which are still too difficult or expensive to measure. For example, waterbodies affected by nitrate pollution caused by farming in their catchments are quite likely to also be exposed to unnaturally high sediment runoff and intermittent pesticide pollution. Likewise, nutrient rich runoff from urban areas will often have high levels of heavy metals, sediment, pesticides and sewage waste.

1.5. What is clean water?

In the Clean Water for Wildlife project we use the term clean water with a specific technical meaning:

What is clean water?

Clean water is defined as water which has a chemistry and biology which would be normal for a given area in the absence of human disturbance. This is commonly referred to as ‘the reference condition’, ‘minimally impaired water quality’ or ‘natural background levels’ (Williams, Biggs and Nicolet, 2010).

This definition of clean water is equivalent to the EU Water Framework Directive (WFD) ‘High’ status.

This natural background water quality is most easily defined by measuring it in waterbodies where there are no, or very slight, impacts from human activity. Often, it is not possible to find present-day examples of ‘natural’ background levels of nutrients: for example, in lowland England there are probably no large rivers with natural nitrate levels.

Fortunately, in these cases, it is often possible to use historical data from rivers or from paleolimnological studies of lake sediments to estimate what the natural levels would be. In the River Thames, for example, there is a 140 year long record of nitrate concentrations upstream of London (Figure 1a). This information gives us an idea of how much lower nitrate levels would have been before the industrialisation of agriculture, and show how they rose through the 20th century as a result of ploughing up grasslands for arable farming and increased use of fertilisers from 1.5-2 mg L^{-1} to around four times that level today. In practice, before widespread agriculture, the natural background nitrate levels were probably even lower – in all probability rarely exceeding 1 mg L^{-1} of NO_3-N.

A similar pattern, although over a shorter period, can be seen in the River Frome in Dorset with steady increases over the last 50 years (Figure 1b). Here concentrations rose from around 2 mg L^{-1} midway through the 20th century to 6 mg L^{-1} in the first decade of the 21st century.

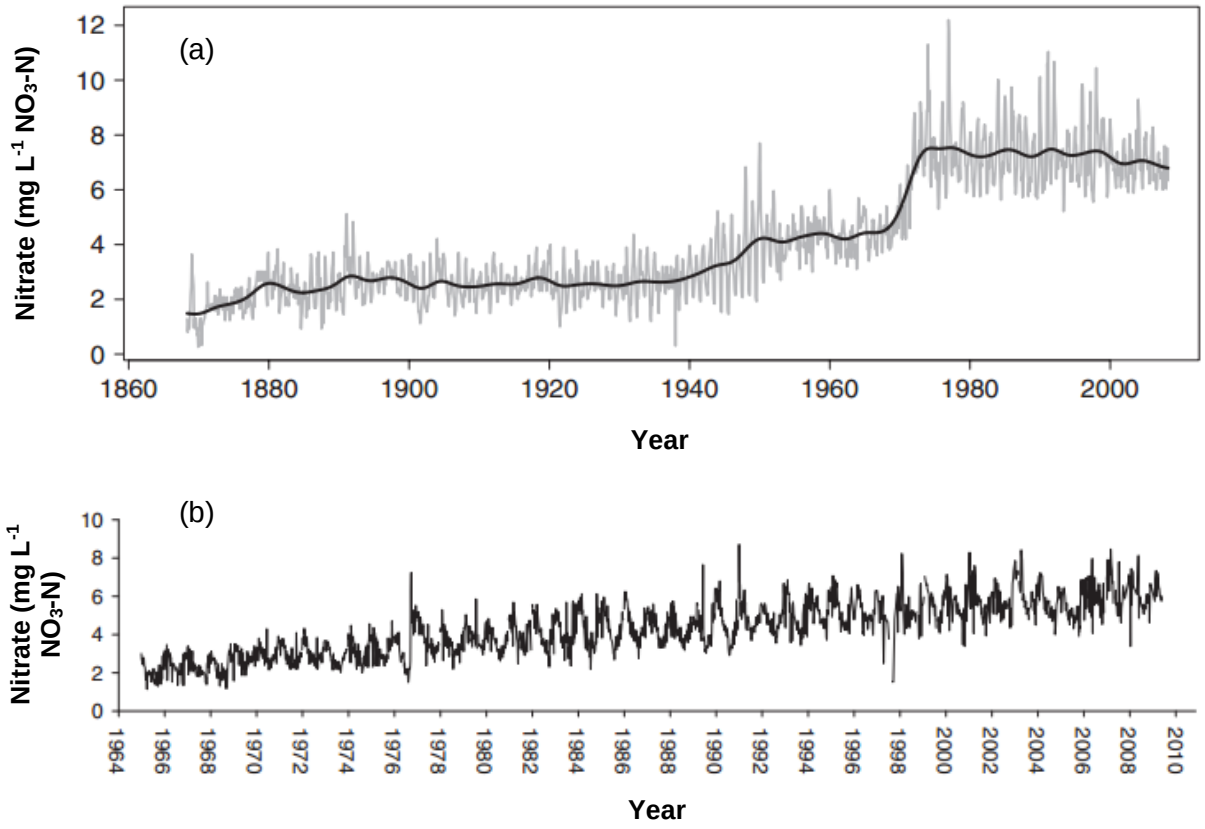


Figure 1. Examples of long-term trends in nitrate pollution: (a) nitrate concentrations in the R. Thames, 1868-2008; (b) nitrate concentrations in the R. Frome, 1965-2009. Sources: Howden *et al.* 2010; Bowes *et al.* 2011).

Even at levels of 1-2 mg L^{-1} NO_3-N, nitrate levels were probably already elevated above true natural background levels but these data at least give an indication of the low concentrations which would occur in 'clean' rivers.

1.6. Clean water thresholds

Appendix Table 1 shows the threshold phosphate and nitrate values **below** which freshwaters habitats are likely to have natural background nutrient levels, and so be regarded as 'clean'. For phosphorus in rivers and lakes, where there is detailed technical knowledge of the geographic variation in natural background levels, a range of values is given reflecting regional variations which are described in detail in the reports of the UK Technical Advisory Group for the Water Framework Directive (UKTAG, 2012 for rivers and streams; UKTAG, 2008 for lakes). Values exceeding those shown in Appendix Table 1 are always indicative of pollution but in some parts of the country the 'clean' phosphorus concentrations will be less than 0.013 mg L^{-1} phosphate-P in rivers and less than 0.005 mg L^{-1} total phosphorus in lakes (Figure 2 – rivers and streams; Table 3 – lakes).

For example, lowland rivers up to 100 m altitude, rich in calcium (with an alkalinity of 100 mg L^{-1} of calcium carbonate) the maximum concentration of phosphate-P consistent with clean water will be between 0.029-0.058 mg L^{-1} depending on the exact altitude and alkalinity. In these areas only sites with **no colour change** in the phosphorus test kit are indicative of clean water. In more upland areas, values are naturally lower: the highest phosphorus concentrations in alkaline upland rivers consistent with clean water are in the range 0.013-0.038 mg L^{-1} depending on exact location and a significant proportion of acid rivers have phosphate levels naturally below 0.020 mg L^{-1}. In these sites, no colour change may be seen with the PackTest kits but phosphate levels may still be unnaturally high. Therefore, caution needs to be exercised in these area in concluding that rivers are 'clean' unless laboratory measurements can be made (Figure 2).

Figure 2. Threshold phosphate-P values for rivers in four different broad alkalinity and altitude combinations in Britain. Values in the table are phosphate-P concentrations in mg L^{-1}. Values in yellow are low altitude acid rivers and streams; blue box values are low altitude alkaline rivers and streams; amber boxes are acid upland rivers and streams; green boxes are alkaline upland rivers and streams. Any site with phosphate-P concentrations above 0.058 µg L^{-1} is polluted but for acid sites, and some alkaline uplands streams and rivers, natural background levels may be half this value or less. To assess alkalinity, calcium carbonate data will need to be obtained from the Environment Agency: in many areas it will be possible to use data available on the EA web site to estimate the alkalinity concentration in your area. Below 0.02 mg L^{-1} PackTest phosphate-P kits would not show any colour change but could still be experiencing phosphate pollution. Sites with natural concentrations to the left of the thick dark line should show no colour change with the PackTest phosphate kit and could still be polluted by phosphorus.

		Alkalinity (mg L^{-1} $CaCO_3$)									
		5	**10**	**20**	**40**	**50**	**75**	**100**	**150**	**200**	**250**
Altitude (m)	**0**	0.013	0.016	0.021	0.027	0.030	0.035	0.040	0.047	0.053	0.058
	20	0.013	0.014	0.019	0.025	0.028	0.033	0.037	0.043	0.049	0.053
	40	0.013	0.013	0.017	0.023	0.025	0.030	0.034	0.040	0.045	0.049
	60	0.013	0.013	0.016	0.021	0.023	0.028	0.031	0.037	0.041	0.045
	80	0.013	0.013	0.015	0.020	0.022	0.025	0.029	0.034	0.038	0.042
	100	0.013	0.013	0.014	0.018	0.020	0.023	0.026	0.031	0.035	0.038
	200	0.013	0.013	0.013	0.013	0.013	0015	0.017	0.021	0.023	0.025
	300	0.013	0.013	0.013	0.013	0.013	0.013	0.013	0.014	0.015	0.017
	350	0.013	0.013	0.013	0.013	0.013	0.013	0.013	0.013	0.013	0.014

For lake phosphorus the situation is a little more complicated because official standards are based on total phosphorus concentrations (Table 3). In addition, the method for calculating natural background levels requires making a specific calculation for each site. However, broadly speaking, unpolluted lakes should have natural background levels no more than 0.035 mg L^{-1}, but in soft water and upland areas the value may be as low as 0.005 mg L^{-1} (Table 3). As there is no precise relationship between total phosphorus levels and phosphate concentrations, it is not possible to predict the total phosphorus levels from the PackTest results.

Table 3. The levels of total phosphorus in lakes which indicate 'clean' pollution free conditions

Lake type	Highest total phosphorus concentration consistent with clean water (mg L^{-1})
High alkalinity, deep	0.016
High alkalinity, deep	0.025
High alkalinity, shallow (Region 1)	0.016
High alkalinity, shallow (Region 2)	0.025
High alkalinity, very shallow (Region 1)	0.023
High alkalinity, very shallow (Region 2)	0.035
Moderate alkalinity, deep	0.008
Moderate alkalinity, shallow	0.011
Moderate alkalinity, very shallow	0.015
Low alkalinity, deep	0.005
Low alkalinity, shallow	0.007
Low alkalinity, very shallow	0.009
Marl, shallow	0.009
Marl, very shallow	0.010

Notes: Definitions of Region 1 and 2, deep, shallow and very shallow and high, moderate and low alkalinity lakes are given in UKTAG (2016).

Overall, for much of lowland England, Wales, Scotland and Northern Ireland, where the concentrations of nitrate and phosphate in waterbodies are above the threshold values given in Table 1, this is likely to have an increasingly detrimental impact on freshwaters systems or individual species (see Section 1.9). As far as practical, the threshold values in Table 1 are based on UK conditions, whilst taking into account the international scientific literature and, where appropriate, national and international legislation. The Water Framework Directive, for example, gives legally defined limits for nutrient levels in rivers, streams and lakes and provides useful boundaries for phosphate in rivers. However, the levels for nitrate defined as acceptable in the Water Framework Directive are currently designed to ensure levels are safe for drinking water, rather than protecting ecosystems. As these concentrations are far higher than the level at which biological impairment can occur they are not used here to define 'clean' water conditions.

For rivers and lakes the information that is available to help determine the natural background nutrient levels is substantial. For those wishing to explore this detailed technical information further a review for lakes by Cardoso *et al.* (2007) provides an entry into the scientific literature of this area. For rivers, a good introduction to recent scientific literature on natural nutrient levels is provided by Dodds and Smith (2016). However, for many small waterbody types (ponds, ditches, springs, flushes) the information currently available remains far more limited.

Table 1. Summary table showing maximum concentrations of phosphorus and nitrogen which can be regarded as 'clean' water in different types of waterbody.

(a) Maximum concentrations of phosphorus which equate to clean water in different water body types. For rivers and lakes a range is given to reflect the natural regional variation which occurs in phosphorus concentrations depending on the geology of the UK landscape. Note that the thresholds are based on different chemical fractions of phosphorus in different waterbody types (see Appendix 1 for explanation).

Waterbody type	Threshold concentration (mg L^{-1} P)	Chemical fraction	Principal source of value
Rivers and streams	0.013-0.058	Soluble Reactive Phosphorus	Water Framework Directive
Lakes	0.005-0.035	Total Phosphorus	Water Framework Directive
Ponds	0.065	Soluble Reactive Phosphorus	FHT National Pond Survey (unpublished data)
Ditches[1]	0.065	Soluble Reactive Phosphorus	Literature sources and FHT data
Canals[1]	0.073	Total Phosphorus	SNIFFER 2012

Note that the PackTest kits measure phosphate, not Total Phosphorus, so values in this table are not directly comparable with the levels measured by PackTest kits.

(b) Maximum concentrations of nitrogen which equate to clean water in different water body types; all values below the threshold values are clean. Regional ranges are not given for nitrogen because there is less technical knowledge of the precise boundaries. Note that the thresholds are based on different chemical fractions of nitrate in different waterbody types (see Appendix 1 for explanation).

Waterbody type	Threshold concentration (mg L^{-1} N)	Chemical fraction	Source of value
Rivers	0.9	Total Nitrogen	Literature sources
	0.7	Total Inorganic Nitrogen/Dissolved Inorganic Nitrogen	Literature sources
Lakes	1	Total Nitrogen	Literature sources
Ponds	0.5	Total Oxidised Nitrogen	FHT National Pond Survey (unpublished data)
Ditches[1]	1	Total Nitrogen	Literature sources
	0.5	Nitrate	Literature sources
Canals[1]	2.6	Total Oxidised Nitrogen	SNIFFER 2012

Note 1. It is assumed that canals and ditches would, in the absence of pollution, have similar nutrient concentrations to waterbodies formed by natural processes. For example, although human-created, ditches are analogous ecologically either to smaller permanent and seasonal streams in the headwaters of river systems or the larger natural networks of seasonal and permanent channels that would be found naturally on river floodplains and in wetlands.

1.7. Clean water thresholds for the PackTest kits

In setting the clean water thresholds we have attempted to provide a single set of values for different types of freshwaters Britain: ponds, lakes, streams, rivers, ditches and canals. In doing this we have summarised values for 'natural background' levels of nutrients from a wide range of technical information on different waterbody types into a single scale, which inevitably leads to some oversimplification. A summary of the sources used is given in Appendix 1.

We have divided the PackTest kit categories into four groups to provide a simple indication of areas with (i) 'no evidence of pollution', (ii) 'some nutrient pollution', (iii) 'high levels of pollution' or (iv) 'very high levels of pollution' This works well for nitrate but for parts of the country with naturally low levels of phosphorus, and particularly for lakes, the PackTest results will often be too lenient. Where phosphate concentrations are naturally below the 0.02 mg L^{-1} phosphate value, the PackTest kits may incorrectly classify waters as clean. Lake phosphorus levels in particular require care in the interpretation of the PackTest results. Official standards for lake phosphorus levels in the UK are based on measurements of total phosphorus – the phosphorus both dissolved in the water and associated with any particles, including algae, collected in unfiltered water samples. The main reason for this is that soluble phosphorus dissolved in the water is often used up by algae and large water plants in the summer and, considered alone, can suggest that a system is suffering from less phosphorus pollution than is actually the case. As the PackTest kits measure the dissolved phosphate component, they inevitably underestimate the total phosphorus concentration in the system. For example, at Sowley Pond close to the south coast in Hampshire, the phosphate levels are unnaturally high but do not exceed the 0.05 mg L^{-1} level at any time of the year (Table 2a). However, total phosphorus levels show that phosphorus concentrations are three or four times the natural background level. In practice this means that any colour change in a phosphorus PackTest sample at Sowley is indicative of pollution. Similarly, in Bassenthwaite Lake in the Lake District (Table 2b), which is suffering from the effects of elevated phosphorus levels, concentration of total phosphorus are roughly double the natural level for this system i.e. it is not a clean water lake. However, phosphate levels (measured as orthophosphate) would not be detectable at any time by PackTest kits. The kits would, therefore, incorrectly indicate the status of this lake as clean.

Table 2. Examples of differences in total phosphorus and phosphate concentrations: (a) Sowley Pond, Hampshire (b) Bassenthwaite Lake, Cumbria

(a) Water quality data for Sowley Pond, Hampshire

	2007 Mean	2008 Mean	2009 Mean	2010 Mean	2011 Mean	2012 Mean
Alkalinity (pH 4.5)	56	50	55	55	57	47
Chlorophyll a	57.8	59.9	48.4	49.5	43.1	70.3
Conductivity	241	235	255	231	303	237
pH	7.49	7.48	7.46	7.04	7.44	7.79
Nitrate (as N)	-	0.76	0.76	0.86	0.71	1.00
Total Nitrogen (as N)	1.83	1.95	1.48	1.70	1.65	1.83
Orthophosphate P	-	32	37	50	41	33
Total Phosphorus (as P)	139	158	138	146	179	157

(b) Water quality data for Bassenthwaite, Lake District

	Mean 2005	Mean 2006	Mean 2007	Mean 2008	Mean 2009	Mean 2010	Mean 2011	Mean 2012
Alkalinity (Gran)	-	-	10.0	10.9	9.5	13.2	14.1	14.6
Chlorophyll a	11.98	11.68	8.95	5.59	7.22	8.37	6.02	5.02
Conductivity	-	-	66.5	66.3	60.8	71.2	62.7	65.3
pH	-	-	7.09	7.09	7.40	7.32	7.40	7.18
Nitrate (as N)	0.27	0.40	0.33	0.32	0.29	0.29	0.26	0.25
Nitrogen (as N)	-	-	0.55	0.48	0.46	0.41	0.41	0.42
Orthophosphate	1.6	4.9	2.9	3.1	2.9	2.5	2.8	2.5
Total Phosphorus (as P)	22.4	22.8	6.9	16.1	19.4	15.9	17.6	16.0

Overall therefore:

- Where PackTest phosphate concentrations are less than 0.02 mg L^{-1} and nitrate concentrations are less than 0.2 mg L^{-1} there is probably no pollution, particularly if the water body is in a naturally fertile catchment.

- Where PackTest phosphate concentrations are between 0.02-0.05 mg L^{-1} and nitrate concentration are between 0.2-0.5 mg L^{-1} some pollution may still be possible, particularly if the water body is not in a naturally fertile catchment.

Values above this are nearly always indicative of pollution although there are some natural situations – such as ponds in ancient woodlands with a lot of leaf litter – where high phosphorus levels may be natural. As a general principle, where nutrient pollution is likely to be more subtle, as in regions with naturally very low nutrient levels, detailed laboratory chemistry will still be essential to detect the full range of waterbodies affected by nutrient pollution.

Although it is easy to view mild levels of pollution as far preferable to high levels of pollution, in fact critical ecological damage, especially loss of rare and sensitive species, occurs when water quality degrades from clean to even mildly polluted. For example, in Finnish rivers invertebrate biologists found that up to 45% of invertebrate species could be lost in the transition from High status associated with clean water down to so-called 'Good' status in the Water Framework Directive (Aroviita *et al*., 2010). Hence the clean/polluted threshold is a very important one.

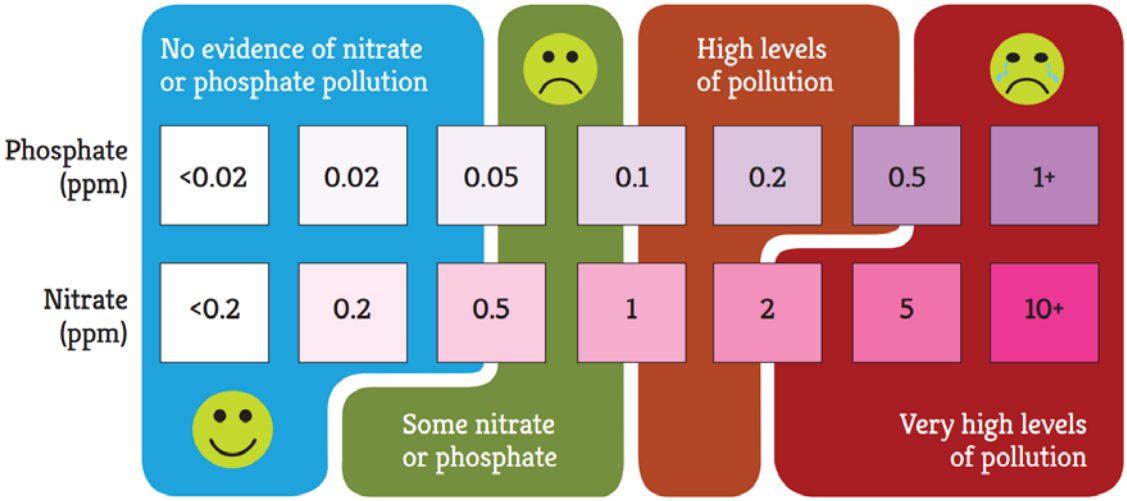


Figure 4. The Clean Water for Wildlife pictogram summarising the levels of pollution indicated by different phosphate and nitrate results.

1.8. Current understanding of the impact of phosphate and nitrate on the freshwater environment

In the human modified environment, nutrients tend to be present in substantially greater abundance than would occur naturally. Over the last century, human derived nutrient sources have increased to such an extent that they dwarf natural nutrient sources (Vitousek *et al*. 1997, Bennett *et al*. 2001).The problem is worldwide, occurring in virtually all areas with industrial agriculture and human settlements. In the United Kingdom around 90% of lowland surface freshwaters like rivers, streams and ponds have ecologically damaging levels of either nitrogen, phosphorus or both (Biggs *et al*. 2014). Groundwaters are similarly widely impacted (Wang *et al*. 2016).

The effects of high concentrations of nutrients are wide-ranging and have been the subject of thousands of scientific studies, particularly in lakes. The book by international experts David Schindler and John Vallentyne (2008) provides a detailed introduction to the effects of nutrients on lakes. There is also a substantial body of information on the effects of nutrients on rivers but rather less detailed studies on ponds or ditches, although the same broad principles clearly apply.

In summary, excess nutrients cause algae, fungi, bacteria and some tolerant water plants to grow more rapidly and become more abundant than they would naturally. The consequences of this are that intolerant species are smothered, outcompeted or directly poisoned resulting in many species becoming rarer. Often, high levels of nutrients lead to the loss of whole communities of large water plants, which has important knock-on consequences for animals that would normally live amongst those plants: their habitat and food sources disappears, and therefore so do they. Commonly in lakes and ponds this leads to toxic blue-green algal blooms, biodiversity loss, and changes in biological community structure and ecosystem functioning (Jeppesen *et al.* 2012). More subtle effects are also coming to light: new evidence suggests that by accelerating the breakdown of dead leaves and wood in the water, nutrient pollution causes a significant loss of woodland-derived

carbon from stream ecosystems, reducing the ability of streams to support aquatic life (Rosemond *et al.* 2015).

As a result of nutrient enrichment, small standing waters like ponds and slow-flowing ditches, often become covered in a surface sheet of duckweeds or filamentous algae which blocks out light. In the dark and oxygen-poor environment below, with no plants to provide habitat structure, the animal community is simplified to a few tolerant creatures such as water slaters, flatworms and American freshwater shrimps. In lakes, large submerged water plants disappear and are replaced by dense populations ('blooms') of algae. This can turn clear-water lakes into waterbodies that are green and turbid. In rivers and streams, nutrient pollution leads to the extinction of sensitive water plants and an excess growth of algae, again simplifying habitats and causing fluctuations in oxygen levels which, in extreme conditions, can cause invertebrates and fish to die.

Although nutrient pollution is one of the most studied environmental impacts, there are still important unknowns. In lakes, despite intensive study over 50 years, the relative importance of nitrogen and phosphorus as pollutants is still an area of active research (Moss *et al.* 2013). Traditionally it believed that phosphorus is the most important ('limiting') nutrient in freshwater (see, for example, Schindler et al. 2016), and nitrogen less important. This is because there is usually less phosphorus available to plants and algae than nitrogen, so it runs out first when plants take both phosphorus and nitrogen from the water to grow.

However, newer evidence suggests the true situation is more complex (Paerl *et al*. 2016). In the past, before much human activity, levels of nitrogen and phosphorus were naturally more equal and so co-limitation is likely to have been much more typical. Both nitrogen and phosphorus cause similar enrichment responses, and there is also a large synergistic effect (i.e. the effect of both nutrients together is greater than the nutrients alone) (Elser *et al.* 2007). Hence, controlling the effects of pollution requires attention to both pollutants, rather than focussing on a single pollutant (phosphorus) as is common practice in much of Europe at present. There is also evidence that increased nitrogen concentrations have a direct impact on macrophytes, leading directly to the loss of stoneworts, and possibly also emergent vegetation (Lambert and Davy, 2011).

In rivers and streams, understanding the effects of nutrient pollution have lagged behind that in lakes, perhaps because the effects in lakes were initially more obvious, and the main pollution problem in rivers was long presumed to be organic pollution from sewage. However, there is a growing range of evidence that nutrient pollution in streams affects both plants and invertebrates, including the food web associations that depend on bottom-living shredding insect communities (Evans-White *et al.* 2009, Prater *et al.* 2015).

Inevitably, a few pollution-tolerant species, including coarse fish, may benefit from the fertiliser effect of nutrient pollution, and the early stages of nutrient enrichment can sometimes appear beneficial – especially if it results in a richer community. However, such increases in richness are usually associated with loss of sensitive and uncommon plants and animals, and overall there are no known examples of freshwater communities as a whole benefitting from nutrient pollution.

2. Testing the PackTest kits

2.1. Background

To assess the viability of the PackTest phosphate and nitrate kits we tested them in two ways: (i) by comparing them with laboratory 'standard' nutrient solutions, (ii) by comparing them with field collected 'natural' water samples. In the laboratory, both sets of samples were analysed using standard water analysis methods (see Sections 2.2.1 and 2.3.3).

Laboratory standard solutions[1] were used to assess the underlying ability of the kits to detect the nutrients in the absence of other chemicals or sediments that are present in 'natural' water bodies in the countryside.

Comparison with natural water samples gave a more realistic impression of the reliability of the kits because they allowed us to test the effect of the natural factors that can interfere with the accuracy of the kits. The trial was undertaken in a programme of monthly sampling over one year covering five waterbody types: ponds, lakes, streams, rivers and ditches (Table 2). Samples were collected in two contrasting areas of southern England (Figure 3)[2]:

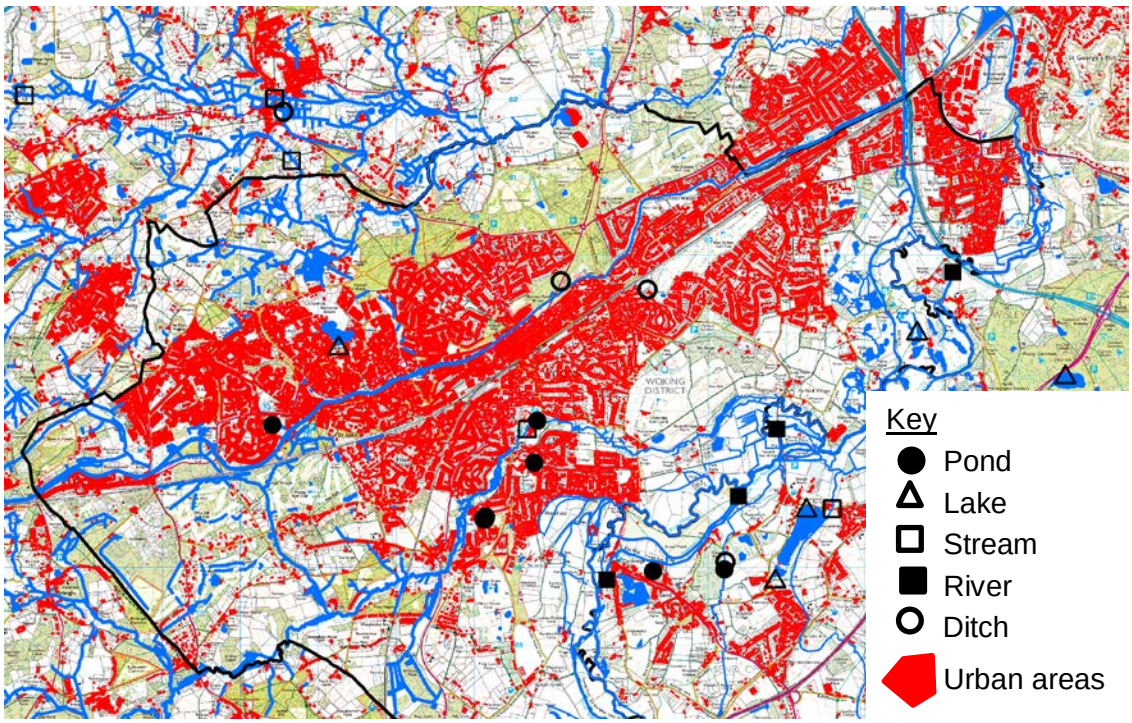
(i) Woking in Surrey, an area exposed to a fairly typical range of water pollution problems, and with a wide range of land use types including industrial farmland (grassland and arable), woodland, urban areas, and some semi-natural vegetation types (e.g. heathland). Treated sewage is discharged to rivers and streams, as well as other licensed effluent discharges.
(ii) the New Forest in Hampshire, an area with large numbers of high quality waters, with little exposure to nutrient pollution. This is mainly an area of extensive heathland, wetland and woodlands, and one of the largest uncultivated areas in lowland Western Europe. It is largely free from the impacts of industrial agriculture but some waterbodies are polluted by sewage effluents. In each region, five waterbodies were sampled from each waterbody type.

Table 2. Definitions of waterbody types used in the trial

Waterbody type	Description
Ponds	Waterbodies between 25 m^2 and 2 ha in area which may be permanent or seasonal. Includes both man-made and natural waterbodies.
Lakes	A body of water >2 ha in area. Includes reservoirs and gravel pits.
Streams	Small running waterbodies created mainly by natural processes. Marked as a single blue line on 1:25,000 Ordnance Survey (OS) maps and defined by the OS as being <8.25 m in width. Streams differ from ditches by (1) usually having a sinuous planform, (2) not following field boundaries, or if they do, pre-dating boundary creation, and (3) showing a relationship with natural landscape contours e.g. running down valleys
Rivers	Larger running waterbodies, created mainly by natural processes. Marked as a double blue line on 1:25,000 OS maps and defined by the OS as>8.25 m in width.
Ditches	Man-made channels created primarily for agricultural purposes, and which usually: (i) have a linear planform, (ii) follow linear field boundaries, often turning at right angles, and (iii) show little relationship with natural landscape contours.

[1] Solutions created in the lab to provide a series of water samples where the level of nutrients is already-known.
[2] This trial was funded under Earthwatch's FreshWater Watch, HSBC-funded, Water Programme.

(a) Location of waterbodies surveyed in the Woking district



(b) Location of waterbodies surveyed in the New Forest

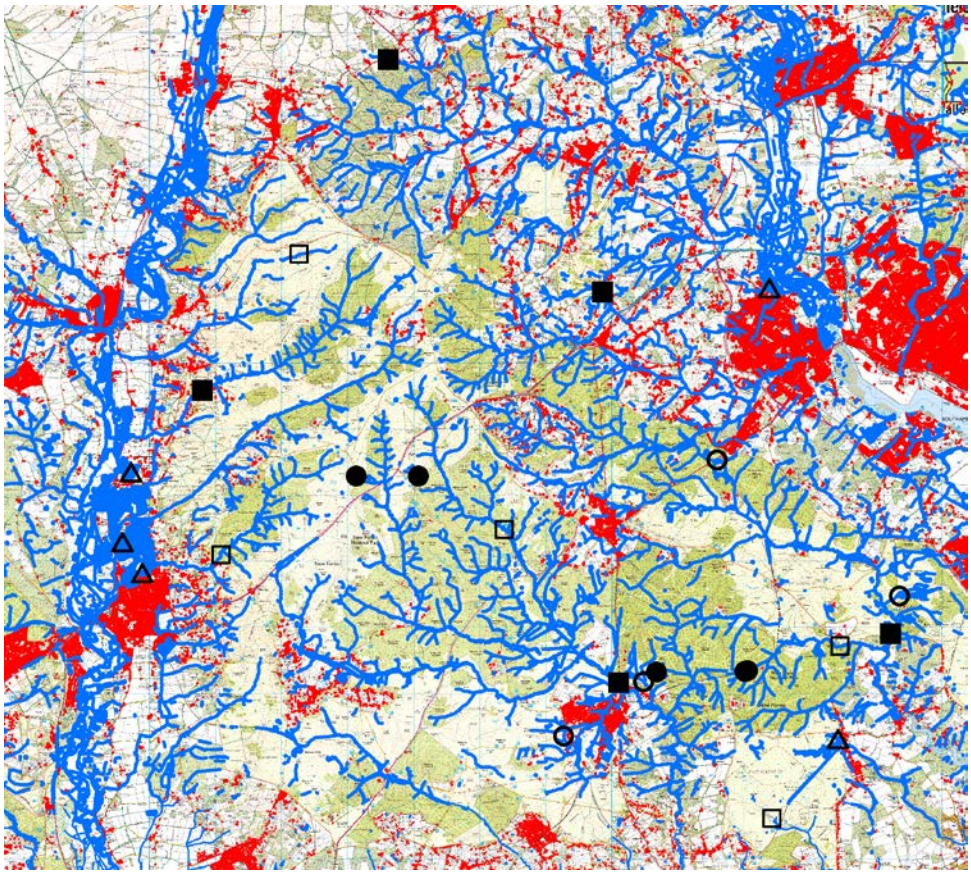


Figure 3. Location of waterbodies surveyed during testing of the PackTest kits (a) Woking district and (b) New Forest

2.2. Phosphate: effectiveness of the PackTest kits in detecting phosphate (PO_4-P)

2.2.1. Comparison with laboratory standard solutions

Methods

Standard solutions were prepared, with phosphate concentrations of: 0 mg L^{-1}, 0.06 mg L^{-1}, 0.12 mg L^{-1}, 0.18 mg L^{-1}, 0.25 mg L^{-1} and 0.31 mg L^{-1}. For each standard, five replicates were analysed. In order to directly compare the laboratory and test kit results, values from the laboratory analysis were converted to the midpoint of the PackTest kits (e.g. values in the range 0.02-0.05 mg L^{-1} were converted to 0.035 mg L^{-1} for analysis). The range of values tested (0-0.31 mg L-1) was chosen to reflect the relatively low concentrations which are of relevance to the Clean Water for Wildlife project.

The PackTest phosphate kit uses the inosine enzymatic reaction (Berti *et al*., 1988) to measure PO_4^{3-} concentrations in seven specific ranges in mg/L (< 0.02, 0.02–0.05, 0.05–0.1, 0.1–0.2, 0.2–0.5, 0.5–1, > 1). In the Clean Water for Wildlife project PackTest kits arer used with unfiltered water samples. Phosphate was measured in the laboratory as soluble reactive phosphorus using a Skalar SAN++ System autoanalyser. In this process the orthophosphate ion ($PO4^{3-}$) reacts with ammonium molybdate and antimony potassium tartrate under acidic conditions to form a complex. This was reduced with ascorbic acid to form a blue complex which absorbs light at 880 nm. The colour produced is proportional to the phosphorus concentration, and was read at 885nm on a spectrophotometer. Analyses were undertaken by Freshwater Habitats Trust staff in Oxford Brookes University laboratories.

Results

PackTest kits compared well with laboratory standard solutions. There was no significant difference between the two methods for phosphate (Wilcoxon signed rank test, p>0.05) (Figure 4).

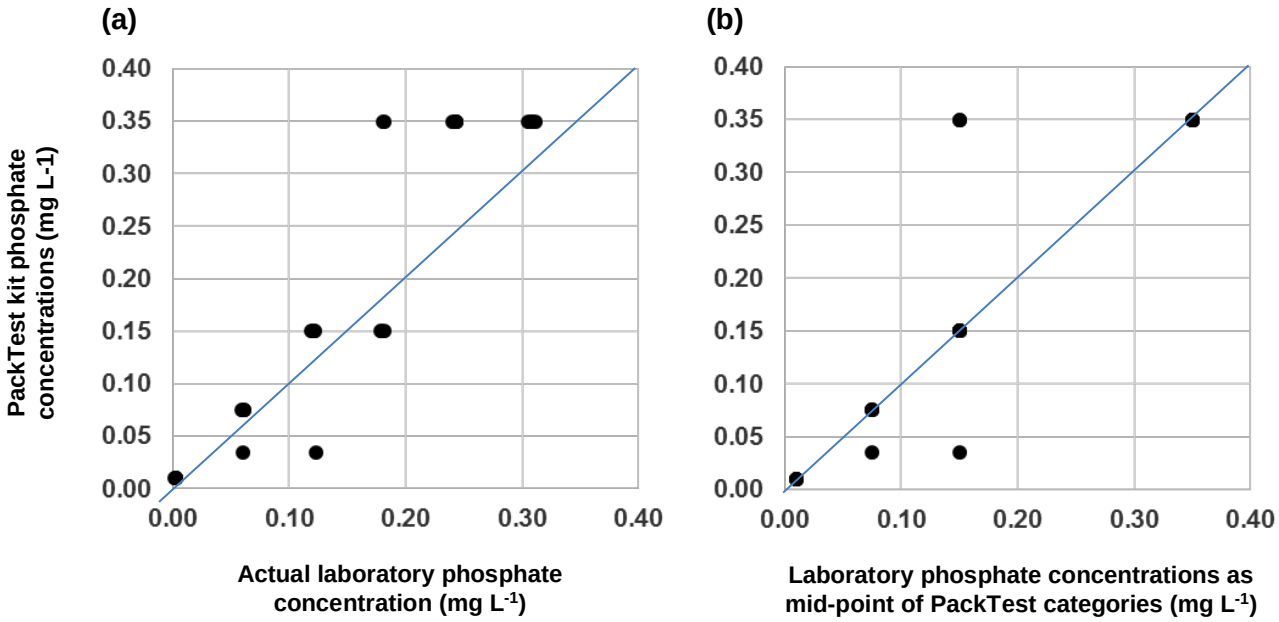


Figure 4. Comparison of PackTest kits and laboratory analysis of laboratory standard phosphate solutions (n = 30). (a) PackTest results compared to actual laboratory values; (b) PackTest and

laboratory results categorised as the mid-point of the PackTest colour chart bars. Lines show the point where all measurements would lie if laboratory and PackTest values were exactly the same. Note that some points on the graphs overlap so not all 30 sites are visible as separate points.

2.2.2. *Comparison with natural water samples*

Methods

The sites described in Section 2.1 were surveyed at monthly intervals from February 2014 to January 2015. At each site a sample was collected for testing using the PackTest low range phosphate test kit and a second sample for laboratory phosphate analysis. A total of 600 site visits were made to collect water quality data. Sites were dry on 26 occasions (mainly ditches and ponds) giving a total of 574 measurements from the waterbodies. PackTest samples were analysed the same day indoors in batches at the home of the surveyor. Samples for the comparative laboratory analysis were returned to the laboratory and maintained at 2-4ºC until they were filtered and analysed, which was within 1-3 days after collection. Phosphate was analysed as described above in Section 2.2.1. The delay prior to analysis may have led to some changes in dissolved phosphate concentrations, although Moore and Locke (2013) found only slight non-significant changes in filtered soluble reactive phosphorus concentrations following 7 days storage at 4ºC. Laboratory samples were filtered, following standard practice for determining soluble reactive phosphorus. PackTest samples were unfiltered before analysis and may therefore be detecting particulate P or even algal bound P. PackTest kits are not normally used with pre-filtration in the field, although this may contribute to some of the differences seen between field and laboratory measurements.

Analysis

To compare PackTest kit and laboratory analysed samples all values were converted to the mid-point of PackTest ranges. As the data are categorical rather than continuous, non-parametric statistical tests were used throughout to assess differences. To assess the overall effectiveness of the PackTest kits, the proportion of sites allocated to each of the seven PackTest categories was compared to the proportions in each category based on the laboratory measured phosphate values.

Results

Overall, the PackTest kits broadly reflected the ‘true’ laboratory measured phosphate concentrations in natural water samples. This can be seen in the box and whisker plot (Figure 5) which shows that in each PackTest category, median laboratory measured phosphate values were close to the mid-point of the PackTest category. The exception was the penultimate category (0.75 mg L^{-1}) where the PackTest kits tended to underestimate the true phosphate value. However, the long ‘whiskers’ and broad interquartile range for this category partly reflect the small number of water samples falling into this phosphate range.

Differences between laboratory measured phosphate concentrations in the two lowest PackTest classes (mid points 0.01 and 0.035 mg L^{-1}) and all higher classes were statistically significant. There were no significant differences in laboratory measured phosphate concentrations in the three higher polluted PackTest classes (mid-points 0.150, 0.350 and 0.750 mg L^{-1}). These findings indicate that the PackTest kits can separate clean from more contaminated water but that differences between immediately adjacent categories should not be relied on at higher levels of phosphate. Statistical analysis of the differences between the categories are shown in Appendix 2a.

Figure 6 plots out the match between the PackTest and laboratory data in more detail. Appendix 3a provides the raw data. For this analysis the laboratory data were placed into the

PackTest categories, a process which tends to emphasise differences[3], so some variability around the 'true' PackTest category is inevitable. The results show that clean water sites with low phosphate were identified well by the PackTest method. However, at most categories above this (i.e. between 0.02 and 1 mg L^{-1}), the kits sometimes underestimated the extent of pollution, indicating that sites were lower in phosphate than they really were.

Overall the findings suggest that if the PackTest shows no colour change, we can be confident that the water is not polluted by phosphate. If the tests show a very slight pink tinge (0.02-0.05 mg L^{-1}) and therefore still fall into the clean water category, the water is probably clean, but there is around a 30% chance the sample may be mildly polluted. If the water sample shows a slightly stronger tinge in the 0.05-0.10 mg L^{-1} range, the site is highly likely to be polluted by phosphorus and may be even more polluted than the kit suggests.

2.2.3. *Practical implications of the relationship between PackTest and laboratory measured phosphate concentrations*

In the Clean Water for Wildlife survey the main objective of using the PackTest kits is to separate clean from polluted water, the key boundary for phosphate being between the 0.02-0.05 and 0.05-0.1 mg L^{-1} categories. A wide body of evidence suggests that for phosphate phosphorus, concentrations around 0.05 mg L^{-1} represent a realistic upper boundary between impaired and unimpaired waterbodies across much of the UK (see detailed comments in Sections 1.6-1.8 above and Appendix Table 1a). Practically, therefore, the main issue of concern in the use of kits for Clean Water for Wildlife is the reliability with which they place sites on either side of the clean water boundary.

As shown in Table 3, kits that show no change in colour (in the 0-0.02 mg L^{-1} category) reliably indicate (95%) water that is low in phosphate, and across most of lowland England and Wales, these sites can be described as 'clean'. In harder rock landscapes where phosphorus is naturally present in water at very low concentrations, some waterbodies classified as 'clean' may still have above natural levels of phosphorus. All waterbodies falling into the polluted categories are highly likely (95% certainty) to be polluted by phosphate. Waterbodies with water that is very slightly tinged pink (in the 0.02-0.05 mg L^{-1} category), are probably clean, but caution should be used because the kits tend to underestimate the level of pollution within this range and up to around a third may have some phosphate pollution.

As noted in Section 1.7, care should also be taken in locations, particularly in upland areas of the north and west of the United Kingdom, and on lowland heathland sites, which naturally have very low nutrient concentrations (e.g. oligotrophic ponds, lakes or rivers), where despite the addition of low levels of polluting nutrients phosphate-P levels may still be below the 0.05-0.1 mg L^{-1} 'polluted' boundary. In such locations laboratory water analysis will be essential to obtain a full understanding of nutrient impacts.

[3]For example a laboratory phosphate reading of 0.51 mg L^{-1} would be placed in a different category to a PackTest colorimetric reading for phosphate that is very close to it, but just below, the 0.5 mg L^{-1} value.

Table 3. Proportions of sites correctly identified as clean or polluted with phosphate by the PackTest kits in each PackTest kit category (data summarises results shown in Figure 6).

	Proportion of sites identified as clean or polluted by laboratory analysis	
PackTest category (mg L^{-1})	**Clean (%)**	**Polluted (%)**
0-0.02	98	2
0.02-0.05	67	33
0.05-0.1	15	85
0.1-0.2	0	100
0.2-0.5	5	95
0.5-1	0	100
>1	0	100

Clean

Polluted

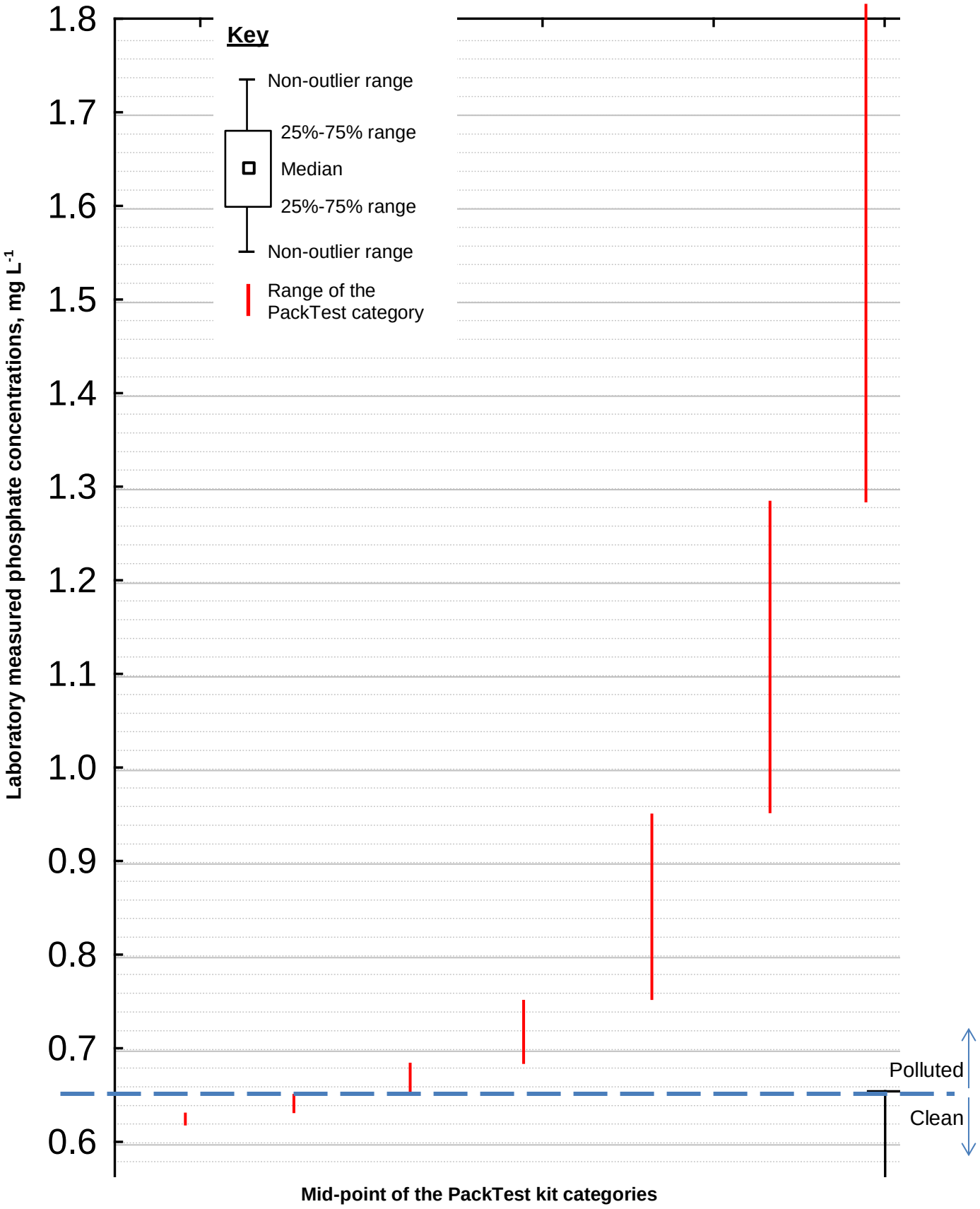
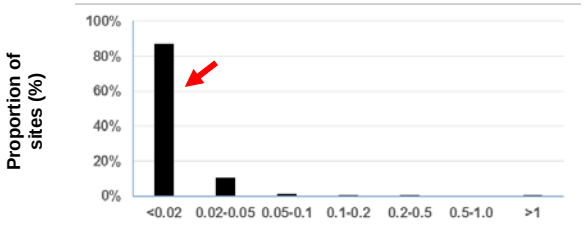


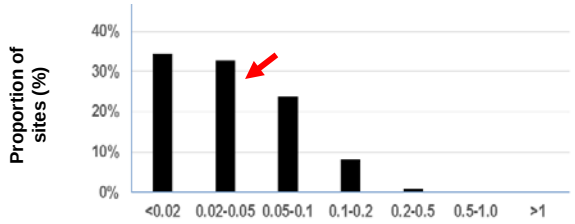
Figure 5. Relationship between PackTest kit categories and the 'true' laboratory measured phosphate values. For water samples within each PackTest category (x axis) the box and whisker plot shows the laboratory measured phosphate values. Red lines show the expected range if there was a perfect match between laboratory and PackTest values. Significant differences between the categories are shown in Appendix 2.

(a) PackTest phosphate category: 0-0.02 mg L^{-1} (n=352)



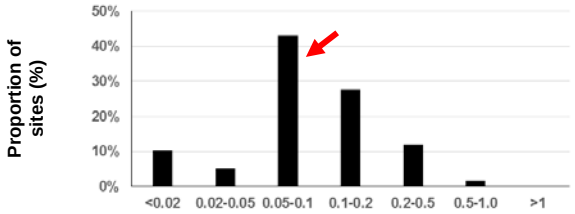
At sites with very low phosphate the majority (87%) of sites PackTest samples were placed in the correct laboratory category. A small proportion (10%) of sites should have been placed in the (still clean water) laboratory category above. Very few (2%) sites were polluted (i.e. greater than 0.05 mg L^{-1})

(b) PackTest phosphate category: 0.02-0.05 mg L^{-1} (n=110)



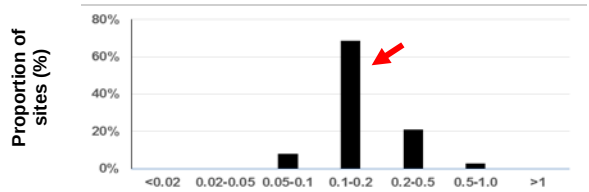
Around a third of PackTest samples in the second of the 'clean' categories were placed in the correct laboratory category. A third of the remainder should have been placed in the clean water category below. And a third should have been placed in polluted categories; mainly (24%) into the adjacent mildly polluted category.

(c) PackTest phosphate category: 0.05-0.1 mg L^{-1} (n=58)



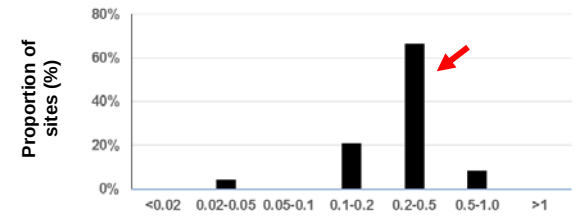
Just over 40% of sites were correctly classified as mildly polluted with phosphate in the range 0.05-0.1 mg L^{-1}. However 40% of the sites were more polluted than this and 16% should have been classified as clean by PackTest.

(d) PackTest phosphate category: 0.1-0.2 mg L^{-1} (n=38)



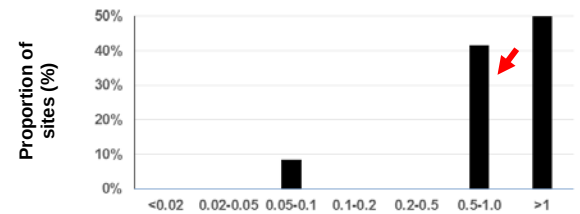
For moderately polluted waterbodies in the 0.1-0.2 mg L^{-1} range almost 70% of the PackTest samples were correctly classified. Just under 30% should have been placed in adjoining polluted categories and 3% in the highly polluted category 0.5-1 mg L^{-1}. None of the highly polluted sites were misclassified as clean.

(e) PackTest phosphate category: 0.2-0.5 mg L^{-1} (n=24)



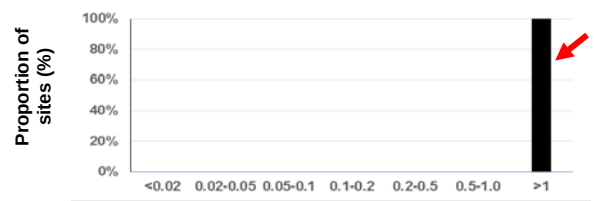
Two thirds of the PackTests were placed in the correct category. Most of the remainder should have been placed in adjacent categories. An anomalous 4% of sites should have been classified as clean. The reason for this is not known.

(f) PackTest phosphate category: 0.5-1.0 mg L^{-1} (n=12)



42% of the PackTest samples that classified sites as having highly polluted water in the 0.5-1 mg L^{-1} range were placed in the correct category. Half of the sites should have been placed in the polluted water category above this. However, in practice, this makes relatively little difference since both categories indicate gross pollution. A small proportion (8%) should have been placed in the mildly polluted category.

(g) PackTest phosphate category: 1+ mg L^{-1} (n=3)



At very high levels of pollution all the PackTest results were correctly classified but note that the sample size was very small for this category.

Figure 6. Proportion of sites in each PackTest nitrate category correctly allocated to the true laboratory measured category. Red arrows indicate laboratory category into which the values should be allocated.

2.2.4. *Results of phosphate field testing: Thames WaterBlitz*

The main comparison of the relationship between PackTest kits and laboratory analysed water samples was undertaken in the year-long study of waterbodies in Woking and the New Forest. However, a small additional study was undertaken as part of the Clean Water for Wildlife project in the catchment of the River Thames on 14th September 2015 as part of the Thames WaterBlitz. In the course of this project samples were collected from 23 sites, all on rivers or streams, which coincided with locations regularly sampled by the Centre for Ecology and Hydrology (CEH) as part of the River Thames Initiative.

Results

The PackTest phosphate samples collected at the same time as the CEH laboratory water samples showed a good match with the results of the laboratory analysed samples (Figure 7). Median laboratory measured phosphate values in each PackTest category were generally within the respective PackTest ranges. Broadly the analysis, undertaken by a different laboratory to that which did the main Woking/New Forest analysis reported above, shows the same or better relationships between PackTest kits and laboratory results reported above.

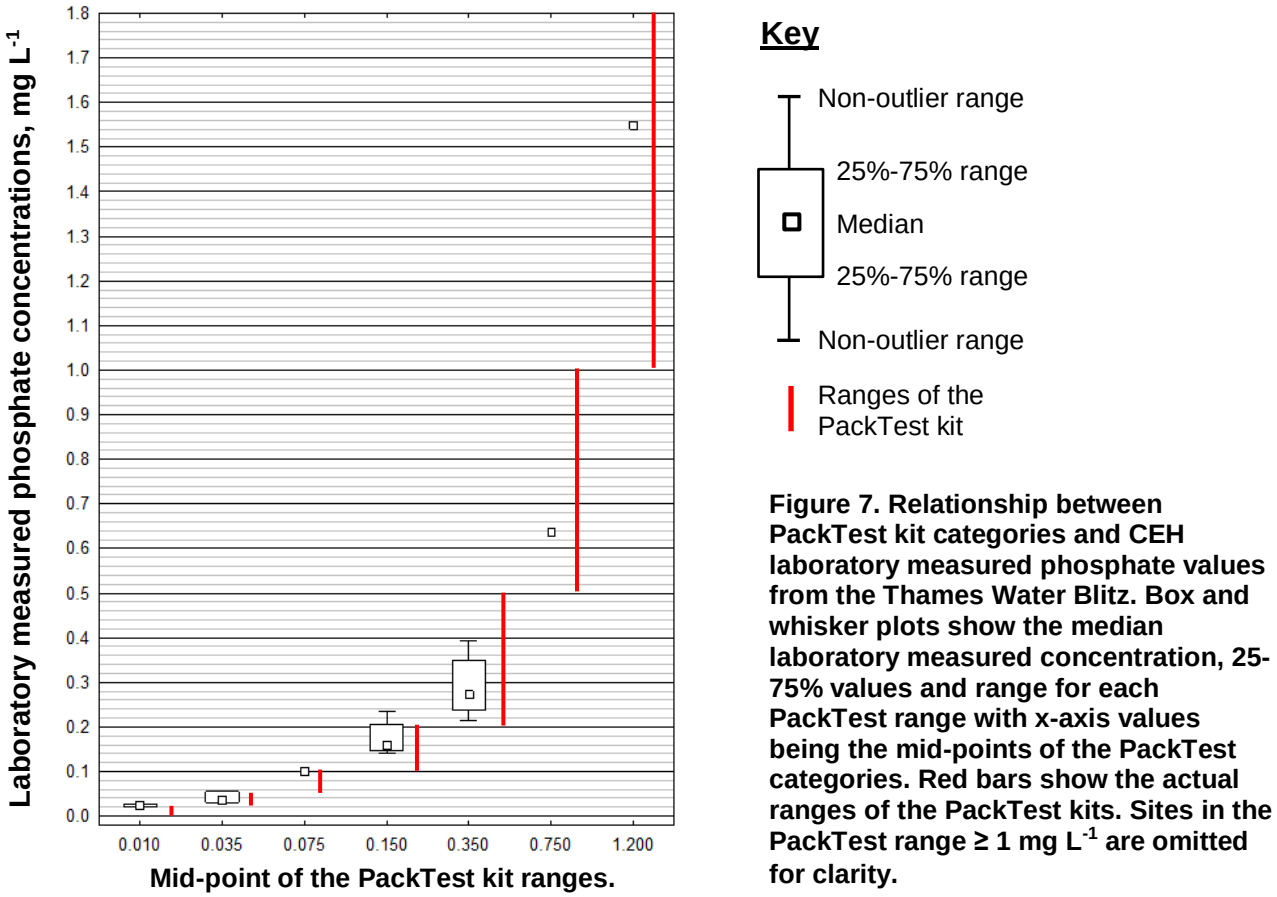


Figure 7. Relationship between PackTest kit categories and CEH laboratory measured phosphate values from the Thames Water Blitz. Box and whisker plots show the median laboratory measured concentration, 25-75% values and range for each PackTest range with x-axis values being the mid-points of the PackTest categories. Red bars show the actual ranges of the PackTest kits. Sites in the PackTest range ≥ 1 mg L^{-1} are omitted for clarity.

2.2.5. *Seasonal variation in phosphate concentrations measured using PackTest kits and laboratory analysed samples*

The nutrient levels in waterbodies often vary across the year (see Section 4.1). Figures 8 and 9 summarise the results of monthly measurement of phosphate concentrations using PackTest kits and standard laboratory water analysis methods. The figures show actual laboratory measured phosphate values, compared to the mid-point values of PackTest categories.

In the cleaner waters of the New Forest (Figure 8) there was very little detectable seasonal change at most sites with very low, near natural, background nutrient concentrations throughout the year. As a result of this, PackTest and laboratory samples were closely matched. At only one site, River Blackwater, was there pronounced seasonal variation in phosphate concentrations. At this site, PackTest and laboratory measured phosphate values were not correlated, with substantial differences between the two measurement methods. It is not clear what caused this discrepancy.

Phosphate levels were generally higher in the Woking area waterbodies (Figure 9). In the sites with pronounced seasonal variation, PackTest and laboratory samples generally showed similar trends in nutrient concentrations, although at three sites (Mayford Pond Small, Papercourt Lake Small and Windle Brook) there were substantial seasonal differences but results from the two methods were not correlated. Like the River Blackwater site in the New Forest, these discrepancies could not be explained. However, they could reflect (a) the inherent variability of the PackTest kits, (b) chemical interference with the PackTest reactions where laboratory values were greater than PackTest values or (c) detection of particulate P by the PackTest kit where PackTest results were higher than laboratory results.

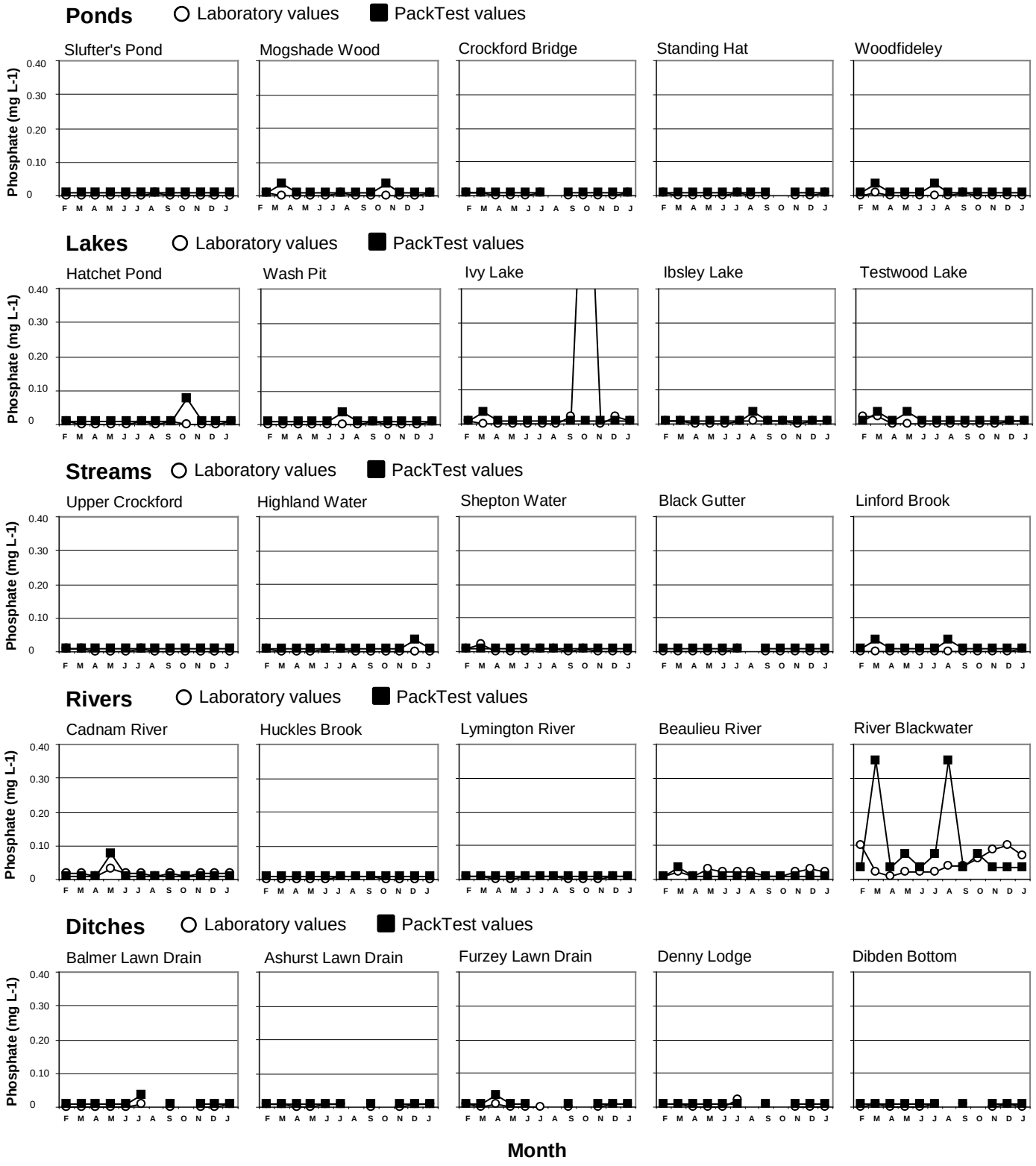


Figure 8. Seasonal variation in phosphate concentration in ponds, lakes, streams, rivers and ditches in the New Forest.

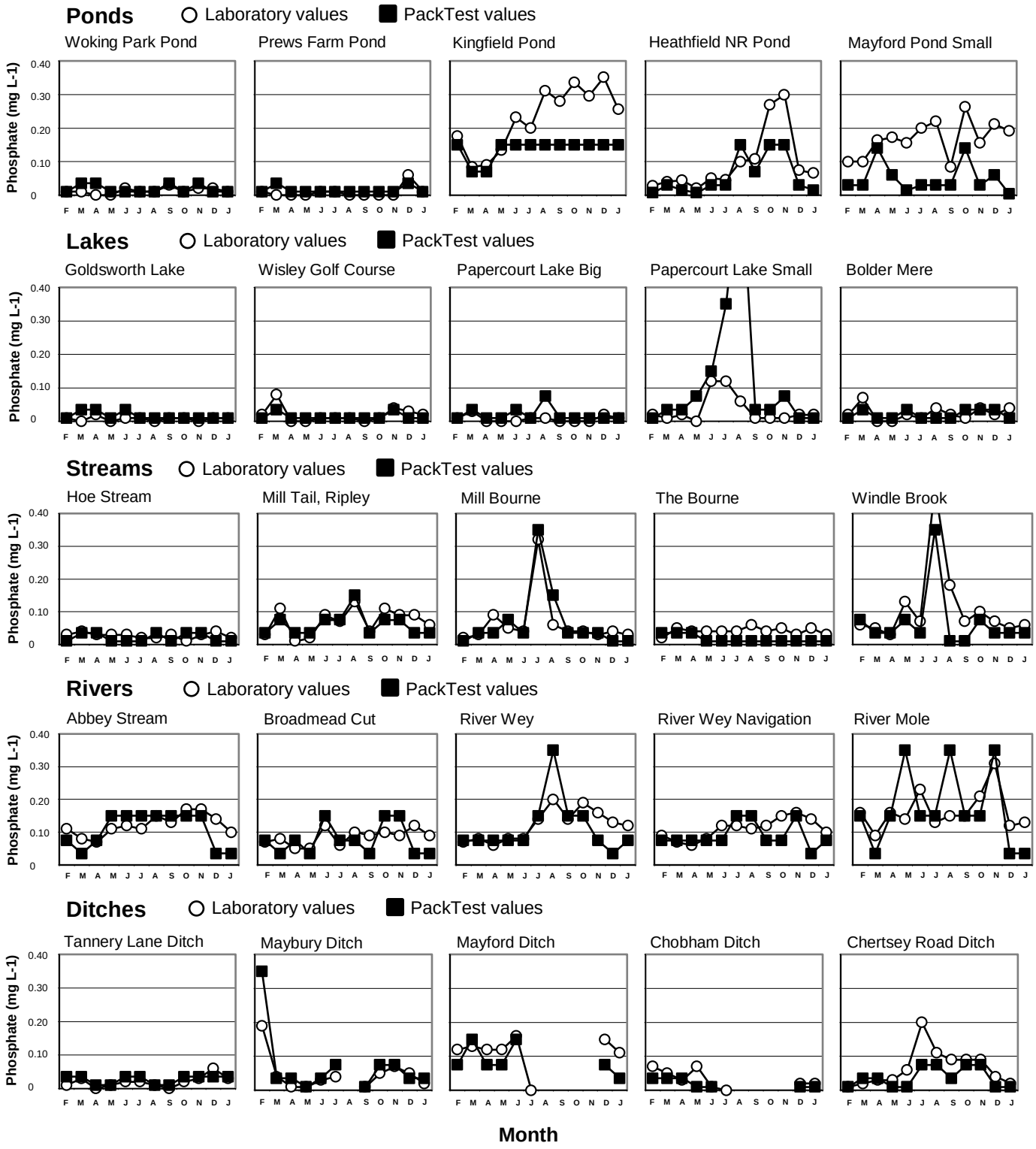


Figure 9. Seasonal variation in phosphate concentration in ponds, lakes, streams, rivers and ditches in the Woking area.

2.2.6. *Additional technical background information for the PackTest phosphate low range test kit*

This section summarises information provided by the PackTest kit manufacturers Kyoritsu. For Clean Water for Wildlife we use Kyoritsu's low range phosphate PackTest which is based on an enzymatic method and does not use strong acid. The manufacturers note that low phosphate concentrations can be measured from samples containing relatively few coexisting substances, such as river water, underground water and drinking water. The low range PackTest kit is not suitable for the analysis of samples collected from water purifier tanks, sewage, industrial waste water or other samples which contain high concentrations of coexisting substances. For more contaminated waters, in which the phosphate level is higher, Kyoritsu recommends the use of the high range Pack Test kit which spans: 0.2-10 mg PO_4^{3-} L^{-1}. There is no published analysis of the method as far as we know and no previous published comparisons of the method with laboratory analysed samples.

Manufacturers information about the method

The phosphate (low range) PackTest kits measures only the dissolved phosphate ion. Hydrolytic phosphorus or Total Phosphorus cannot be measured directly and requires a pre-treatment. The PackTest results can be reported as the range of concentrations of the phosphate ion ($PO4^{3-}$) or phosphate-phosphorus ($PO4^{3-}$-P). Samples should be read in daylight or under a daylight corrected lamp. Kyoritsu note that partially undissolved reagent in the sample tube will not affect the measurement.

Temperature effects

Importantly, the manufacturers suggest that sample temperature should be kept in the range 20-40° C. Lower temperature necessitates longer reaction time. For example: at 10°C, the response time for phosphate is 20 minutes rather than the normal 5 minutes. For the current tests, samples were returned the surveyor's home and left to equilibrate to normal room temperature before testing. An alternative approach in cold weather, or if the water itself is cold, is to collect the water sample in a small bottle and warm it in your hands, or a pocket before testing.

Sampling effects

Importantly, samples should be taken in a manner to be representative of the waterbody being sampled, avoiding effects from sediment or surface water. Care should be taken to reduce contamination from earlier samples. The volume of sample should be 1.5 ml, as higher and lower volumes influence (reduce) colour through dilution and a reduction in reactants.

Interferences with the PackTest phosphate kit

The phosphate kit is recommended for use in the pH range is 6 - 9. The manufacturers suggests that, if necessary, the pH should be adjusted with diluted sulfuric acid or sodium hydroxide solution, although there is no detailed information on how to do this. We have not evaluated the effect of low or high pH on the results reported here.

Coexisting ions can modify the reaction, with potential impacts on the phosphate concentration recorded. The list below, which is provided by Kyoritsu, shows ion concentrations *above* which interference can be significant:

- ≤1000 mg/L: Ba^{2+}, Ca^{2+}, Cl^- , F^-, I^-, K^+, Na^+, NH^{4+}, NO^{2-}, NO^{3-}
- ≤500 mg/L: B^{3+}, Phenol
- ≤200 mg/L: Zn^{2+}

- ≤50 mg/L: $Cu2^{+}$, Mg^{2+}, Ni^{2+}, SO_4^{2-}
- ≤10 mg/L: Al^{3+}, Cr^{3+}, Cr^{6+}, Mn^{2+}
- ≤ 5 mg/L: Fe^{3+}.

In most freshwaters which are not experiencing gross pollution from industrial or sewage sources such concentrations would be unusual but surveyors should be aware that unexpected results might be caused by chemical interferences.

2.3. Nitrate: evaluation of the effectiveness of the PackTest kits in detecting nitrate (NO_3-N)

2.3.1. *Background*

One previous study by Muneoka *et al*. (2014) has compared PackTest kits with laboratory standard analyses. The comparison was undertaken in the Hokkaido province of Japan and two catchments were visited in which levels of nitrate recorded in river water samples were compared (Figure 10).

These authors found a good correlation between PackTest results and laboratory results, but PackTest samples tended to underestimate the nitrate concentration compared to laboratory analyses (Figures 10 and 11).

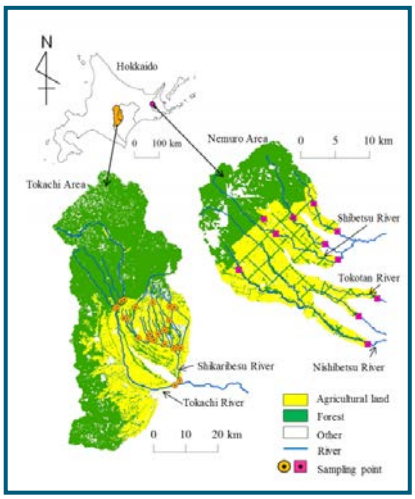


Figure 10. The PackTest nitrate test kit was compared with standard Japanese laboratory methods in the Hokkaido province by Muneoka *et al*. (2014).

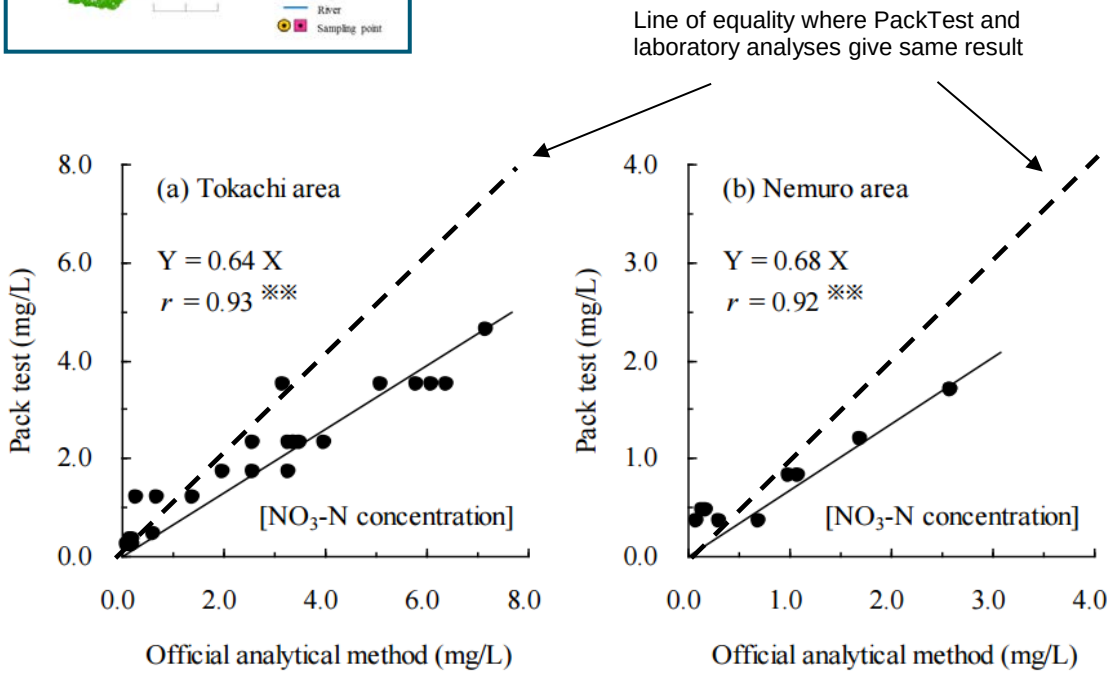


Figure 11. Comparison of PackTest nitrate kits and laboratory nitrate tests of river water samples in the Hokkaido province of Japan (Muneoka *et al*. 2014)

2.3.2. *Approach to testing the nitrate kit*

In the current survey, our approach for testing the nitrate PackTest kit followed the same protocol as the phosphate kits. We compared the quick kits with the results of laboratory analysis using two types of water sample: (i) laboratory standard nutrient solutions and (ii) field collected 'natural' water samples. The laboratory standard solutions provided an assessment of the underlying ability of the kits to detect the nutrients being measured in the absence of other chemicals or sediments that are present in 'natural' water samples. Natural water samples give a more realistic impression of the reliability of the kits because they allow us to take account of elements which can potentially interfere with the accuracy of the kits.

2.3.3. *Nitrate concentration in laboratory standard solutions*

Nitrate was analysed from filtered water samples using a Skalar autoanalyser based on a cadmium reduction, where the solution is passed through cadmium to reduce the nitrate to nitrite. The nitrite diazotises with sulphanilamide producing a-naphthyl-ethylenediamine dihydrochloride to form an azo dye which is read on a spectrophotometer 540 nm. Analyses were undertaken by Freshwater Habitats Trust staff in Oxford Brookes University laboratories. In order to directly compare the laboratory values with the PackTest kits, the laboratory values were converted to the midpoint of the PackTest kit ranges.

The PackTest analysis

Results

As with phosphate, the PackTest kits generally compared well with laboratory standard solutions: pairwise analysis indicated that there was no significant difference between the two methods (Wilcoxon signed rank test, p=0.58) (Figure 12).

When the PackTest and laboratory results were categorised as the mid-point of the PackTest colour chart bars, there is an apparent mismatch in the middle of the range (Figure 12b). This can be attributed to laboratory values which fell very close to the threshold between two ranges. This is always a risk when simplifying values into a single range value, and although visually there is a difference, this was not statistically significant.

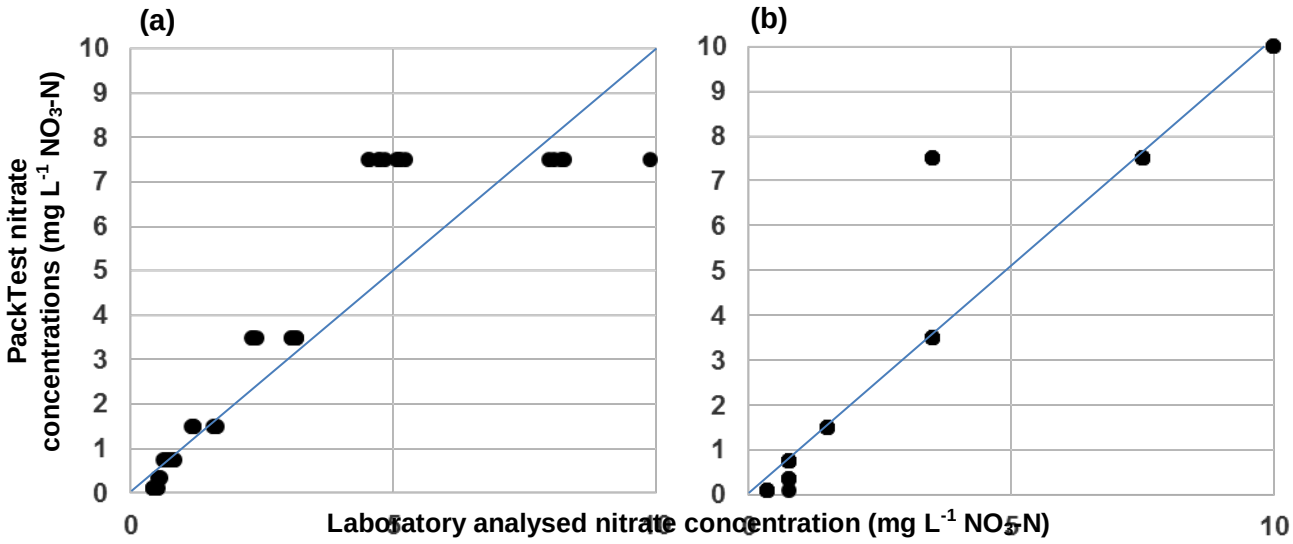


Figure 12. Comparison of PackTest kits and laboratory analysis of laboratory standard nitrate solutions (n = 70). (a) PackTest results compared to actual laboratory values; (b) PackTest and laboratory results categorised as the mid-point of the PackTest colour chart bars.

2.3.4. *Nitrate concentrations in natural water samples*

Waterbodies in the New Forest and Woking area (described in Section 2.1) were sampled at monthly intervals from February 2014 to January 2015. At each site a sample was collected for testing using the PackTest low range nitrate test kit and a second sample for laboratory nitrate analysis. A total of 600 site visits were made to collect water quality data. Sites were dry on 26 occasions (mainly ditches and ponds) giving a total of 574 measurements on the waterbodies. PackTest samples were analysed the same day indoors in batches at the home of the surveyor. Samples for laboratory analysis were returned to the laboratory and maintained at 2-4ºC until analysed, which was within 1-3 days after collection. In the laboratory, nitrate was analysed as described above in Section 2.3.3. Storage in the laboratory prior to analysis may be associated with change in nitrate concentrations. For example, Moore and Locke (2013) found that storage of samples for 7 days at 4ºC led to increases of around 50% in nitrate concentration compared to control samples analysed within 4 hours of collection.

Results

The PackTest kit nitrate values in natural waterbodies (ponds, streams, ditches, lakes and rivers) were correlated with laboratory measured nitrate concentrations. However, within the range 0-2 mg L^{-1}, the PackTest kit values were consistently lower than those measured in the laboratory (Figures 13, 14). For example, in the PackTest nitrate category 0.5-1 mg L^{-1} the laboratory measured median value of sites in this category was 1.22 mg L^{-1} i.e. above the upper bound of the PackTest category. In the two highest concentration categories, the true median value lay within the PackTest range.

Differences between laboratory measured nitrate concentrations in lowest PackTest classes were statistically significant (Figure 13; Appendix Table 2b). Thus the two lowest (clean) PackTest kit classes, 0.1 and 0.35 mg L^{-1} nitrate, were significantly lower than those in the polluted PackTest classes (0.75, 1.50, 3.50 and 7.50 mg L^{-1}). Laboratory measured nitrate concentrations at sites which fell into the 0.75 mg L^{-1} PackTest class were also significantly lower than those in the 7.5 mg L^{-1} PackTest class. There were no significant differences in laboratory measured nitrate concentrations in the three higher polluted PackTest classes (mid-points 1.50, 3.50 and 7.50 mg L^{-1}). These findings indicate that the PackTest kits can separate clean from more contaminated water but that differences between immediately adjacent categories should not be relied on, particularly where there is evidence of nitrate pollution above 0.5 mg L^{-1}.

Overall, the results reported here broadly reflect the same pattern seen by Muneoka *et al.* 2014 who found that PackTest kits underestimated laboratory measured nitrate values (Section 3.3.1) by up to 30%. However, in contrast to the present study they found that differences were more pronounced at high nitrate concentrations.

Table 4. Median laboratory measured nitrate concentrations in the six PackTest nitrate classes in waterbodies in the Woking district and the New Forest (n=574)

PackTest nitrate class: mid-point and range (mg L^{-1} nitrate-N)	Median laboratory nitrate concentration for the PackTest class (mg L^{-1} nitrate-N)
0.1 (range 0-0.2)	0.33
0.35 (range 0.2-0.5)	0.54
0.75 (range 0.5-1)	1.22
1.5 (range 1-2)	2.82
3.5 (range 2-5)	3.59
7.5 (range 5-10)	5.86

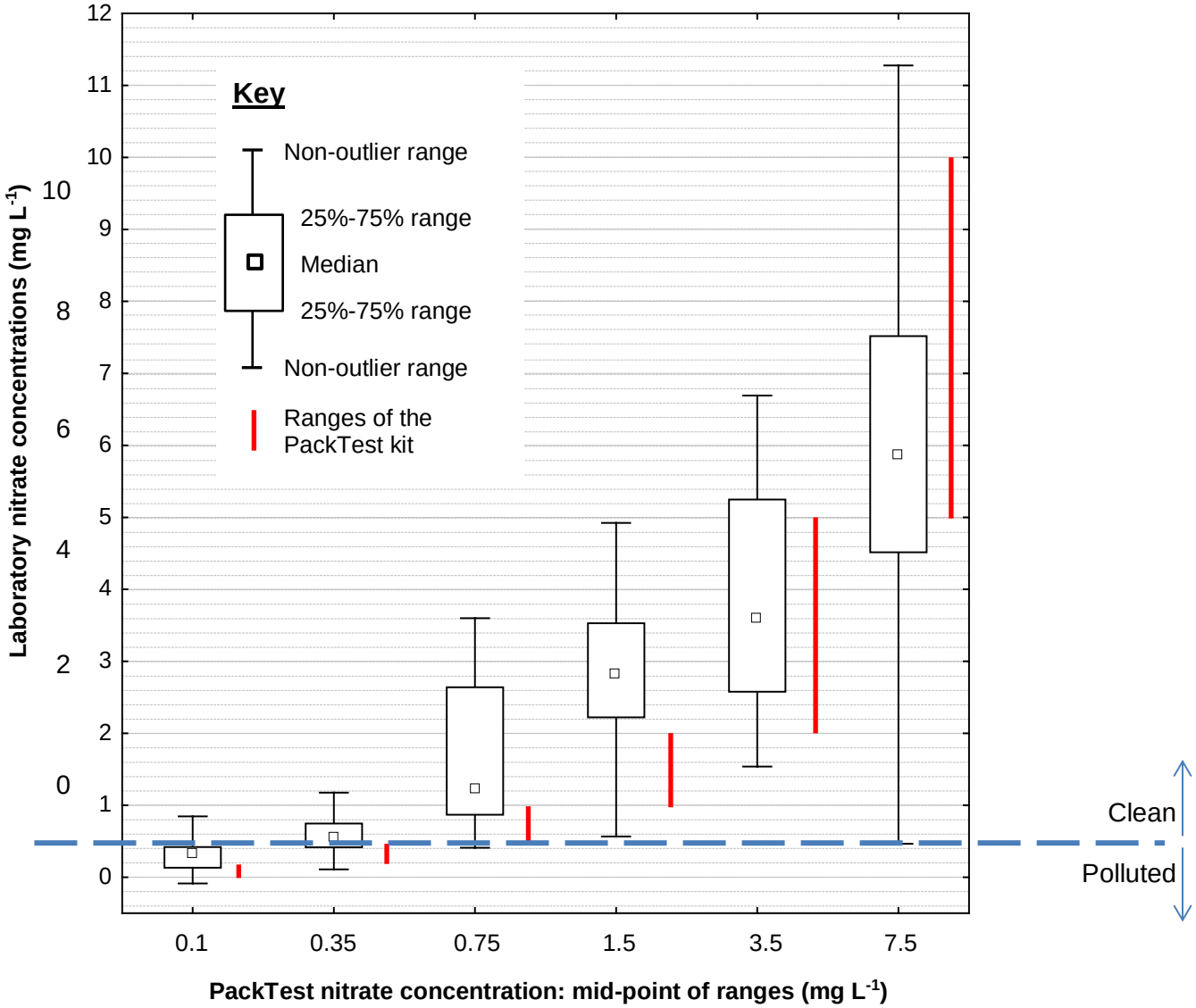


Figure 13. The relationship between PackTest nitrate categories and laboratory measured nitrate concentrations.

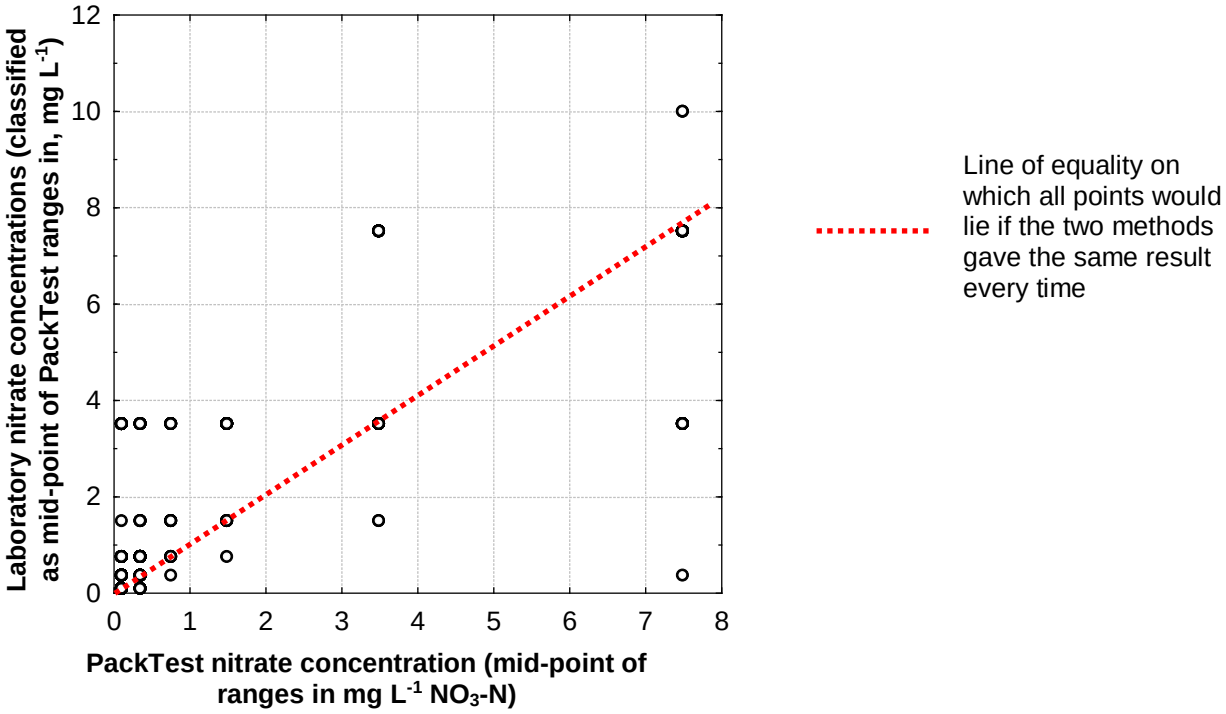


Figure 14. The correlation between PackTest and laboratory nitrate measurements when laboratory results are categorised using the six PackTest classes. The correlation is significant at p<0.001 (Spearman rank correlation, R = 0.71, n=573).

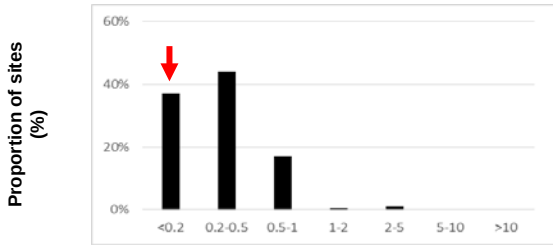
The underestimation of nitrate concentrations by the PackTest kits is plotted in more detail in Figure 15. Note that for this analysis the laboratory data were placed into the PackTest categories, a process which tends to emphasise differences[4], so some variability around the 'true' PackTest category is inevitable.

In this figure, graphs a - d show that the PackTest kits consistently underestimated the laboratory measured nitrate value when nitrate is present in the water at low to moderate levels (i.e. <2.0 mg L^{-1}). At higher levels of pollution, above 2.0 mg L^{-1}, this trend was less evident and the majority of laboratory measured values fell into the same category as the class identified by PackTest sampling.

Overall the findings suggest that if the PackTest shows no colour change, then the water is likely to be clean, although there is still a 1 in 6 chance that the water could be mildly polluted by nitrate. If the tests show a very slight pink tinge (0.2-0.5 mg L^{-1} NO^3-N) there is a roughly 50:50 chance that the water is not polluted by nitrate. If the PackTest reading is above 0.5 mg L^{-1} NO^3-N the water is highly likely to be polluted, and may be one to two pollution classes higher in nitrate than the test shows.

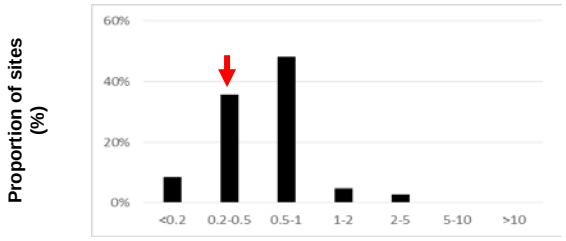
[4] Placing 'continuous' laboratory data into categories exaggerates differences. For example a laboratory phosphate reading of 0.51 mg L^{-1} would be placed in a different category to a PackTest colorimetric reading for phosphate that is very close to it, but either at, or just below, the 0.5 mg L^{-1} value.

(a) PackTest nitrate category: <0.2 mg L^{-1} NO_3-N (n-274)



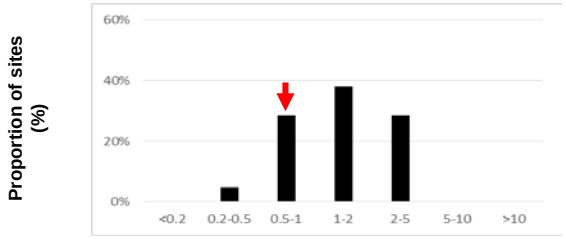
Of the samples that PackTest placed in the lowest nitrate category, c.37% were placed in the correct laboratory category. Most (44%) should have been placed in the clean water category above (i.e. 0.2-0.5 mg L^{-1}). A smaller proportion (18%) belonged in the polluted laboratory categories (i.e. greater than 0.5 mg L^{-1}).

(b) PackTest nitrate category: 0.2-0.5 mg L^{-1} NO_3-N (n=106)



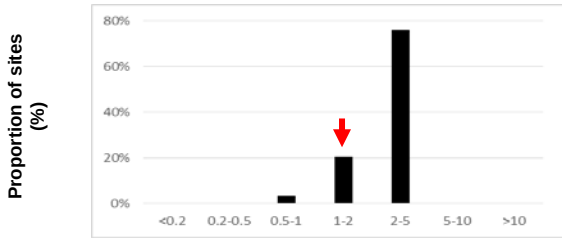
Again PackTest tended to underestimate the true level of nitrate pollution in waterbodies. Just over a third (36%) of PackTest samples recorded in the second of the 'clean' categories were placed in the correct laboratory category. Almost half (48%) should have been placed in the mildly polluted category and 8% in moderately polluted categories.

(c) PackTest nitrate category: 0.5-1.0 mg L^{-1} NO_3-N (n=21)



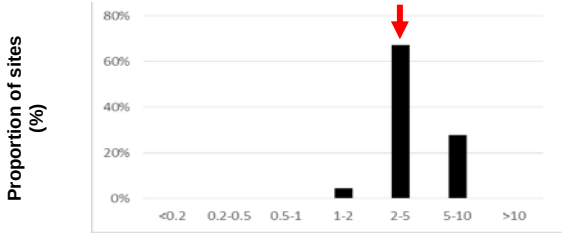
The PackTests again tended to underestimate how polluted sites were by nitrate. Only 29% of sites were correctly classified as mildly polluted with nitrate in the range 0.5-1 mg L^{-1}. Most of the remaining 67% should have been placed in more polluted categories.

(d) PackTest nitrate category: 1.0-2.0 mg L^{-1} NO_3-N (n=29)



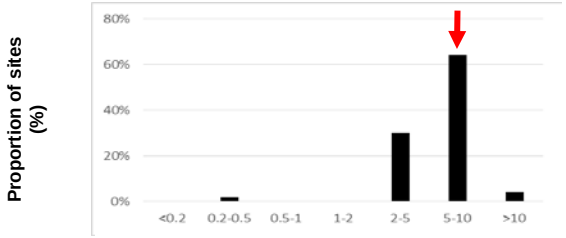
For waterbodies that PackTest classified as moderately polluted in the 1-2 mg L^{-1} range, 21% of the samples were correctly classified, but in the majority of cases (76%) PackTest underestimated the level of pollution, and should have placed sites in adjoining higher nitrate pollution categories.

(e) PackTest nitrate category: 2.0-5.0 mg L^{-1} NO_3-N (n=43)



The PackTests placed the majority (67%) of sites in the correct 2-5 mg L^{-1} range. Most of the remaining third of sites should have been placed in the more polluted 5-10 mg L^{-1} category.

(f) PackTest nitrate category: 5.0-10.0 mg L^{-1} NO_3-N (n=50)



At high levels of nitrate pollution the PackTest kits placed 64% of sites in the right pollution category. Most of the remainder (30%) were placed in the category below. An anomalous 2% of sites were placed in the clean water category.

Figure 15. Proportion of sites in each PackTest nitrate category correctly allocated to the true laboratory measured category. Red arrows indicate laboratory category into which the values should be allocated.

2.3.5. *Practical implications of the relationship between PackTest and laboratory measured nitrate concentrations*

In the Clean Water for Wildlife survey the main objective of using the PackTest kits is to separate clean from polluted water, the key boundary for nitrate being between the 0.2-0.5 and 0.5-1 mg L^{-1} categories. Current evidence suggests that for nitrate-nitrogen, concentrations around 1 mg L^{-1} represent a realistic upper boundary between impaired and unimpaired waterbodies across much of the UK (see Appendix Table 1b). Practically, the main issue of concern in the use of kits for the Clean Water for Wildlife survey is the reliability with which they place sites on either side of this clean water boundary.

Our results (Table 5) show that if a moderate colour change occurs (above 0.5 mg L^{-1}) it is highly likely (>98%) that the site is polluted. If the nitrate kits show no colour change then the water is probably clean (81%), but if there is a hint of colour (0.2-0.5 mg L^{-1}), care needs to be taken because around half of the sites are likely to be mildly polluted with nitrate.

As noted in Section 1.7, care should also be taken in locations, particularly in upland hard rock landscape of the north and west of the UK which naturally have very low nutrient concentrations where, despite the addition of nitrogen from a range of sources (atmospheric deposition, sewage works, land runoff), nitrate concentrations may still remain below the 0.5-1 mg L^{-1} 'polluted' boundary. In such locations, any colour change may be regarded as a sign of pollution and laboratory water analysis will be essential to obtain a full understanding of nutrient impacts.

Table 5. Proportions of sites correctly identified as clean or polluted by the PackTest kits, according to PackTest kit category

	Proportion of sites identified as clean or polluted by laboratory analysis		
PackTest category (mg L^{-1})	**Clean (%)**	**Polluted (%)**	
0-0.2	81	19	Clean
0.2-0.5	44	56	
0.5-1.0	5	95	Polluted
1.0-2.0	0	100	
2.0-5.0	0	100	
5.0-10	2	98	
>10	0	100	

2.3.7. *Seasonal variation in nitrate concentrations measured using PackTest kits and laboratory analysed samples*

The results of monthly measurement of nitrate concentrations using PackTest kits and standard laboratory water analysis methods are shown in Figure 18 and 19. The figures show actual laboratory measured nitrate values, compared to the mid-point values of PackTest categories.

In the New Forest (Figure 17), with the exception of the River Blackwater, there was little detectable seasonal change at most sites with very low, near natural, background nutrient concentrations throughout the year. PackTest and laboratory samples were closely matched at all sites.

Nitrate levels were generally higher in the Woking area waterbodies, particularly in the streams and rivers. In the sites with pronounced seasonal variation PackTest and laboratory samples generally showed similar trends in nitrate concentrations (Figure 18). Although most sites showed a reasonable match between methods, the kits sometimes failed to reflect all of the seasonal variation evident in the laboratory analysed samples (Heathfield NR Pond, River Wey Navigation, Abbey Stream).

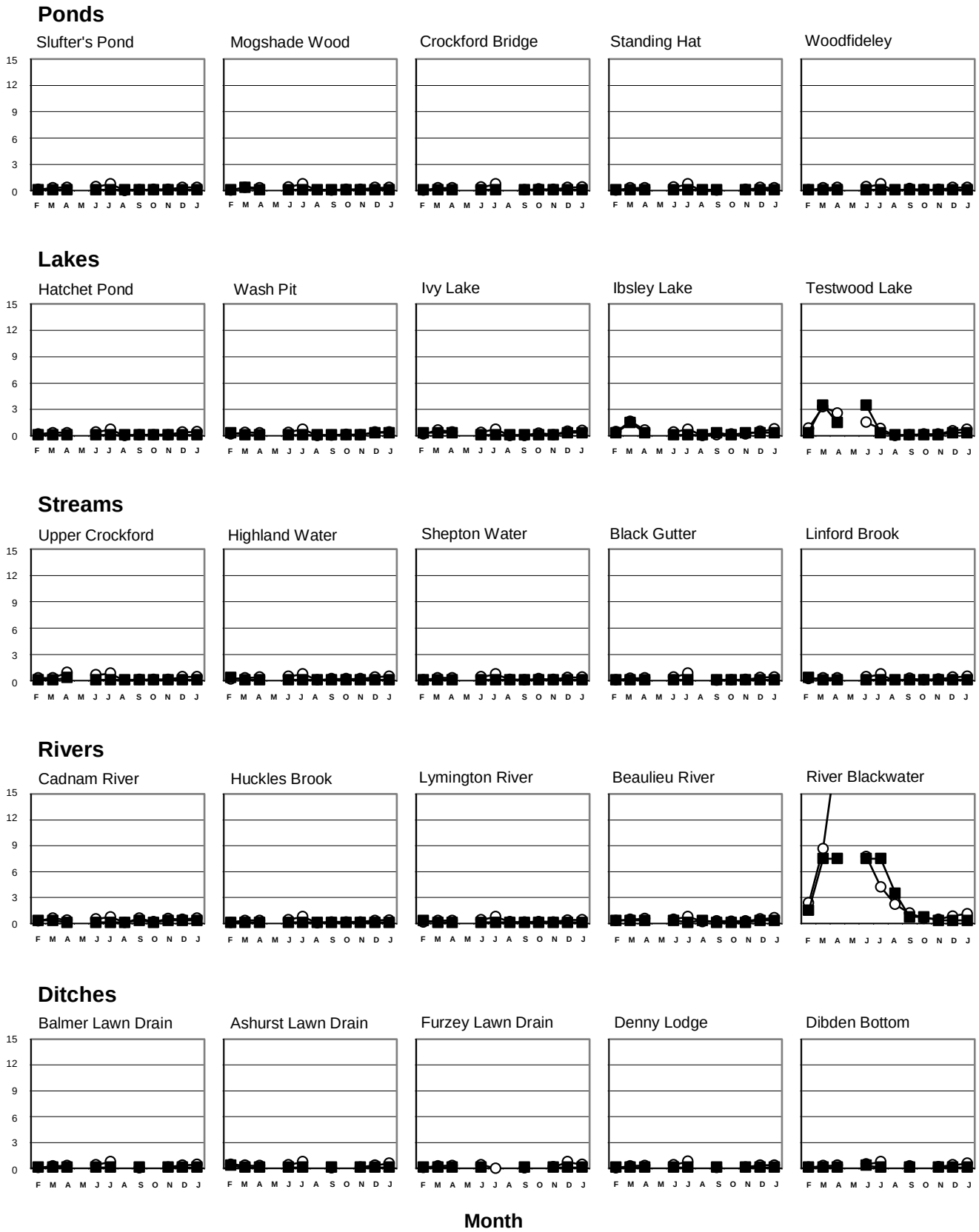


Figure 17. Seasonal variation in nitrate concentration in ponds, lakes, streams, rivers and ditches in the New Forest.

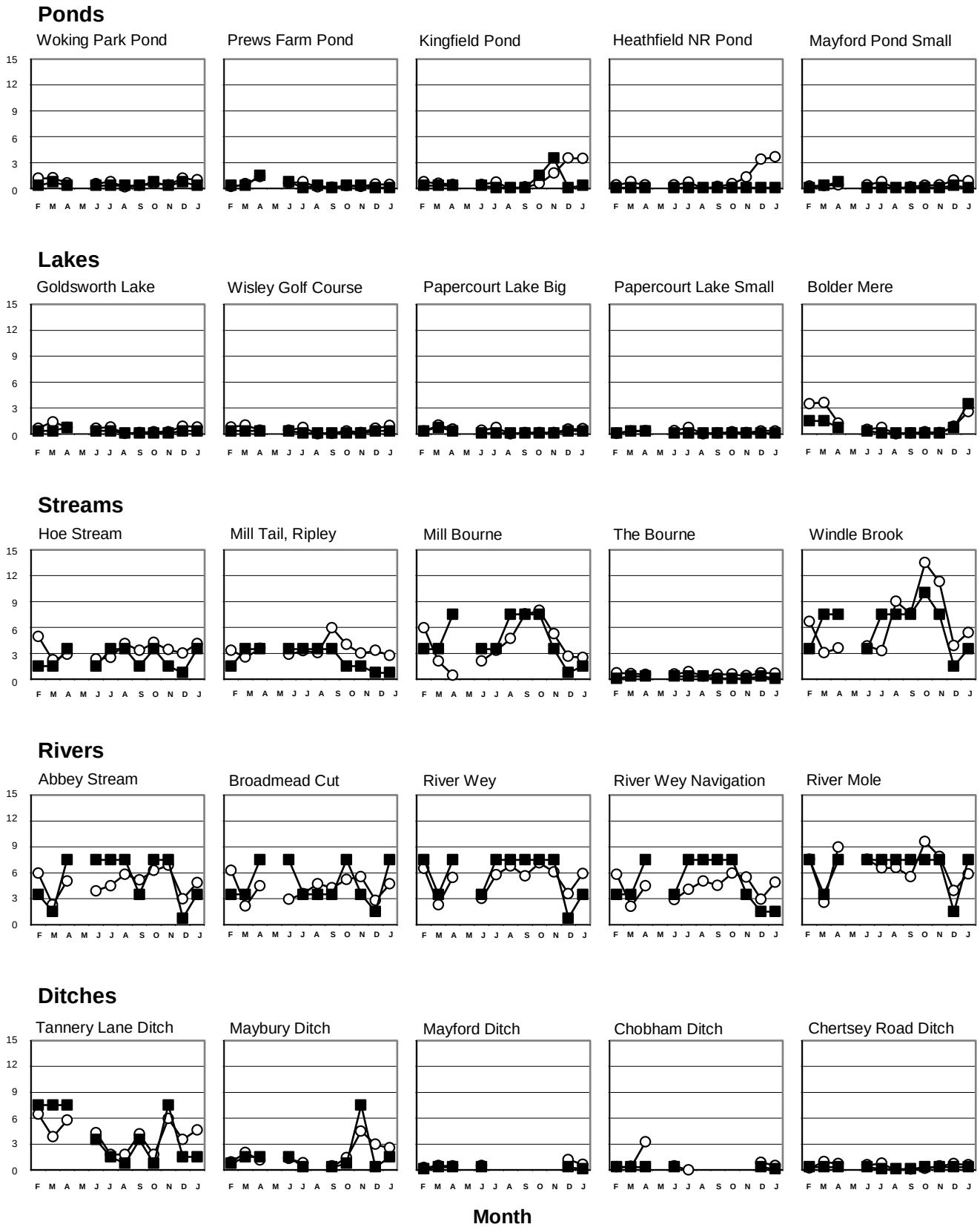


Figure 18. Seasonal variation in nitrate concentrations in ponds, lakes, streams, rivers and ditches in the Woking area.

2.3.8. *Additional technical background information for the PackTest nitrate test kit*

This section summarises information provided by the PackTest kit manufacturers Kyoritsu. For Clean Water for Wildlife we use Kyoritsu's standard nitrate PackTest (not the high range) which is based on a reduction by zinc to nitrate and the naphthylethylenediamine colour method.

Manufacturer's information about the method
Samples should be read in daylight, not in direct sunshine or under a daylight corrected lamp.

Temperature effects
Importantly, the manufacturers suggest that sample temperature should be kept in the range 15-40°C. Lower temperature necessitates longer reaction time though no details are given. For the current tests, samples were returned the surveyor's home and left to equilibrate to normal room temperature before testing. An alternative approach in cold weather, or if the water itself is cold, is to collect the water sample in a small bottle and warm it in your hands or in a pocket before testing.

Interferences with the PackTest nitrate kit
The nitrate kit is recommended for use in the pH range is 2 – 9, and is described by Kyoritsu as best between 6 and 7. The manufacturers suggests that, if necessary, the pH should be adjusted with diluted sulfuric acid or sodium hydroxide solution, although there is no detailed information on how to do this. We have not evaluated the effect of low or high pH on the results.

According to the information provide by Kyoritsu a range of coexisting ions can modify the nitrate test reaction colour. The list below indicates the concentrations at which interferences become significant:

≤ 1000 mg/L: Al^{3+}, B^{3+}, Ba^{2+}, Ca^{2+}, Cl^{-}, CN^{-}, F^{-}, Mg^{2+}, Mn^{2+}, Na^{+}, NH_4^{+}, PO_4^{3-}, SO_4^{2-}, Zn^{2+}, Phenol

≤ 250 mg/L: K^{+}

≤ 100 mg/L: Co^{2+}, Cr^{3+}

≤ 50 mg/L: Fe^{2+}, Ni^{2+}

≤ 20 mg/L: Fe^{3+}

≤ 5 mg/L: I^{-}

≤ 2 mg/L: Cd^{2+}, Residual Chlorine

≤ 1 mg/L: Cr^{6+}

Sub-ppm level: Cu^{2+}, Hg^{2+}, NO^{2-}, Sn^{2+}, Protein, Surfactant

In the samples that we have evaluated, data to assess potential interferences were available only for PO_4^{3-} which was below the 1000 mg/L level in all the samples tested. Although we have not tested the remaining potentially interfering ions specifically, it is unlikely that most would be above the limit values based on levels generally seen in freshwaters in southern England. Within Kyoritsu's 'sub-ppm[5]' group it is potentially possible that Cu^{2+} and NO^{2-} could be present in the waters tested. 'NO^{2-} is most likely to be present in sites receiving treated sewage effluents but there was no consistent evidence to suggest that this was the case. It seems possible that 'proteins' could be present in samples at sub-ppm levels, however this is not a routinely monitored variable.

[5] Ppm = parts per million

3. Synthesis: probability that sites are correctly identified as clean or polluted by the PackTest kits

Overall, the results of the PackTest kits suggest they are broadly reliable as a tool for identifying clean and polluted water. As shown in Figure 19, for sites which have phosphate values in the lowest category there is a high probability (98% or more) that the site is correctly identified as clean. About two thirds of sites in the 0.02-0.05 mg L^{-1} phosphate category are likely to be clean. Sites categorised by PackTest as having 'some phosphate pollution', are mostly correctly identified as polluted but about one in six sites will actually clean in terms of phosphate. Sites in the Highly or Very Highly polluted phosphate categories have a high probability of being correctly identified as polluted (at least 96%).

Nitrate tests placed in the lowest category are reasonably likely to be clean, with 81% correctly identified as unpolluted. Some caution is needed in interpreting the sites which fall into the 0.2-0.5 'clean' nitrate category as less than half of these are actually likely to be unpolluted. Sites which are identified as falling into the 'some', high' or 'very high' nitrate pollution categories are correctly identified at least 95% of the time.

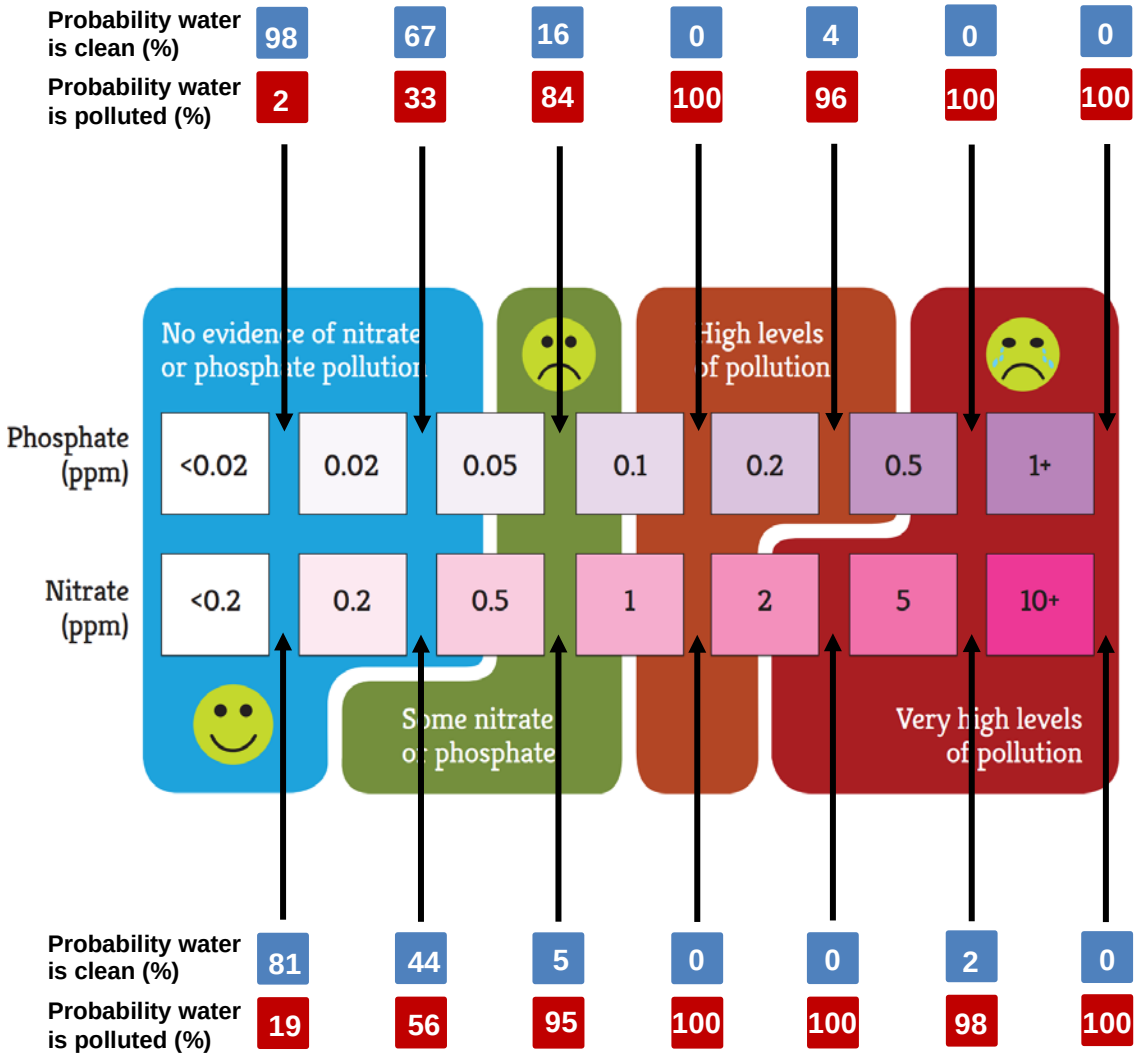


Figure 19. Probability that sites allocated to a particular PackTest phosphate or nitrate class are correctly classified as either clean or polluted

4. Interpreting the kit results and minimising errors

4.1. Seasonal effects

Nutrient levels in many waterbodies vary across the year. Nutrient peaks often occur as a result of seasonal inputs of pollutants from, for example, run-off from fertilizer application and muck spreading, particularly after rain. In running waters polluted by farm runoff and sewage works, nitrate concentrations often rise in winter when there is more runoff from the land, whereas phosphorus pollution is diluted, rising again in summer when there is less flow. In contrast, in lakes and ponds nutrient levels often decline in summer as water plants (including algae) take them up and build them into plant matter, before releasing the nutrients back into the water again as the plants die and decay in winter. These seasonal variations can make it difficult to interpret the results of both laboratory and kit nutrient information from a single visit to a site.

The PackTest kits measure soluble phosphorus and nitrate nitrogen, which are the most bioavailable and thus normally a good indicator of pollution. However, where phytoplankton (floating algae) and larger water plants do develop in substantial quantities it is possible for most of the soluble phosphorus and nitrate to be taken up by plants. In these conditions the PackTest kits will suggest “clean” water conditions, even though waterbodies are highly polluted by nutrients. This is why the lake standards (Appendix Table 1) use total phosphorus, which includes the phosphorus contained in the algal cells. For lakes and ponds nutrients are usually most likely to be detected in the winter when algal and plant growth is lowest. Less is known about seasonal variation in ditch nutrient concentrations but it seems likely that similar broad principles apply. For further information on the different fractions of phosphorus and nitrogen see Appendix 1.

4.2. Avoiding methodological errors

PackTest kits are simple but there are ways in which errors can creep in. Being aware of these errors, and reducing them through practice or training, is important for consistent results to be achieved. The main errors to take care avoiding are:

Using old kits
Kyoritsu recommend that PackTest kits should be used within 12 months after purchase. They should be stored in a cool, dry and dark place. The age of the kits can be determined from the batch numbers on the outside of the package. In practice, a test of our kits showed that some become less sensitive with age even before the 12 month due date (Figure 20). In practice we would recommend using all kits within the same season that they are received e.g. within 3 months and at least within 6 months.

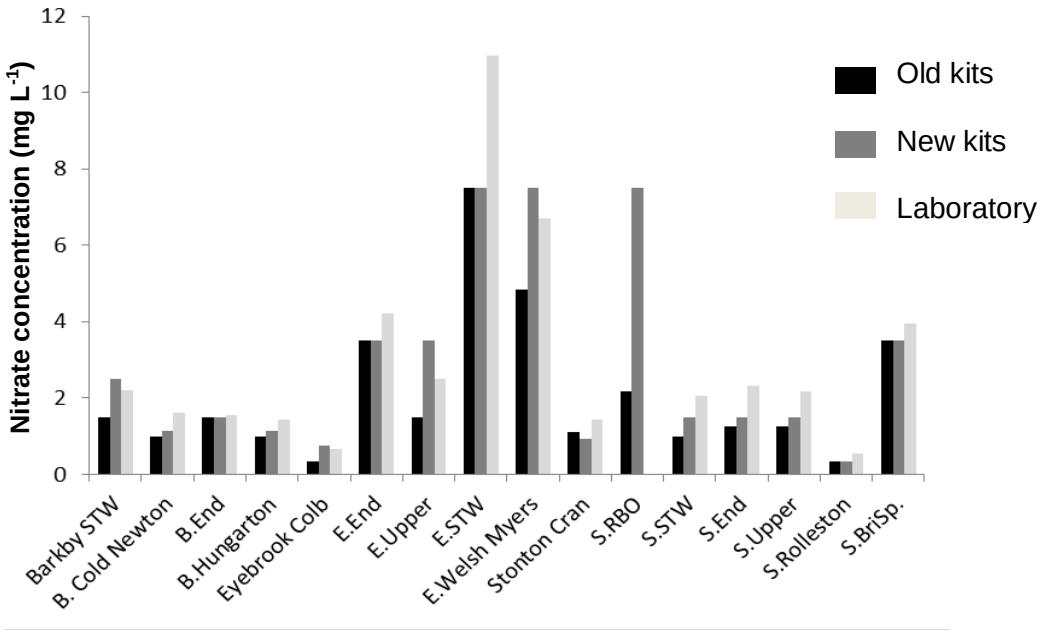


Figure 20. Comparison of old and new PackTest nitrate kits, compared to laboratory derived nitrate data. The survey sites are all rural streams in the Loddington area of Leicestershire.

Colour

The colour charts show a change in the intensity of colour, rather than a colour change, so people who are colour-blind are still able to use the kits. However colour intensity may look different in different light, so the tubes should be viewed in reasonably bright daylight, but not direct sunlight. If samples are analysed indoors, they can be viewed at a window or, as the manufacturer recommends, using a daylight type lamp.

It is useful to do the first few trials together with one or two other people to provide confidence that there is agreement on the correct colour category.

Temperature

Care needs to be taken to ensure that the water sample is warm enough when it is tested. **The manufacturers specify a range between 20-40°C for phosphate and 15-40°C for nitrate**. As the temperature drops the colour change is slower. At 10°C the manufacturers recommend that a phosphate test would take 20 minutes, rather than 5 minutes for the correct colour to develop. One solution is to take water samples home and let them warm up to room temperature. Warming the sample tubes in your hand before filling them is also possible though it is more difficult to get the right temperature. We did not systematically test for the effect of temperature on the analysis. As far as possible we have aimed to run analyses at toom temperature i.e. around 20°C.

Timing

As well as getting the right temperature, it is important to time the reactions correctly (3 minutes for nitrate, 5 minutes for phosphate) because the kits will continue to darken in colour after this time.

Recording the PackTest result

For the Clean Water for Wildlife survey results are reported by deciding which two colour bars the sample lies between. In the example shown in Figure 21 the colour lies between the 5 and 10 mg L^{-1} colour bars. Strictly the sample lies in the range 5-10 mg L^{-1}. However, to analyse the samples (which cannot easily be done with ranges) we arbitrarily convert this range to the mid-point i.e. 7.5 mg L^{-1}. For samples which are colourless we have created a class of no colour change. For samples which appear to be more intensely coloured than the darkest colour we have assumed that there is created an additional 10+ category for nitrate and 1+ category for phosphate.

Where the colour of the sample exactly matches the colour bar, so theoretically could be in either the higher or lower range, the sample should be assigned to the **higher** range, for consistency.

In practice, for Clean Water for Wildlife, differences between the very polluted categories are of limited importance. Much of the critical damage to wildlife occurs as waterbodies across the clean/moderately polluted water threshold.

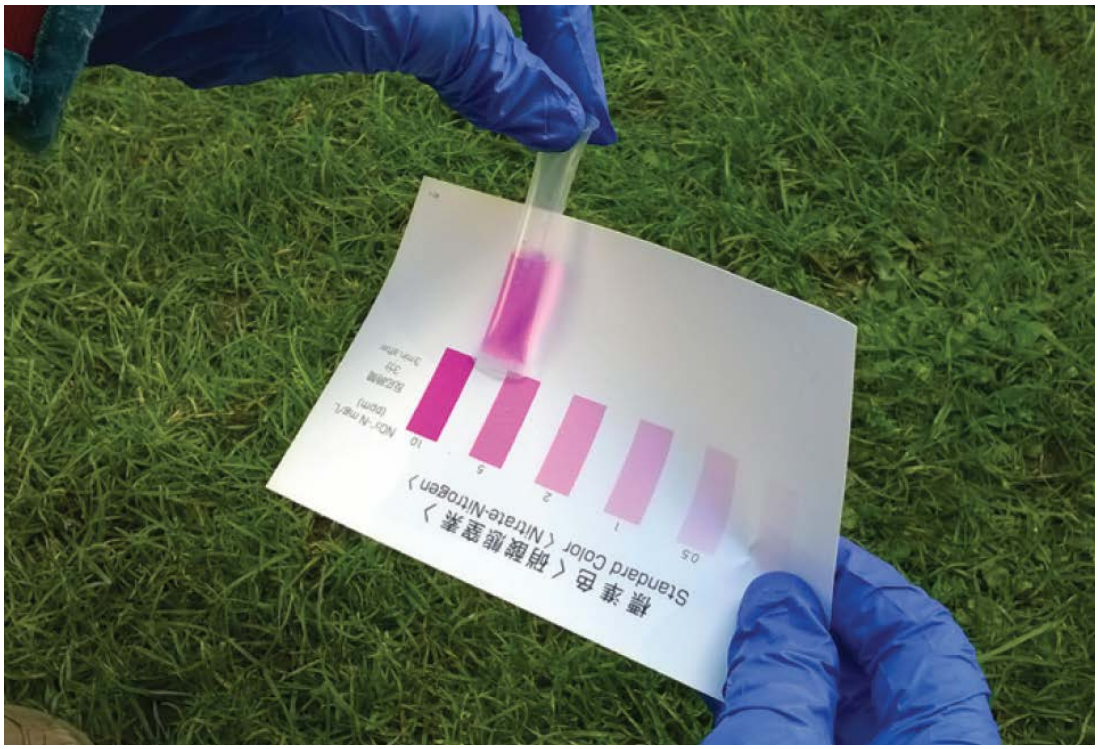


Figure 21. The PackTest nitrate kit in use

5. References

Aroviita, J., Mykrä, H., Hämäläinen, H., 2010. River bioassessment and the preservation of threatened species: towards acceptable biological quality criteria. Ecological Indicators, 10: 789–795.

Bennett, E.M., Carpenter, S.R. and Caraco, N.F. (2001). Human impact on erodable phosphorus and eutrophication: a global perspective. Bioscience 51: 227-34

Berti, G., Fossati, P., Tarenghi, G., Musitelli, C., & Melzideril, G. V. (1988). Enzymatic colorimetric method for the determination of inorganic phosphorus in serum and urine. Journal of Clinical Chemistry Clinical Biochemistry, 26, 399–404.

Biggs, B.J.F. (2000). Eutrophication of streams and rivers : Dissolved nutrient-chlorophyll relationships for benthic algae. Journal of the North American Benthological Society, 19: 17–31.

Bowes, M.J., Smith, J.T., Neal, C., Leach, D.V., Scarlett, P.M., Wickham, H.D., Harman, S.A., Armstrong, L.K., Davy-Bowker, J., Haft, M. and Davies, C.E. (2011). Changes in water quality of the River Frome (UK) from 1965 to 2009: Is phosphorus mitigation finally working? Science of the Total Environment 409: 3418–3430.

Brahney, J., Mahowald, N., Ward, D.S., Ballantyne, A.P. and Neff, J.C. (2015). Is atmospheric phosphorus pollution altering global alpine lake stoichiometry? Global Biogeochemical Cycles 29, 1369−1383.

Cardoso, A.C., Solimini, A., Premazzi, G., Carvalho, L., Lyche, A. and Rekolainen, S. (2007). Phosphorus reference concentrations in European lakes. Hydrobiologia 584: 3-12.

Dodds, W.K., Jones, J.R. and Welch, E.B. (1998). Suggested classification of stream trophic state: Distributions of temperate stream types by chlorophyll, total nitrogen, and phosphorus. Water Research, 32(5), 1455–1462.

Dodds, W. and Smith, V.H. (2016) Nitrogen, phosphorus, and eutrophication in streams. Inland Waters 6:155-164.

Elser, J.J., Bracken, M.E.S., Cleland, E.E., Gruner, D.S., Harpole, W.S., Hillebrand, H., Ngai, J.T., Seabloom, E.W., Shurin, J.B. and Smith, J.E. (2007). Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. Ecology Letters 10: 1135–1142.

Evans-White, M.A., Dodds, W.K., Huggins, D.G. and Baker, D.S. (2009). Thresholds in macroinvertebrate biodiversity and stoichiometry across water-quality gradients in Central Plains (USA) streams. Journal of the North American Benthological Society 28: 855-868.

Gonzalez Sagrario, M. A., Jeppesen, E., Goma, J., Sondergaard, M., Jensen, J.P., Lauridsen, T. and Landkildehus, F. (2005). Does high nitrogen loading prevent clear-water conditions in shallow lakes at moderately high phosphorus concentrations? Freshwater Biology, 50: 27–41.

Howden, N.J.K, Burt, T.P., Worrall, F., Whelan, M.J. and Bieroza, M. (2010). Nitrate concentrations and fluxes in the River Thames over 140 years (1868–2008): are increases irreversible? Hydrological Processes 24: 2657-2662.

James, C., Fisher, J., Russell, V., Collings, S. and Moss, B. (2005). Nitrate availability and hydrophyte species richness in shallow lakes. Freshwater Biology, 50: 1049–1063.

Lambert, S. J. & A. J. Davy, 2011. Water quality as a threat to aquatic plants: discriminating between the effects of nitrate, phosphate, boron and heavy metals on charophytes. New Phytologist 189: 1051–1059.
Miltner, R.J. and Rankin, E.T. (1998). Primary nutrients and the biotic integrity of rivers and streams. Freshwater Biology, 40(1), 145–158.

Muneoka, T., Yamazaki, Y., Wakou, S. and Kimura, M. (2014). Evaluation of nitrate pollution in river water at agricultural watershed. International Journal of Environmental and Rural Development, 5: 51-56.

Janse, J.H. and Van Puijenbroek, P.J.T.M. (1998). Effects of eutrophication in drainage ditches. Environmental Pollution, 102: 547–552.

JIS (2013). JIS K0102-1993 43.2.3.

Joint Nature Conservancy Council. (2005). Common standards monitoring guidance for canals. doi: ISSN 1743-8160.

Jeppesen, E., Søndergaard, M., Lauridsen, T.L., Davidson, T.A., Liu, Z., Mazzeo, N., Trochine, C., Ozkan, K., Jensen, H.S., Trolle, D., Starling, F., Lazzaro, X., Johansson, L.S., Bjerring, R., Liboriussen, L., Larsen, S.E., Landkildehus, F., Egemose, S. and Meerhoff, M. (2012). Biomanipulation as a restoration tool to combat eutrophication: recent advances and future challenges. Advances in Ecological Research 47: 411-487. Elsevier, New York.

Liu, C., Kroeze, C., Hoekstra, A.Y., Gerbens-Leenes, W., 2012. Past and future trends in grey water footprints of anthropogenic nitrogen and phosphorus inputs to major world rivers, Ecological Indicators 18, 42-49.

Mainstone. (2010). An evidence base for setting nutrient targets to protect river habitat. Natural England Research Reports, Number 034. Natural England, Sheffield.

Mekonnen, M.M. and Hoekstra, A.Y. (2015). Global gray water footprint and water pollution levels related to anthropogenic nitrogen loads to fresh water, Environmental Science and Technology, 49, 12860−12868.

Miltner, R.J. and Rankin, E.T. (1998). Primary nutrients and the biotic integrity of rivers and streams. Freshwater Biology, 40: 145–158.

Moore, M.T. and Locke, M.A. (2013). Effect of storage method and associated holding timeon nitrogen and phosphorus concentrations in surface water samples. Bulletin of Environmental Contamination and Toxicology, 91:493-498.

Moss, B., Jeppesen, E., Søndergaard, M., Lauridsen, T.L. and Liu, Z. (2013). Nitrogen, macrophytes, shallow lakes and nutrient limitation: resolution of a current controversy? Hydrobiologia 710: 3–21.

North/South Consultants Inc. (2006). Literature review related to setting nutrient objectives for Lake Winnipeg. Winnipeg, Manitoba.

Paerl, H.W, Scott, J.T., McCarthy, M J., Newell, S.E., Gardner, W.E., Havens, K.E., Hoffman, D.K., Wilhelm, S.W., and Wurtsbaugh, W.A. (2016). It Takes Two to Tango: When and Where Dual Nutrient (N & P) Reductions Are Needed to Protect Lakes and Downstream Ecosystems. Environmental Science and Technology, 50: 10805–10813.

Portielje, R. and Van der Molen, D.T. (1999). Relationships between eutrophication variables: from nutrient loading to transparency. Hydrobiologia, 409, 375–387.

Prater, C, Norman, E.J. and Evans-White, M.A. (2015). Relationships among nutrient enrichment, detritus quality and quantity, and large-bodied shredding insect community structure. Hydrobiologia, 753: 219-232

Rosemond, A.D., Benstead, J.P., Bumpers, P.M., Gulis, V., Kominoski, J.S., Manning, D.W.P., Suberkropp, K. and Wallace, J.B. (2015). Experimental nutrient additions accelerate terrestrial carbon loss from stream ecosystems. Science, 347:1142–1145.

Schindler, D. and Vallentyne, J. (2008). The algal bowl. Overfertilization of the world's freshwaters and estuaries. The University of Alberta Press, Earthscan.

Schindler, D.W., Carpenter, S.R.; Chapra, S.C., Hecky, R.E. and Orihel, D.M. (2016). Reducing phosphorus to curb lake eutrophication is a success. Environmental Science and Technology, 50: 8923–8929.

SNIFFER (2008). Development of a system for classifying the ecological potential of UK and Irish canals. Project WFD61c. SNIFFER, Edinburgh.

UKTAG. (2008). UK Technical Advisory Group on the Water Framework Directive. UK environmental standards and conditions (phase 2). Final report.

UKTAG (2012). A revised approach to setting Water Framework Directive phosphorus standards (pp. 1–35).

USEPA. (2000a). Ambient water quality criteria recommendations. Information supporting the development of state and tribal nutrient criteria. Rivers and Streams in Nutrient Ecoregion VI. doi: EPA 822-B-00-017. United States Environmental Protection Agency, Washington.

USEPA. (2000b). Ambient water quality criteria recommendations. State and tribal nutrient criteria. Lakes and reservoirs in nutrient ecoregion XIII. doi: EPA 822-B-00-014. United States Environmental Protection Agency, Washington.

USEPA. (2000c). Nutrient criteria technical guidance manual. Rivers and streams. EPA-822-B-00-002. United States Environmental Protection Agency, Washington.

Van Liere, L., Janse, J.H. and Arts, G.H.P. (2006). Setting critical nutrient values for ditches using the eutrophication model PCDitch. Aquatic Ecology, 41(3), 443–449.

Veraart, A. J. (2012). Denitrification in ditches, streams and shallow lakes. Thesis, Wageningen University, Wageningen, NL.

Vitousek, P.M., Mooney, H.A., Lubchenco, J. and Melillo, J.M. (1997). Human domination of Earth's ecosystems. Science 277: 494–499.

Vollenweider, R.A. and Kerekes, J. (1982). Eutrophication of waters. Monitoring assessment and control. Paris (France): OECD Cooperative programme on monitoring of inland waters (eutrophication control); Environment Directorate.

Wang, L., Stuart, M.E., Lewis, M.A., Ward, R.S., Skirvin, D., Naden, P.S., Collins, A.L. and Ascott, M.J. (2016). The changing trend in nitrate concentrations in major aquifers due to historical nitrate loading from agricultural land across England and Wales from 1925 to 2150. Science of the Total Environment 542, 694–705.

Wheeler, B.D. and Giller, K.E. (1982). Status of aquatic macrophytes in an undrained area of fen in the Norfolk broads, England. Aquatic Botany, 12, 277–296.

Wilby, N. (2012). SNIFFER, Edinburgh. A system for classifying the ecological potential of UK and Irish canals. WFD61c. SNIFFER, Edinburgh.

Williams, P., Biggs, J. and Nicolet, P. (2010). Comment: New clean-water ponds—A way to protect freshwater biodiversity. British Wildlife 22: 77-85.

Appendix 1. Overview of the nutrient threshold values that represent clean water in ponds, lakes, streams, rivers and ditches.

This appendix briefly summarises the technical information used to identify clean water sites based on nutrient chemistry threshold values. These clean water sites are analogous to reference condition, minimally impaired water quality or natural background levels (Williams, Biggs, & Nicolet, 2010). The thresholds are also intended to encompass those sites identified as being at 'High' status in the Water Framework Directive which are those with 'no or very low human pressure'. However, although most European countries operating under the Water Framework Directive have freshwater monitoring programmes for rivers, streams and larger lakes, and 'reference' conditions are already defined for these, regular monitoring of headwaters, small lakes, ponds and ditch systems is rare, and no phosphorus standards currently exist for these smaller water bodies. In addition, there are no biologically relevant nitrogen standards under the Water Framework Directive for surface freshwaters (although there are human health related drinking water limits), and even internationally nitrogen thresholds are less common, with phosphorus considered to be the critical limiting nutrient for most water bodies (Schindler *et al*. 2016). It is often difficult to determine which of the two nutrients (nitrogen or phosphorus) is limiting or more important to plant and algal growth, largely because both nutrients tend to increase concurrently.

Conservative thresholds approach

Our approach to setting threshold values for defining clean water has been to set a single, quite lenient, value above which levels always indicate pollution. For example under the Water Framework Directive typology approach, we have selected the highest 'unimpacted' boundary value to define a single clean water threshold. Because of this some impacted waterbodies, particularly those with naturally low nutrient concentration naturally, may be incorrectly classed as clean. However, this approach is necessary in order to provide a practical rapid assessment system with the PackTest kits.

Phosphorus

The revised UK Technical Advisory Group values for phosphorus in rivers are in line with the threshold values suggested by other authors working on large scale studies of river systems both within the UK and internationally (Appendix Table 1a). There are slight differences between authors; however, as outlined by UKTAG working group (UKTAG, 2012), comparison across authors for setting of standards is complicated by a range of typologies and approaches. In addition, few other studies are as comprehensive as the review carried out for setting the Water Framework Directive standards. Our threshold value of 0.050 mg L^{-1} soluble reactive phosphorus applies to low altitude high alkalinity rivers, and represents the 95th percentile of the standard. This value is the highest allowable soluble reactive phosphorus level for any river type; that is, any value above this could be considered to be impacted. However, under Water Framework Directive, lower soluble reactive phosphorus values apply to rivers of different alkalinity and altitude. We draw on the Water Framework Directive threshold levels for high status rivers for determining our clean water thresholds in rivers, by using the maximum allowable soluble reactive phosphorus value for any river type as a conservative (perhaps better described as lenient) threshold for clean water, 0.058 mg L^{-1} soluble reactive phosphorus (Appendix Table 1a).

For lakes, total phosphorus is the established indicator as it is relatively easy to measure (UKTAG, 2008) and phosphorus levels are generally given as total phosphorus, as opposed to soluble reactive phosphorus. The highest allowable threshold value for any lake type

under the UKTAG 2106 classification is 0.035 mg L^{-1} total phosphorus, comparable to the values for the Habitats Directive. In a pivotal piece of work, Vollenweider and Kerekes (1982) proposed a more stringent total phosphorus value for lakes with a 0.010 mg L^{-1} total phosphorus threshold value (Appendix Table 1a). However, they did not use a typology approach, and many of the UKTAG lake typologies have comparable threshold values. The UKTAG consultation is a much more recent piece of work and for this reason we have used it as our threshold for clean lake water. Similar to the rivers, this value of 0.035 mg L^{-1} total phosphorus is the highest allowable of any lake type, and lower values apply to specific Water Framework Directive type lakes (Appendix Table 1a).

Smaller water bodies are not currently accounted for under Water Framework Directive monitoring so threshold values for these water bodies are derived from various sources, including data collected by Freshwater Habitats Trust. Pond data were collected during the National Pond Survey of the early 1990s which surveyed ponds located in semi-natural landscapes where impacts from pollution on ponds were minimal. Soluble reactive phosphorus values were measured at a reasonably large number of sites (c. 200) which had a mean concentration of 0.065 mg L^{-1} soluble reactive phosphorus. Although there are no other datasets available with which to compare this information, some Dutch work on lakes and ponds indicates that a value of less than 0.05 mg L^{-1} total phosphorus, in conjunction with a total nitrogen value of less than 1.35 mg L^{-1} prevents the dominance of cyanobacteria (Portielje & Van der Molen, 1999). As such these National Pond Survey values form our threshold values for clean water ponds. In Countryside Survey 2007 we adopted a slightly more lenient cutoff of 0.12 mg L^{-1} which was the 90%-ile of National Pond Survey soluble reactive phosphorus concentration (Williams *et al.* 2010). However, we have opted for the tighter standard for ponds in the present work as the original dataset included some sites, which, on further assessment appear to be suffering from phosphorus pollution, leading us to over-estimate the natural background phosphorus levels.

Similar to ponds, there are few standards for nutrient levels in ditches. The Dutch, although lacking official standards for ditches, generally regard 0.15 mg L^{-1} soluble reactive phosphorus as the critical value for duckweed dominance. This is based on values used for shallow lakes. There has been a great deal of modelling work done on Dutch ditches to ascertain critical levels, and the critical level of 0.15 mg L^{-1} seems to be applicable to highly impacted agricultural ditches, in order to prevent the dominance of duckweed (van Liere, Janse and Arts, 2006). However, just using duckweed as an indicator is insufficient for determining clean water quality. It could be inferred that anything above this value is detrimental for submerged ditch flora but values far below this are likely applicable in order to protect the ecological integrity of these systems. For example work by Wheeler *et al.* (1982) indicated that the phosphorus levels at very clean sites on the Norfolk broads, with a high abundance of the nutrient sensitive *Utricularia* species, had maximum values of 0.050 mg L^{-1} soluble reactive phosphorus. Additionally work by Veraart (2012) found that Dutch ditches in nature reserves and bogs had SRP values of between 0.012 and 0.021 mg L^{-1} soluble reactive phosphorus. Both of these pieces of research are small scale, and very localised, so generalisation is difficult. However, logically, values in ditches should not be dissimilar to threshold values for shallower slow moving or standing waterbodies, such as ponds and shallow lakes. Under UKTAG (2008) the highest values of 0.055 mg L^{-1} total phosphorus applies to shallow lakes, and the average value for ponds from the soluble reactive phosphorus survey is 0.065 mg L^{-1} soluble reactive phosphorus. Thus it seems appropriate that a maximum value which matches that set for ponds could also be applied to ditches, and as such we set our threshold value at 0.065 mg L^{-1} soluble reactive phosphorus. There are few ditches left which are have the low nutrient levels typical of natural waters, and most drain heavily agricultural land, so it is likely that these values will frequently be surpassed in today's landscape.

Canals are defined as artificial water bodies under the Water Framework Directive and because of this the reference condition is set at 'maximum ecological potential'. Maximum ecological potential is determined by the best available biology, given the level of boat traffic (SNIFFER, 2008). Determining classification boundaries for canals is difficult owing to the small data set, and as such must be approached and interpreted with caution (SNIFFER, 2008). There is an extreme paucity of data linking biology with physiochemical characteristics for canals. Additionally canals are complicated in that they have features similar to both rivers and lakes, but comparison of canals with the Water Framework Directive values for either of these systems would mean the even the highest quality canals would be likely to fail when nutrient enrichment was being assessed (SNIFFER, 2008). Values for soluble reactive phosphorus and total oxidised nitrogen were determined using the Macrophyte Fertility Index (MFI), which is analogous to the Average Score Per Taxon (ASPT) for macroinvertebrates (Wilby, 2012). Of all the metrics tested, MFI was most strongly correlated with phosphorus levels. The MFI-phosphate model was developed for rivers, and a similar relationship exists for canals, albeit with a much greater level of uncertainty owing to the small number of sites (Wilby, 2012). This work proposed a maximum value of 0.073 mg L^{-1} soluble reactive phosphorus as the threshold for high status canals, a value which is slightly higher than those for lakes and rivers with similar alkalinity. Similar to rivers and lakes, canals at lower alkalinities have values more stringent than this with a 0.029 mg L^{-1} limit for the canals with alkalinities of 50 mg L^{-1} calcium carbonate. This threshold is similar to the 0.020 mg L^{-1} limit proposed by Natural England for sites of special scientific interest (SSSI) canal sites (Joint Nature Conservancy Council, 2005). In the present guide we have adopted the 0.073 mg L^{-1} soluble reactive phosphorus proposed by the SNIFFER (2012) model.

Nitrogen

For streams, the USEPA study (2000c) is one of the most comprehensive studies available surveying and reviewing river total nitrogen levels. Similar to the Water Framework Directive approach for setting phosphorus thresholds, it gives an acceptable range based on ecotype, or region within the US. These criteria are intended as a starting point for the development of state standards and were derived from actual conditions measured in low impact streams within each of the aggregate ecoregions. Nitrogen levels in reference rivers vary considerably across the ecoregions, the highest of which, 2.19 mg L^{-1} total nitrogen, applies to only one ecoregion: aggregate ecoregion VI, the corn-belt and northern Great Plains which are characterized by nutrient-rich soils (USEPA, 2000a). As such this area is significantly different in geographical terms to any region in the UK. The next highest level outside of this ecoregion is 0.9 mg L^{-1} total nitrogen, which is more applicable as a threshold level for total nitrogen in UK rivers and streams. The work by Dodds *et al*. (1998) gives confidence to this value. They used records from over 1000 rivers, ranging in size and naturalness, from North America, New Zealand and a small number in Europe and applied a frequency distribution method to assign thresholds between trophic classes. Their work, based on temperate rivers, highlights an oligo to mesotrophic threshold value of 0.7 mg L^{-1} total nitrogen, relatively similar to the 0.9 mg L^{-1} total nitrogen level from the USEPA work. Miltner and Rankin (1998) found a similar threshold for total inorganic nitrogen (TIN), with deleterious effects on fish occurring over 0.65 mg L^{-1} NO_3-N. Although there are no large scale studies of this type for Europe, values for other studies should be broadly applicable to the UK, and as such we suggest a threshold value for total nitrogen of 0.9 mg L^{-1}, in agreement with the second highest allowable level suggested by the USEPA, and encompassing levels suggested by Dodds *et al.* (1998). For total/dissolved inorganic nitrogen the value should logically fall below the threshold proposed for total nitrogen, and as such a level of 0.7 mg L^{-1} is suggested as a DIN/TIN threshold in agreement with work done by Miltner and Rankin (1998) and Biggs (2000). Note that this approach, identifying thresholds between oligotrophic, mesotrophic and eutrophic rivers, is not exactly comparable to the approach used in the Water Framework Directive thresholds for High status. However,

in the absence of more precise data it does give an indication of the levels of nitrogen associated with unimpaired conditions.

For lakes, the same USEPA (2000c) study applied the same aggregate ecoregional approach to lakes, and the total nitrogen maximum value was 1.27 mg L^{-1}. Once again this high total nitrogen value occurs in a region not applicable to the UK, ecoregion XIII, which lies in sub-tropical/tropical Florida (USEPA, 2000b). The next highest value is 0.8 mg L^{-1}. Other studies have focused more on ecological tipping points, distinct from a clean water threshold, such as Gonzalez Sagrario *et al.* (2005) who found that values greater than 2 mg L^{-1} NO_3-N caused a shift to a turbid state with low plant coverage. One pertinent, albeit small scale, UK study by James *et al.* (2005) found reasonably species-rich macrophyte assemblages at winter nitrate values of 1-2 mg L^{-1} NO_3-N. Without broader European studies to draw on a certain amount of extrapolation is necessary, thus drawing on the work by the USEPA (2002) and also guided by James et al (2005) we propose a total nitrogen threshold value of 1 mg L^{-1}.

Pond data sets are largely unavailable in the literature, besides those gathered by Freshwater Habitats Trust during the National Pond Survey. Values for total nitrogen are based on a relatively small sample size of 45 sites, but would suggest a total nitrogen level of 2.9 mg L^{-1} mean or 1.5 mg L^{-1} median value for unimpacted pond sites. This mean value appears inflated compared with the thresholds suggested for other waterbodies, and as the sample size is small the median value of 1.5 mg L^{-1} has been utilised as our threshold, as it is more in keeping with the values suggested for rivers and lakes, which should not be too dissimilar to ponds. This threshold agrees with a study published by Portielje and Van der Molen (1999) on Dutch ponds and lakes which, while not entirely relevant for threshold setting, did demonstrate that with values less than 1.35 mg L^{-1} NO_3-N, in combination with a low total phosphorus value, cyanobacterial dominance was prevented. Within the National Pond Survey study, the mean total oxidised nitrogen value was 0.5 mg L^{-1} NO_3-N, and was based on a more robust 158 sites. As such we use this value for our threshold.

Ditches are again difficult to ascribe thresholds to owing to the paucity of studies assessing their nutrient status and biological impact. There have been some Dutch studies on ditches, but they tend to focus more on ecological tipping points, such as duckweed dominance at values greater than 1.5 mg L^{-1} total nitrogen (van Liere *et al*. 2006, Janse and Van Puijenbroek, 1998). As outlined for total phosphorus, these values, although interesting, are not applicable for protecting the ecological integrity of these sites. For total phosphorus thresholds in ditches we have applied the same threshold values as those used for shallow lakes and ponds. Following that rationale, but bearing in mind the limitation of the small sample size for the total nitrogen values for ponds, the threshold value for lakes of 1 mg L^{-1} total nitrogen is proposed as the threshold value for ditches. As the sample number for nitrate values in ponds is much greater and thus more reliable, the nitrate threshold for ditches is based on the pond value of 0.5 mg L^{-1} NO_3-N.

There has been some work on canals to identify nitrogen concentrations associated with ‘clean’ high status sites (e.g. SNIFFER, 2008; Wilby, 2012). Wilby identified 165 reference canal sites in England, Wales, Scotland and Northern Ireland and then modelled the relationship between macrophyte fertility index (MFI) and total oxidised nitrogen concentrations to identify the levels associated with clean, unimpaired conditions. This work proposed a maximum value, based on modelling, of 3.0 mg L^{-1} total oxidised nitrogen at alkalinities of 250 mg L^{-1} $CaCO_3$, dropping to 1.3 mg L^{-1} total oxidised nitrogen at alkalinities of 50 mg L^{-1} $CaCO_3$. The actual mean total oxidised nitrogen concentration of reference (i.e. minimally impaired) sites was 1.27 mg L^{-1} NO_3-N. It is noticeable that the canal ‘clean water’ nitrate concentrations are apparently somewhat higher than those in other freshwater systems. This may reflect the fact that, with a comparatively small set of sites to choose from, reference

sites are, in fact, impacted by nitrogen pollution. Thus some canals which would be treated as polluted in terms of nitrate according to the PackTest categorisation (page 5) would technically fall inside the High status (the maximum/good boundary) proposed by Wilby (2012).

Dealing with nutrient fractions

Nutrients can occur in several different forms in water: organic, inorganic and total. These are often referred to in different ways, including soluble, insoluble and bioavailable. Total forms provide a comprehensive assessment of the nutrients in the entire system. For example, in relation to phosphorus, the total phosphorus will assess how much phosphorus there is dissolved in the water, in addition to that contained in organic forms such as algae and sediment. In order to do this, for both total phosphorus and total nitrogen, the sample must be first digested, to release that contained within the organic fraction in order to quantify it. In contrast soluble reactive phosphorus is that fraction of phosphorus which is soluble and inorganic, the form directly taken-up by plant cells. It is also referred to as inorganic phosphorus. A reading for soluble reactive phosphorus will not include the fraction of phosphorus which is already locked up in algae and other plant or animal matter. There is also evidence that organic phosphorus may also be bioavailable in addition to soluble reactive inorganic forms.

For nitrogen the forms reported in the literature are a little more complex. Similar to total phosphorus, total nitrogen gives a comprehensive estimate of the amount of nitrogen in both the organic and inorganic forms. The most relevant forms for this study are 1) total nitrogen (TN), 2) nitrate, 3) total inorganic nitrogen (TIN), and total soluble nitrogen (SIN) and 4) total oxidised nitrogen (TON). TN is the sum of nitrate, nitrite, ammonia and organically bonded nitrogen. TIN is essentially the same as SIN, and constitutes nitrate, nitrite and ammonia. Total oxidised nitrogen is a measure of nitrate and nitrite, without ammonia or other forms of nitrogen. Nitrite levels are generally inconsequential in freshwater, as it is quickly oxidised to nitrate, thus measures of TON are relatively comparable to nitrate based standards.

It is difficult to extrapolate inorganic fractions from total fractions, or vice versa, as there is a great deal of site specific variation, and constant natural flux. For waterbodies with long residence times soluble reactive phosphorus concentrations can greatly under-estimate phosphorus flux into the plant community due to high uptake rates (Mainstone, 2010). In addition biological process quickly convert one form of nitrogen into another via the nitrogen cycle, and the fractions of N are in constant flux.

Where nutrients are used as the basis for categorisation, the USEPA (2000c) recommends the use of total nutrient fractions, rather than inorganic pools. This is owing to the rapid depletion and recycling of the inorganic fractions of nutrients in stream ecosystems. This is further compounded by the fact that inorganic fractions do not incorporate nutrients contained in the benthic biomass as outlined above, whereas total nitrogen and total phosphorus incorporate nutrients contained in phytoplankton biomass (North/South Consultants Inc., 2006). The literature cited in this report refers to several nutrient fractions, with total fractions, inorganic fractions or other combinations used. While it is not ideal to have thresholds based on different fractions, it is unavoidable given the predominance of total phosphorus threshold work for lakes and soluble reactive phosphorus work on rivers, in addition to the paucity of nitrogen thresholds for all water bodies, and severe lack of threshold studies on smaller water bodies. A certain amount of downward extrapolation can be employed based on the logic that, in all cases, the total fraction will give the highest value. Thus, if a threshold is set for a total fraction, then all other fractions should fall within that limit. For example, the total phosphorus threshold for lakes is 0.055 mg L^{-1} PO_4-P. If the soluble reactive phosphorus value for this lake is also analysed, it would have to be less than the total phosphorus level, thus < 0.055 mg L^{-1}. Similarly for nitrogen total nitrogen must

be greater than total inorganic nitrogen, which must in turn be greater than total oxidised nitrogen and nitrate.

Appendix Table 1a. Phosphate threshold values

Author	System	Range/ value	Fraction	n	Description
Rivers and streams					
UKTAG 2012	UK rivers and streams	0.013-0.058 mg L^{-1} PO_4-P	SRP	Substantial	Threshold between high-good status. Value changes depending on typology
Dodds et al, 1998	Temperate stream sites from US, NZ and few EU	0.025 mg L^{-1} P	TP	1366	Frequency curve analysis to indicate threshold between oligo and mesotrophic streams
Miltner and Rankin, 1998	Wadeable Ohio streams	0.060 mg L^{-1} P	TP	655	Above this threshold fish metric declined
Lakes					
(UKTAG, 2008, 2016)	UK lakes	0.005-0.035 mg L^{-1} P	TP	Substantial	Threshold between high-good status. Value changes depending on typology
(Vollenweider & Kerekes, 1982)	International lakes	0.010 mg L^{-1} P	TP	Substantial	Threshold of oligo-mesotrophic lakes. OECD model.
Ponds					
FHT data from the National Pond Survey	Minimally impacted UK ponds	0.077 mg L^{-1} P = median 0.190 mg L^{-1} P= mean	TP	49	Mean and median levels of minimally impacted ponds
FHT data from the National Pond Survey	Minimally impacted UK ponds	0.003 mg L^{-1} PO_4-P = median 0.065 mg L^{-1} PO_4-P = mean	SRP	162	Mean and median levels of minimally impacted ponds
Portielje and van der Molen 1999	Lakes and ponds in the Netherlands	0.050 mg L^{-1} P	TP	231	When mean summer TP was lower than this, in conjunction with TN<1.35 mg/l, then cyanobacteria dominance disappeared
Ditches					
Veraart 2012	Dutch ditches in national reserve and bog	0.012-0.021 mg L^{-1} PO_4-P	SRP	7	
Van Liere et al, 2000	Dutch ditch models	0.150 mg L^{-1} PO_4-P	SRP		Critical value for duckweed dominance
Wheeler et al, 1982	Lowland Broad drainage ditch, UK	0.050 mg L^{-1} PO_4-P	SRP	1 broad system	Values for dystrophic *Utricularia* species
Canals					
Wilby 2012	Reference level UK canals	0.010-0.073 mg L^{-1} PO_4-P	SRP	200	Dependent on alkalinity
Joint Nature Conservancy Committee, 2005	SSSI canals, UK	0.020-0.06 mg L^{-1} P	TP		If canal expected to naturally be mesotrophic i.e. fed from upland or hard rock areas target is 0.02; if naturally eutrophic e.g. fed from lowland, soft geology target is 0.06.. Canal CSM targets currently under-review.

Appendix Table 1b. Nitrogen threshold values

Author	System	Range/ value (mg L^{-1})	Fraction	n	Description
Rivers and streams					
USEPA 2002 (summary table)	US rivers	0.1-2.18	TN	Substantial	Ecoregion approach for setting N thresholds. These values represent the baseline values of unimpacted waters
Dodds et al (1998)	Streams in US, New Zealand and Europe	0.7	TN	1070	Frequency distribution model work of temperate streams.
Miltner and Rankin (1998)	Ohio streams	0.61	TIN	1657	Deleterious effect on fish communities above this level
Biggs *et al.* (2000)	New Zealand streams	0.7	SIN	30	Threshold level for acceptable chlorophyll a levels
Lakes					
USEPA 2002	US lakes	0.12-1.27	TN	Substantial	Ecoregion approach for setting N thresholds. These values represent the baseline values of unimpacted waters
James et al (2005)	Shallow lakes in UK and Poland	1-2	Nitrate	60	Reasonably diverse macrophyte species richness at values less than these
(Gonzalez Sagrario *et al.* 2005)	Danish lakes and mesocosm	>2	TN	24 enclosures and 204 lakes	Above this level there is a shift to a turbid state
Ponds					
FHT NPS survey	UK high quality ponds	0.01 (median) and 0.5 (mean	TON	158	Median and mean values for unimpacted ponds
FHT NPS survey	UK high quality ponds	1.5 (median) and 2.9 (mean)	TN	45	Median and mean values for unimpacted ponds
Portielje and van der Molen 1999	Lakes and ponds in the Netherlands	1.35	TN	231	When mean summer TN was lower than this, in conjunction with TP 0.05 ug/l, then cyanobacteria dominance disappeared
Ditches					
Van Liere et al, 2000	Dutch ditch models	1.5	TN	Modelled	Critical value for duckweed dominance
Janse 1998	Dutch ditch models	1.5	TN	Modelled	Critical value for duckweed dominance
Canals					
Wilby 2012	Reference level UK canals	Modelled: 0.4 3.0 mg L^{-1} NO^3-N Actual mean: 1.27 mg L^{-1} NO^3-N	TON	165	Dependent on alkalinity

Appendix 2. Statistical significance of differences between laboratory nutrient concentrations classified into the PackTest classes

Appendix 2a. Statistical significance of differences between laboratory measured phosphate concentrations of samples classified into six PackTest classes (Figure 5)

PackTest class	0.010 mg L^{-1}	0.035 mg L^{-1}	0.075 mg L^{-1}	0.150 mg L^{-1}	0.350 mg L^{-1}	0.750 mg L^{-1}
0.010 mg L^{-1}	-	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001
0.035 mg L^{-1}		-	p<0.01	p<0.001	p<0.011	ns
0.075 mg L^{-1}			-	ns	ns	ns
0.150 mg L^{-1}				-	ns	ns
0.350 mg L^{-1}					-	ns
0.750 mg L^{-1}						-

Overall test of difference amongst groups: Kruskal-Wallis test: H (4, N= 520) = 277.5209; p <0.001. Note that the 0.750 mg L^{-1} category has few sites (n=12).

Appendix 2b. Statistical significance of differences between laboratory measured nitrate concentrations of samples classified into six PackTest classes (Figure 14)

PackTest class	0.1 mg L^{-1}	0.35 mg L^{-1}	0.75 mg L^{-1}	1.5 mg L^{-1}	3.5 mg L^{-1}	7.5 mg L^{-1}
0.1 mg L^{-1}	-	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001
0.35 mg L^{-1}		-	p<0.05	p<0.001	p<0.001	p<0.001
0.75 mg L^{-1}			-	ns	ns	p<0.05
1.50 mg L^{-1}				-	ns	ns
3.50 mg L^{-1}					-	ns
7.50 mg L^{-1}						-

Overall test of difference amongst groups: Kruskal-Wallis test: H (5, N= 505) = 325.6506 p<0.001.

Appendix 3. Proportion of sites correctly allocated to the laboratory measured nutrient category by PackTest kits

Appendix 3a. Proportion of sites correctly allocated to the true laboratory measured phosphate category (red text) by PackTest kits

Lab measured phosphate category (mg L^{-1})	PackTest kit phosphate categories (mg L^{-1})						
	<0.02	0.02-0.05	0.05-0.1	0.1-0.2	0.2-0.5	0.5-1	>1
<0.02	87.2%	34.5%	10.3%	0.0%	0.0%	0.0%	0%
0.02-0.05	10.5%	32.7%	5.2%	0.0%	4.2%	0.0%	0%
0.05-0.1	1.4%	23.6%	43.1%	7.9%	0.0%	8.3%	0%
0.1-0.2	0.3%	8.2%	27.6%	68.4%	20.8%	0.0%	0%
0.2-0.5	0.3%	0.9%	12.1%	21.1%	66.7%	0.0%	0%
0.5-1.0	0.0%	0.0%	1.7%	2.6%	8.3%	41.7%	0%
>1	0.3%	0.0%	0.0%	0.0%	0.0%	50.0%	100%

Appendix 3b. Proportion of sites correctly allocated to the true laboratory measured nitrate category by PackTest kits

Lab measured nitrate category (mg L^{-1})	PackTest kit nitrate categories (mg L^{-1})					
	<0.2	0.2-0.5	0.5-1.0	1.0-2.0	2.0-5.0	5.0-10
<0.2	37%	8%	0%	0%	0%	0%
0.2-0.5	44%	36%	5%	0%	0%	2%
0.5-1.0	17%	48%	29%	3%	0%	0%
1.0-2.0	0.4%	5%	38%	21%	5%	0%
2.0-5.0	1%	3%	29%	76%	67%	30%
5.0-10	0%	0%	0%	0%	28%	64%
>10	0%	0%	0%	0%	0%	4%