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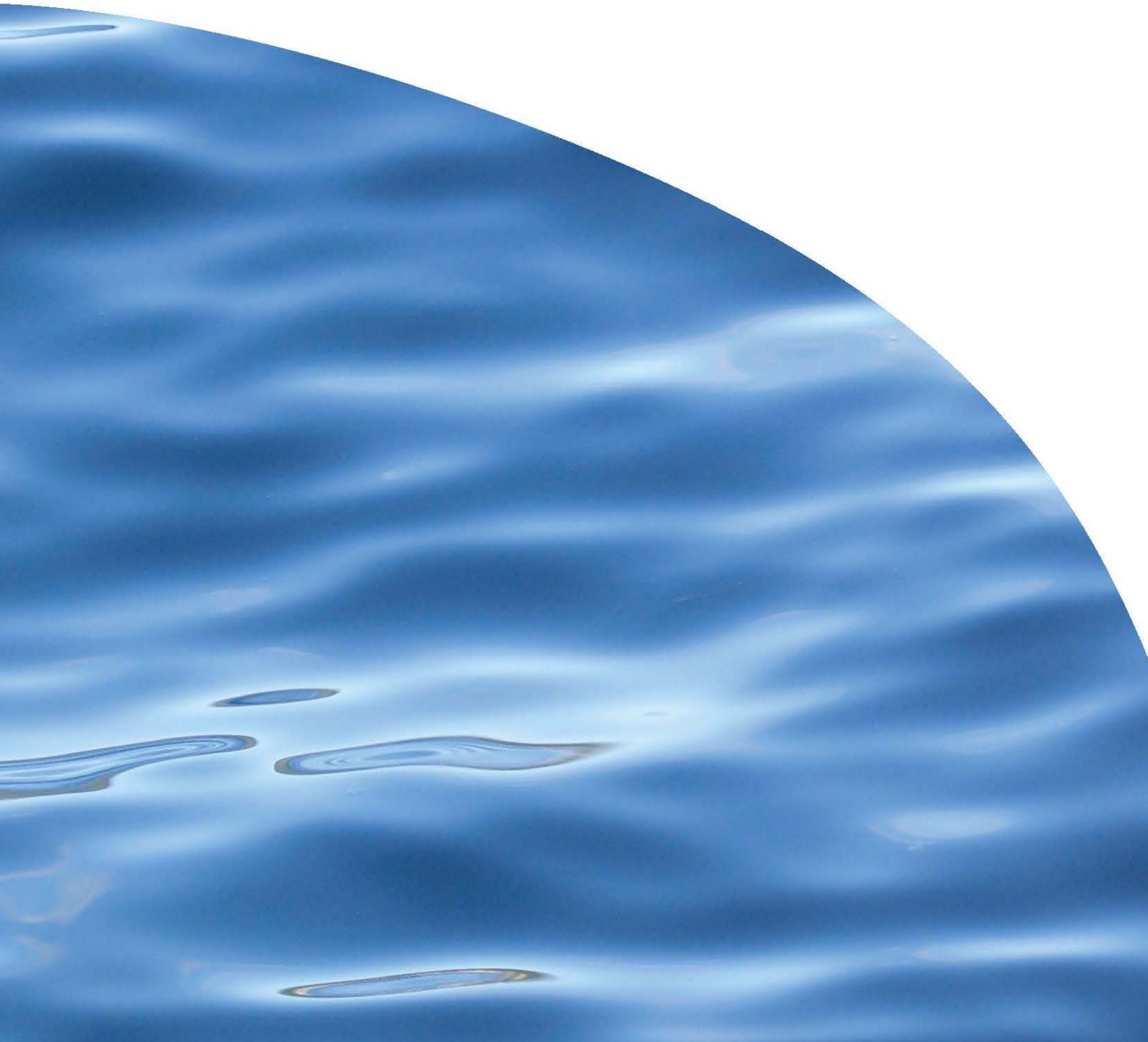
REVIEW OF FUNCTIONAL AND MACROINVERTEBRATE SAMPLING METHODS FOR NON-WADEABLE RIVERS



REVIEW OF FUNCTIONAL AND MACROINVERTEBRATE SAMPLING METHODS FOR NON-WADEABLE RIVERS

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Prepared for Marlborough District Council.

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EXECUTIVE SUMMARY

Non-wadeable rivers are defined here as waterways where water depth and/or velocity restrict or prevent the sampling of biota from representative habitats using standard protocols. Sampling of non-wadeable waterways for bioassessment purposes can require increased equipment, personnel and time to adequately differentiate biotic and functional responses to human pressure from the variety of channel features and habitat types present in larger waterways. There are currently no nationwide biomonitoring protocols for sampling macroinvertebrates in non-wadeable rivers, and consequently they are often left out of monitoring networks. A survey of unitary authorities revealed that few routinely monitored non-wadeable rivers for either macroinvertebrates or functional indicators and only three included them in state of the environment (SOE) monitoring.

The international literature presents a range of methods that have been employed to sample macroinvertebrates in non-wadeable waterways. Techniques include sampling near-shore littoral habitats with essentially wadeable techniques, such as sweeps and kick netting, using a boat to access large woody debris, and sampling deep water habitats with dredges or airlift samplers. Artificial substrates have also been used to sample deep, often fast flowing rivers, the two most commonly employed being Hester-Dendy multiplate samplers and rock baskets. Sampling in New Zealand has employed a similar range of techniques, albeit to a lesser extent. Sampling protocols employed by bioassessment agencies in the USA tend to use a littoral sampling approach that targets potentially rich near-shore habitats, including snags which may be accessed by a boat. Alternatively, field trials in the United Kingdom and Ireland have indicated that littoral sampling can be effectively complemented with deep water samples collected with an airlift sampler.

How much to sample is a key consideration for agencies wishing to accurately assess the quality of non-wadeable waterways. Habitat units can become larger and the distances between them increase, and one way to address this is to use a sample reach length which becomes larger with increasing river size, *i.e.* using a multiple of the mean wetted width of the reach of interest. However, direct comparisons have shown that using a fixed length sample reach (*e.g.* 500 m) can be effective, whilst providing a standard approach that can potentially reduce the sampling effort required.

There are standardised functional indicators available for monitoring ecosystem processes in non-wadeable rivers. Ecosystem metabolism and organic matter assays have been applied in New Zealand's rivers and provide data directly comparable to a growing national and international data set. Criteria exist for interpreting ecosystem metabolism values, although further knowledge of how natural environmental variability distinguishes between wadeable and non-wadeable river metabolism is warranted. Range in baseline conditions for cotton decomposition is currently unknown. There is growing international support for using nitrogen isotope values of in-stream biota as an indicator of nitrogen processes in rivers. Standardised methodology for sample collection and processing exist, but knowledge of reference condition for New Zealand is currently unknown.

Recommendations

Sampling of near-shore littoral zones is likely to be a component of macroinvertebrate protocols for non-wadeable rivers that do not experience excessive flow variability. Sampling deep water habitats where they are extensive, for example using an airlift sampler will increase the diversity of macroinvertebrate taxa and may provide additional information for quantifying responses to human pressure in some rivers. The use of boats to sample large non-wadeable rivers means that such methods can be employed while also providing better access to a greater proportion of near-shore habitats. Ecosystem metabolism measurements are recommended for whole system assessments of river function and can be readily obtained by deploying a continuous dissolved oxygen logger. Most established hydrology sites provide suitable conditions for logger deployment in flowing water.

Gaps

The following further considerations are necessary to develop a comparable, effective and efficient protocol for collecting macroinvertebrate samples in non-wadeable rivers: (i) sampling intensity between habitat types, (ii) defining number of replicates required, (iii) determining the most responsive metrics to human pressures,(iv) differentiating the influence of natural environmental variability at multiple scales, and (iv) developing an approach to provide a benchmark against which to assess impacts. Similarly, further investigation of how ecosystem metabolism differs between wadeable and non-wadeable rivers is needed to develop universal assessment criteria. Further definition of reference condition is required before the application of cotton strips and $\delta^{15}N$ of primary consumers as functional indicators in non-wadeable rivers.

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1. WHAT IS A NON-WADEABLE WATERWAY?

The progression from small to large rivers is continuous and there is no distinct geographic characteristic/s that will consistently delineate where and when a waterway will shift from being wadeable to non-wadeable (Figure 1, Flotemersch *et al.* 2006a). The ability to wade and sample effectively and representatively across a range of desired habitats will also vary between river systems, as well as a result of hydrological and seasonal cycles within a river system (Wilhelm *et al.* 2005). As state of the environment (SOE) sampling generally occurs during warmer months, when flows are often lower, summer is likely to be the most important time for determining whether a waterway is considered non-wadeable for sampling purposes. Although an applied definition of a non-wadeable waterway will inevitably be somewhat subjective and some target habitats may be accessible by 'wading' at a range of flows (*e.g.* near-shore littoral zones), other considerations may also apply. For example, gaining a representative sample across a range of potential habitats becomes increasingly difficult as river size increases. Further, existing methodologies for quantifying and describing ecological condition in a standardised fashion (generally well-developed for wadeable streams) may not be appropriate for larger rivers in general (including those with wadeable sections or habitats).

Definitions as to what constitutes a non-wadeable waterway vary. There are those that specifically refer to the ability to wade a river, have defined non-wadeable reaches where an investigator cannot wade from bank to bank (Edsall *et al.* 1997) or from one end of the reach to the other (Meador *et al.* 1993), and where a boat is needed to access sampling habitats (Fitzpatrick *et al.* 1998). Other studies refer to 'large rivers' as having at least 3 km of contiguous river channel that is too deep to be sampled effectively by wading (Lyons *et al.* 2001). A survey of European agencies responsible for bioassessment of waterways demonstrated that the majority of respondents considered a 'deep water' site as one where a reliable kick and/or sweep net sample could not be collected (Jones *et al.* 2005). In addition, sites were predominantly considered to be 'deep' when either the depth of the main channel or that of the entire width of the site exceeded 100 cm, although it was also noted that the critical depth is potentially related to the height of the sampler (Jones *et al.* 2005).

The above definitions may rely on a decision being made upon arrival at a site. An alternative and potentially more useful approach is to use operational classifications based on more predetermined riverine characteristics applied *a priori* to screen rivers that are likely to have non-wadeable reaches. For example, Wilhelm *et al.* (2005) defined non-wadeable rivers in Michigan as those that are ≥ 5th order based on the Strahler concept, and with a basin area ≥ 1600 km^2, a mainstem length ≥100 km, and a mean annual discharge ≥ 15 m^3/s. Another approach is to score selected parameters such as those above and calculate an average score which can then be used to classify sites as likely non-wadeable (*e.g*. Grafe 2002).

Flotemersch *et al.* (2006a) provide a narrative ('sampleability') definition for use in North American of a non-wadeable river or river reach as:

"River reaches where boats are always necessary to access sample points; occasionally necessary to pull boats through shallow areas."

Such sites are generally considered to have a catchment area of 800-40,000 km^2 and be 4^{th} – 8^{th} order. Flotemersch *et al.* (2006a) also identify smaller and larger river classes that overlap with this definition which are worthy of note (see Figure 1). Notably, those with a catchment area of 500 – 1000 km^2 are 3^{rd} – 5^{th} order and are considered to be 'transitional waterways', defined as:

"Contains both wadeable and non-wadeable segments with a mosaic of habitat types that shift in quantity and quality in response to prevailing flow conditions. Sampling often requires a combination of methods developed for wadeable streams and large rivers."

Other definitions of large river systems have set thresholds based on stream order or discharge, such as those of 7^{th} order or greater (Vannote *et al.* 1980; Johnson *et al.* 1995) or rivers with a virgin mean annual discharge (VMAD) of ≥ 350 $m^3 s^{-1}$ (Dynesius & Nilsson 1994; Nilsson *et al.* 2005). However these definitions are likely to exclude potentially important portions of non-wadeable river reaches. Very large rivers that are $>8^{th}$ order and have catchments of > 25,000 km^2 ('Great rivers') although not present in New Zealand, are defined by Flotemersch *et al.* (2006a) as:

"River reaches where boats are always necessary to access sample points. Habitat types are frequently large and thus may require the development of habitat specific expectations for biotic assemblages. Consequently, complete assessment may require sampling and assessment of different habitats"

Based on the above and our knowledge of necessary logistical and safety considerations of sampling waterways for ecological assessment in the New Zealand context we developed the following working definition for use in this report:

"A waterway where water depth and/or velocity restrict or prevent the sampling of biota from representative habitats using standard protocols."

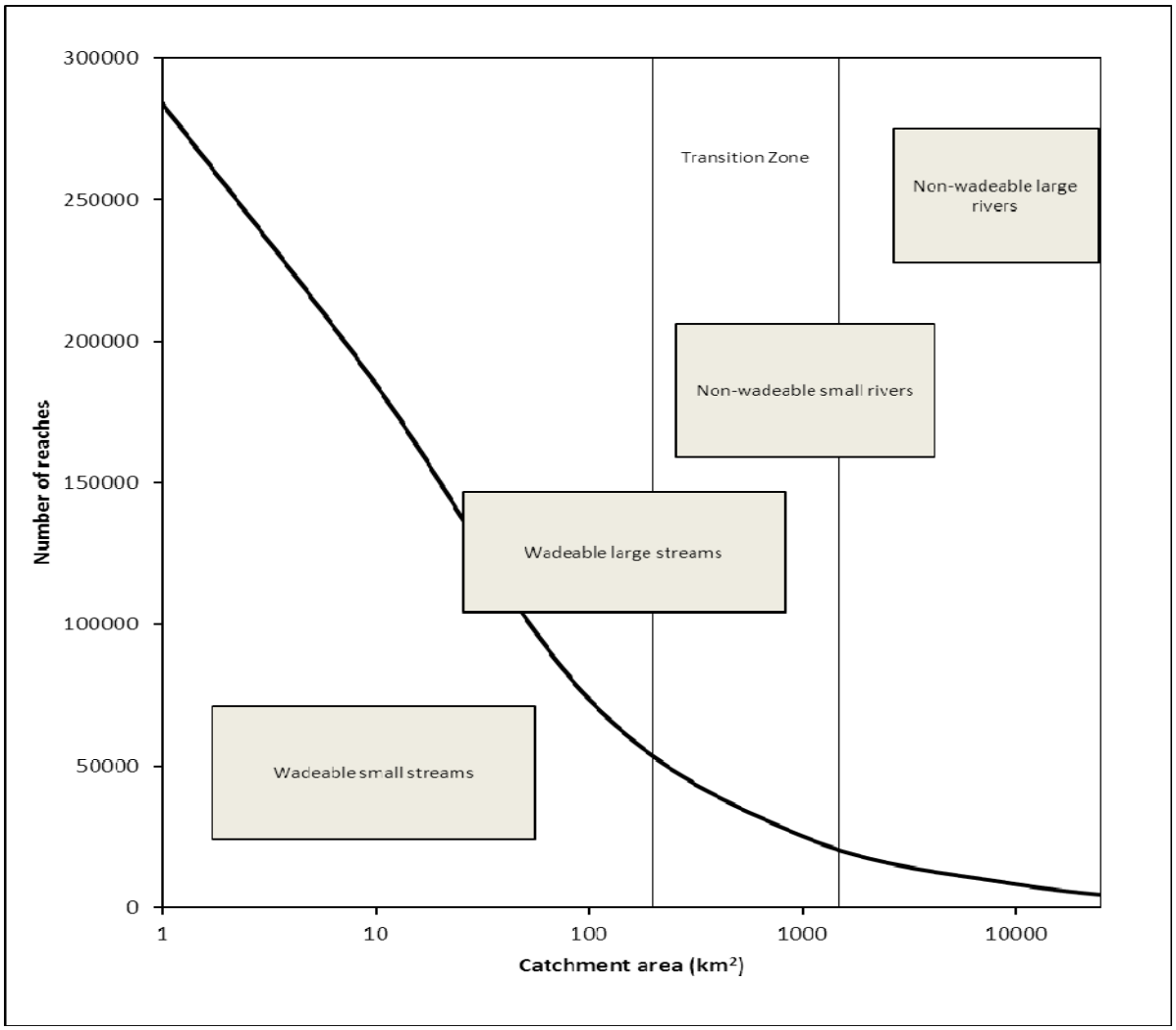

Figure 1. Hypothetical transition from wadeable to non-wadeable waterways based on catchment area of River Environment Classification (REC) reaches. Adapted from Flotemersch *et al.* (2006a).

2. CURRENT NON-WADEABLE RIVER MONITORING IN NEW ZEALAND

In April 2012 we contacted the relevant people at sixteen regional or unitary authorities responsible for ecological monitoring of waterways in New Zealand. Fourteen provided a response. Contacts were asked to complete a short questionnaire regarding how they sampled (or did not sample) non-wadeable waterways in their region. Respondents were first presented with our working operational definition of a non-wadeable waterway and asked whether they had a written alternative. As stated earlier our working definition was as follows:

"A waterway where water depth, velocity, and/or clarity[1] restrict or prevent the sampling of biota from representative habitats using standard protocols."

No other written definitions were reported to be in use by respondents. However, it was noted that sampling was possible in some reaches of non-wadeable waterways under certain conditions using modified wadeable stream protocols. One respondent also questioned whether it was the availability of habitats as opposed to access to them that was more important and whether the definition should focus more on the action of 'wading' (*i.e.* ability to wade across reach or length of reach). It was also questioned whether 'clarity' should be used in the definition.

2.1. Monitoring of non-wadeable waterways

Contacts were asked how many of the sites they monitored for SOE reporting purposes could be considered non-wadeable. Three of the respondents indicated that they sampled non-wadeable waterways as part of SOE monitoring, and on average these sites made up ~13 % of the sites they sampled. From respondents that provided other feedback, it was indicated that non-wadeable waterways in their region were: (i) purposefully not selected for SOE monitoring, (ii) able to be sufficiently accessed, (iii) had only physico-chemical data collected on them, or (iv) were occasionally sampled at low flows for drought monitoring purposes.

In addition, we asked if any monitoring of non-wadeable waterways was undertaken outside of the SOE framework. Of the respondents who did undertake some monitoring of non-SOE sites, one had undertaken sampling at 30 sites for assessment of the utility of habitat quality, functional indicators (metabolism, decay rates) and macroinvertebrates as indicators to determine responses to human pressure, and had conducted sampling at 21 sites along one large river in their region to characterise macroinvertebrate community composition. Two councils collected dissolved oxygen

[1] Clarity was included in our original working definition of a non-wadable waterway, but was removed following consideration of the literature and feedback from regional and unitary authorities.

data at several sites, although for one this information was not currently used as a functional indicator. Two councils also undertook some monitoring of non-wadeable waterways, where they fell within established monitoring programmes. In addition functional indicators and artificial substrates had been trialled in the past in two regions. Where non-wadeable waterways were sampled for SOE or non-SOE monitoring purposes, they were sampled at some point between late spring and early autumn depending on river conditions and sampler availability.

2.2. Methods used to collect macroinvertebrate samples

Contacts were asked which habitats they sampled for macroinvertebrates and what methods were used in non-wadeable rivers. The mesh size of nets used for kick and sweep net collection methods ranged from 0.5 mm to 1 mm, and sweep and kick net collections were generally standardised by time or length of habitat sampled (3 minutes or 3 times 1 m long sweeps). One respondent indicated that sampling occurred along the edges of the channel or habitats until it became unsafe to do so, presumably as a result of water depth and velocity. Sweep and kick netting in near-shore littoral areas were by far the most commonly employed collection methods for both SOE and non-SOE monitoring of non-wadeable waterways (Table 6). An airlift sampler was also used by one respondent.

2.3. Methods used to collect functional indicators

Contacts were asked what methods they used to collect data for functional indicator purposes in non-wadeable rivers. Although some functional information was collected for SOE monitoring purposes, when collected the majority was for non-SOE purposes including biological oxygen demand and ecosystem metabolism (Table 7).

2.4. Concurrent habitat quality information

Where sampling of non-wadeable waterways was undertaken a range of concurrent habitat information was also collected. The following is a summary of the comments of the four respondents that carried out habitat assessments:

1. Macrophyte cover and water clarity, and proportion of habitats sampled were visually estimated.
2. Depth was not recorded as water was too deep. Habitat assessments were carried out using a combination of SHAP protocols 1 and 2 using visual estimates from the bank.
3. Habitat quality was assessed at both functional indicator and macroinvertebrate sampling sites. For macroinvertebrates, sampling depth and substrate were

recorded and occasional water quality measurements for DO, temperature, conductivity and turbidity were made. For functional indicators upstream water depth was recorded at five points on each of five transects over a 500 m reach.

4. Riffle depth, maximum depth, channel width, percentage macrophyte cover, Cyanobacteria presence and filamentous algae percentage cover, habitat sampled (*e.g.* pool, riffle, woody debris, macrophytes), water level, shaded or open site.

3. METHODS FOR SAMPLING MACROINVERTEBRATES IN NON-WADEABLE WATERWAYS

3.1. International examples of non-wadeable river sampling

A range of methods is available for sampling benthic macroinvertebrates in non-wadeable rivers and these are regularly cited in overseas scientific literature and bioassessment monitoring protocols. These methods can be divided into two general approaches, those that are passive (*e.g.* artificial substrates) and those that are active (*e.g.* sweep netting). Each method has certain advantages and disadvantages and these should be considered in light of the objectives of the sampling to be undertaken (Table 1). To determine the frequency of use of these methods, we conducted a keyword search of macroinvertebrate sampling methods in ISI Web of Knowledge ®, using the following terms: Nonwadeable OR non-wadeable OR unwadeable OR large AND river*, stream*, AND *invertebrate* AND method* OR protocol*. Studies were initially limited to 1990 onwards, however some earlier studies of particular methods (*e.g.* airlift samplers) were included in the discussion below where their application was explicitly tested. The reviewed literature was not limited to the hits from the listed search terms and citations in the returned articles were also followed up and incorporated if deemed to be relevant, based on our working definition.

Our international literature review indicated that overall, littoral kick/sweep netting and benthic grabs were the most commonly employed methods for sampling macroinvertebrates in non-wadeable waterways (Table 1). A significant proportion of studies, ~35 %, used complementary collection methods to sample different habitats within rivers (*e.g.* main channel *vs.* littoral zone, macrophytes *vs.* fine sediments). The aims and objectives for collecting macroinvertebrates in non-wadeable waterways found in the reviewed literature include:

- Development and testing of metrics and indicators (*e.g.* Haybach *et al.* 2004; Jackson *et al.* 2010; Weigel & Dimick 2011)
- Development and testing of standard sampling protocols (*e.g.* Flotemersch *et al.* 2006b; Blocksom & Flotemersch 2008)
- Comparisons of sampling methods (*e.g.* Blocksom & Flotemersch 2005)
- Discerning environmental effects, such as long term climatic factors, habitat restoration or point source pollution outfalls (*e.g*. Daufresne *et al.* 2004; Applegate *et al.* 2007; Bij De Vaate *et al.* 2007).

Table 1. Summary of active and passive methods for collecting benthic macroinvertebrates in non-wadeable waterways and their disadvantages and advantages, summarised from Flotemersch *et al.* (2006a). Number of reviewed published studies that employed each category of techniques and their percentage occurrence is also presented.

Method	Example	Advantages	Disadvantages	No. of studies	Percentage occurrence
Passive methods	**Artificial substrates** *e.g.* Hester-Dendy multiplate samplers (Figure 2a) and Rock baskets (Figure 2b)	• Allows quantitative sampling of otherwise inaccessible habitats. • Can be utilised in a range of channel habitats. • Can provide effective reflection of water quality as a result of standardised sample area and substrate. • Potentially easy and quick to deploy and retrieve. • Reduces sampler variability.	• Requires two trips to sample reach and potentially deployment from a boat. • Substrates can be lost, damaged, or rendered useless (*e.g.* vandalism, high or low flows). • The effectiveness of placement and retrieval can affect results. • Measures colonisation as opposed to the resident/*in situ* assemblage at time of sampling.	11	26 %
	Drift nets (Figure 2c)	• Collects drifting invertebrates.	• Performance is reliant on sufficient flow, and the duration and timing (*i.e.* day *vs.* night) of deployment. • Reflects downstream drifting macroinvertebrate assemblage as opposed to the resident/*in situ* assemblage. • Can become clogged or damaged by debris.	3	7 %
Active methods	**Littoral sampling** *e.g.* Fixed area Hess and Surber samplers (Figure 2d and 2e), and snag net (Figure 2f)	• Observable habitats can be targeted. • Dip nets can be used on both stable and unstable habitats. • Effective for collecting resident/*in situ* macroinvertebrate assemblage from habitats which are present.	• Sampling restricted to accessible shallow sites, the extent and characteristics of which can be affected by flow variability. • Difficult or impossible at sites with steep sides or soft sediments.	23	58 %

Method	Example	Advantages	Disadvantages	No. of studies	Percentage occurrence
Active methods… *continued*			• Samples can be variable due to patchy nature of both habitats and macroinvertebrates, thereby increasing number of required replicates. • Potentially different types of littoral habitats sampled at different sites (inter site variation). • Samples often contain large amounts of debris.		
	Bottom samplers *e.g.* Shipek (Figure 3a), Ponar (Figure 3b), and Peterson grabs (Figure 3c), Airlift sampler (Figure 3d), Rallier du Baty-style dredge (Figure 3e) and freeze core samplers	• Require only a single site visit. • Reflects resident/*in situ* assemblage at time of sampling. • Effective for sampling deeper habitats. • Effective for invertebrates that occupy soft sediments and fine gravels. • Results are quantitative and can be standardised.	• Operated without knowing exactly what is being sampled. • Ineffective on hard or rocky substrates. • Organisms can be lost during collection. • Can be cumbersome and problematic to operate (*i.e.* heavy, blockages, leakages, difficult at high flows). • Can fail to adequately collect organisms that have patchy distributions. • Often requires a boat and additional personnel.	22	55 %

3.1.1. Artificial substrates

Artificial substrates can be defined as "devices made of natural or artificial materials of various composition and configuration that are placed in the water for a predetermined period of exposure and depth for colonization" (Klemm *et al.* 1990). Substrates are usually deployed for four to six weeks at depths of 1-3 m, so that samplers will be not become exposed or inaccessible as a result of water level fluctuations, which can hamper the efficacy of sampling and retrieval (Johnson *et al.* 2006). Samplers are carefully retrieved (*e.g.* with a net held downstream or enveloping the sampler if possible), scrubbed (either in the field or upon return to the laboratory), and the resulting sample is sieved and then preserved. Multiple samplers are generally deployed in each sample reach to buffer for the effect of potential loss or vandalism (Johnson *et al.* 2006).

Two of the more commonly employed techniques are Hester-Dendy multiplate samplers and rock baskets. Hester-Dendy multiplate samplers (after Hester & Dendy 1962) consist of several hard plates, often 7.6 mm x 7.6 mm and usually made from Perspex or Masonite, which can be set at different spacing to provide different refuge sizes and flow characteristics within each sampler. Samplers are typically secured to an anchoring device such as a block or pole (Figure 2a). Rock baskets are usually constructed from plastic or non-toxic wire and filled with rocks of similar composition and size (Figure 2b). Although less quantitative than Hester-Dendy samplers, rock baskets provide a more semi-natural substrate for colonisation with a wider variety of irregular surfaces and spaces (Flotemersch *et al.* 2006a).

Hall (1982) compared macroinvertebrate colonisation of Hester-Dendy samplers (with variable plate areas) and rock baskets (cylindrical wire baskets with cement spheres) in the Upper Mississippi River. After being deployed in the river for six weeks the rock baskets contained higher density, biomass and number of macroinvertebrate taxa than the Hester-Dendy samplers. In addition, samples on both types of artificial substrate were dominated by a few taxa with a 49 % similarity in community composition between the two methods. Given their more natural appearance, Hall 1982 recommended the use of rock baskets, particularly where rocks were a naturally occurring benthic substrate. It was acknowledged, however, that the colonisation of Hester-Dendy samplers in this study may have been affected by using a 2 mm standard gap between plates.

The condition and material of artificial substrates can also affect their efficiency. Valenty & Fisher (2012) compared previously deployed Hester-Dendy artificial substrate samplers with unused ones. Their results indicated that unused samplers were colonised by significantly less Ephemeroptera, Plecoptera and Trichoptera (EPT) taxa and tended to be dominated by more tolerant species compared to used samplers, possibly due to increased plate roughness on used samplers or residual oils and toxins leaching from unused plates and deterring more sensitive taxa. Therefore, these authors recommended that (i) new and old samplers should not be

used in conjunction as they produced different results, and (ii) new substrates should be soaked under natural conditions prior to deployment for monitoring purposes (Valenty & Fisher 2012).

Using data collected over 10 years with modified Hester-Dendy samplers, Applegate *et al.*2007 investigated temporal and spatial changes in the benthic macroinvertebrate assemblages across a range of sites in the Ohio River. Additional sampling was also undertaken around (above and below) industrial and municipal wastewater outfalls where macroinvertebrate community responses to river flow and reduced water quality were detected using Hester-Dendy samplers (Applegate *et al.* 2007). These samplers were also used by Weigel & Dimick (2011) to determine the role of disturbances in non-wadeable Wisconsin rivers because the objective of the study was to compare between sites using a systematic and standardised approach, and not to compare macroinvertebrate communities among habitats. Notably, the authors commented that despite having to visit each site twice (deployment and retrieval), sampling took a similar amount of time and effort as more intensive transect approaches where sites are usually visited only once because of reduced time spent processing samples (Weigel & Dimick 2011).

Rock baskets and Hester-Dendy multiplate samplers are not the only artificial substrates available. For example, Daufresne *et al.* (2004) used cylindrical artificial substrates (30 cm diameter x 15 cm high) deployed in both the lentic and lotic zones to study temporal patterns of the invertebrate fauna of the upper Rhône River in France, and relate them to environmental variables.

3.1.2. Drift netting

Drift nets can be deployed in flowing water to collect drifting invertebrates as they are carried downstream (Figure 2c). They require sufficient flow to be effective and often collect fewer invertebrates than other methods (Blocksom & Flotemersch 2005). Drift nets are most suitable for looking at diel and seasonal activity patterns rather than the macroinvertebrate fauna as a whole (Cellot 1989). To sample invertebrate drift in a large river, Cellot (1989) deployed both drift nets (500 µm) and baskets of rope as an artificial substrate suspended at three depths near the left bank and in the centre of the channel in the Rhône River. A later study by Cellot (1996) used the same method to investigate the effect of side arms on invertebrate drift in the Upper Rhône River, and the author concluded that side-arms did indeed affect the community structure of drifting macroinvertebrates both directly through movement out of side arms and indirectly by increasing the food supply for filter feeders downstream of confluences.

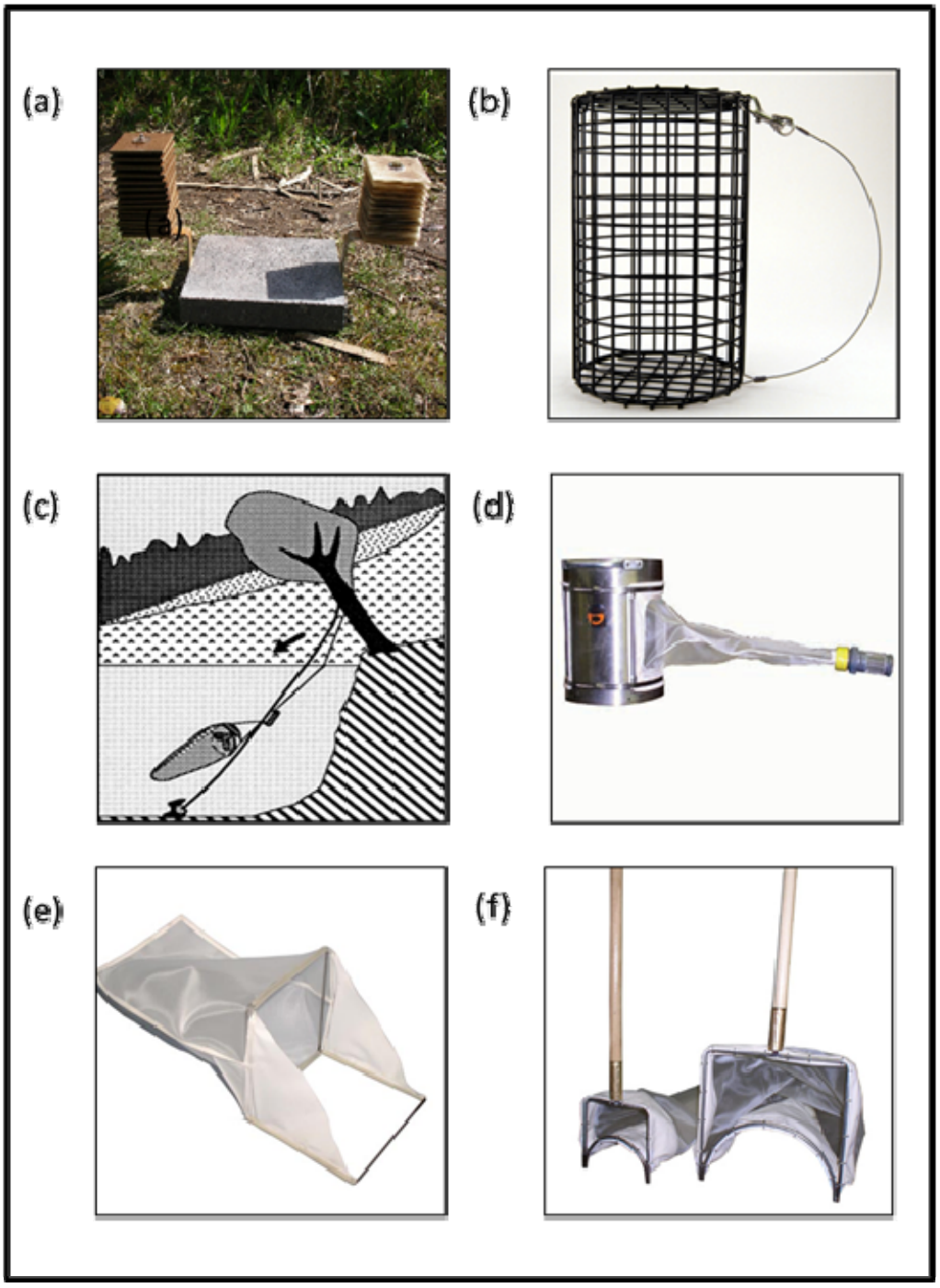

Figure 2. (a) Hester-Dendy multiplate artificial substrate samplers made from tempered hardboard (left) or Perspex (right) attached to a concrete weight, (b) Rock basket artificial substrate http://www.wildco.com/Artificial-Substrate-Basket.html, (c) Diagram of drift net deployment in the water column (reproduced from Cellot (1996), (d) Quantitative Hess sampler http://www.wildco.com/Hess-Sampler-Nitex-500A-m.html, (e) Quantitative Surber sampler http://www.wildco.com/Mini-Surber-Sampler-500um-No-carry-case-Nitex-500A-m.html, (f) Modified snag nets http://www.wildco.com/Complete-Snag-Net-Large-2-1-8-Replacement-net-only-Nitex-500-microns.html.

3.1.3. Near-shore littoral sampling

Although the littoral zone along river margins generally accounts for only a small proportion of the entire channel in larger rivers, it is usually considered to be where much of the macroinvertebrate productivity takes place. Sampling is often restricted to these littoral habitats because deeper benthic habitats further out in the channel are more difficult to sample and benthic organisms are usually lower in abundance, especially in large, sandy rivers (Angradi *et al.* 2006). Consequently some methods developed for wadeable streams may be useful in large rivers where littoral zone habitats can be accessed and provide suitable conditions for sampling (see later). Conventional D-frame hand nets can be used to sample littoral habitats such as macrophytes, under banks or as a kick net where velocity is sufficient and benthic substrates are suitable (Johnson *et al.* 2006). Sampling can be qualitative, semi-quantitative or quantitative by using timed and/or habitat targeted or fixed area samples such as Hess (Figure 2d) and Surber samplers (Figure 2e) (Johnson *et al.* 2006).

In their study of benthic macroinvertebrate assemblages in a range of waterways in south-western British Columbia, Reece and Richardson (2000) used a 400 µm kick net to collect samples from the river edge to as deep as possible in the river channel, sampling < 20 % of the entire channel width. Although their samples may have been biased by their proximity to the rivers' edge, taxa richness and relative abundances differed from those in smaller streams, being dominated by collector-gatherers (up to 85 %), mostly Chironomidae (Reece & Richardson 2000). Likewise, Hughes *et al.* (2012) sampled near-shore habitats of raftable rivers in the Pacific Northwest using a 500 µm kick net. Wessell *et al.* (2008) sampled non-wadeable rivers in Michigan, using a 500 µm 'dipnet' to sample all available habitats, including snags which were scrubbed into the nets, on 11 transects evenly spaced over 2000 m using timed 15 second sweeps or kicks. Angradi *et al.* (2009b) collected samples in mid-continent American rivers using two 30 second kick or sweep nets (depending on flow) at each of 11 transects spaced along a 500 m sample reach.

Sweep and/or kick netting methods have also been employed in studies outside of North America. Storey & Lynas (2007) collected macroinvertebrates from a discontinuous 10 m length of each available mesohabitat in the lower Ord River (Australia) using a 250 µm sweep net. Similarly, several major Italian rivers have been sampled by Lucadamo *et al.* (2008) using a proportional sweep and kick net (250 µm mesh) approach from representative habitats. They observed that representative taxa changed with water/habitat quality, such that EPT taxa were most abundant in good quality river sections and poorer quality sections were dominated by collector taxa (Lucadamo *et al.* 2008). Cortes *et al.* (2008) sampled the River Douro (Portugal) using kick and sweep netting approaches (500 µm mesh), whereby all habitats present at six transects spread over 50 m were sampled in proportion to occurrence. Mustow (2002) sampled four waterways in the River Ping system in northern Thailand to test the application of a biological monitoring approach. Samples were collected using

timed near-shore kick net samples (6-8 at 3-minute samples, 400 µm mesh), or where water was too deep at the bank by using a hand operated dredge sampler thrown from the bank (five samples, 500 µm mesh); replicates were combined for each method (Mustow 2002). The author determined from this study that on average six combined 3-minute kick net samples were necessary to collect 90 % of the available taxa.

Different methods are also used where different substrates are present. For example, Pan *et al.* (2011) used a 420 µm kick net to sample stony substrates while a weighted Peterson grab with the same mesh size was used to sample clay, sand and silt substrates. Quantitative area samplers can also be used to sample the littoral zone on non-wadeable waterways, as used by Mérigoux *et al.* (2009) who sampled macroinvertebrates using a Hess type sampler with a 200 µm mesh to develop an instream habitat model of hydraulic preferences for a range of taxa. They predicted that habitat and flow restoration would have a range of positive and negative effects on various taxa depending on their hydraulic requirements (Mérigoux *et al.* 2009).

Although not always present in the littoral zone, large woody debris (or snags) is an important habitat in some non-wadeable rivers, particularly many large North American rivers. Snag sampling is covered in this section as logs trapped along shorelines are usually sampled using a modified sweep netting approach (Figure 2f) (*e.g.* Angradi *et al.* 2009a), although some studies have used a specially designed sieve to remove cut sections of submerged logs for analysis in the laboratory (*e.g.* Benke *et al.* 2001). Angradi *et al.* (2009a) compared littoral zone and snag macroinvertebrate assemblages using a combination of kick and sweep nets, and a modified 'snag' net respectively (all 500 µm). The snag net used was a modified kick net with the ground bar replaced by a length of chord; the net was placed on a snag and an area of ~1m in front (upstream) of the net was scrubbed by a second person (a boat was used to access and sample from snags). The authors concluded that, although sampling of snags may not be cost effective for assessments where a standard sample area is required or in rivers where their distribution is patchy, they present several possible advantages where they are abundant including, (i) snags provide a naturally occurring hard semi-standard substrate for macroinvertebrate colonisation, and (ii) snag sampling may allow greater precision of assessments as they are less affected by local variation than littoral habitats (Angradi *et al.* 2009a).

3.1.4. Deepwater sampling

A variety of devices for sampling non-wadeable river bottom substrates, which are often fine and mobile, have been developed, including benthic grabs/dredges, freeze corers, and airlift type samplers. Airlift samplers powered by compressed air can be used to suspend loose substrates and associated biota and carry them up a suction pipe with bubbles of air before belching them into a mesh bag (Figure 3d). Freeze corers have been used to sample macroinvertebrates from bottom substrates in deep

rivers (Humpesch & Nederreiter 1993), whereby an insulated core is first driven into the sediment using a sledge hammer and liquid nitrogen is poured down the core to freeze benthic sediments and biota which can then be extracted. This method requires a boat large enough to carry a winch and requires stable anchoring in the current, so is likely to be only suitable in slow-flowing sections of large rivers.

A range of bottom grab/dredge type samplers is available, and although each has slightly different strengths and weakness related to their individual designs, they all use a similar approach (Flotemersch *et al.* 2006a). Examples include Ponar, Peterson and Eckman grabs (Figure 3a-c). The choice of sampler depending on the predominant substrate at a site (*e.g*. Zilli *et al.* 2008). Grabs are generally lowered onto the substrate from a boat, or pushed into the substrate using a pole. The mechanical jaws of the grab are then closed and a sample with a known surface area is retrieved and sifted through a sieve with the desired mesh size.

Using a Ponar grab deployed from a boat to sample benthic invertebrates from mud, sand and gravel (small to large) substrates, Thorp (1992) demonstrated that large geomorphic features within the channel, such as islands can have a positive effect on the diversity and density of macroinvertebrates, presumably, by creating flow refuges, increasing the potential area of the littoral zone and adding other habitats such as large woody debris to the river. Samples were collected at six points along each of six transects that spanned the channel from above to below the selected island (Thorp 1992). Although this approach meant that distinct substrates and their associated biotic assemblages were not sampled, it allowed systematic error to be reduced. Bartsch *et al.* (1998) also used a Ponar grab to collect samples which were then sieved through a 595 µm mesh in the field. Sampling locations were chosen using a stratified random design that allowed channel border and contiguous backwaters areas to be sampled at three locations per site. Royer *et al.* (2001) also used a Ponar grab (Petite), to sample sites that were deep and had fine sediments, these were complemented with near-shore samples collected using a modified Surber sampler (500 µm mesh).

Masson *et al.* (2010) sampled macroinvertebrates in the sediments of the St Lawrence River using a Shipek grab sampler (Figure 3a), which sampled a 400 cm^2 area of sediment which was then washed through a 500 µm mesh sieve. They noted responses of macroinvertebrate taxa to heavy metals and other pollutants, although these were variable within taxa (Masson *et al.* 2010). Bij De Vaate *et al.* (2007) used an Eckman grab (and a 500 µm sieve) to sample bottom assemblages and a 500 µm sweep net approach to sample bank and vegetation assemblages in the Rhine delta forelands. They also demonstrated a positive response between taxa richness and restoration due to improvement of habitat quality (Bij De Vaate *et al.* 2007). Schweiger *et al.* (2005) also used a combination of sampling methods to sample deeper, open water (bottom grab) and shoreline sites (kick net). Bady *et al.* (2005) investigated the effect of sampling effort on biomonitoring using invertebrate traits in three large European rivers. River bottom samples were collected using a Peterson grab (200 µm

mesh), while other microhabitats were sampled with Surber samplers (Figure 2e) (10 samples per habitat, 500 µm mesh). It was determined that more than 90 % of the functional diversity of the assemblage was collected in the first five samples and only ~10 % by the following five and that taxa richness was strongly dependent on sampling effort (Bady *et al.* 2005).

Tamura grabs (clam shell buckets) have been employed mainly in large South American rivers. De Drago *et al.* (2004) used a Tamura grab sampler deployed from an anchored boat to collect samples from central channel and near bank sites in the lower Paraguay River, as did Marchese *et al.* (2005) on the upper Paraguay River. Samples washed through a 200 µm sieve showed a distinct difference between the two site types and significant correlations between sediment type and biotic parameters. Tamura grabs have also been used on the Paraná River by Blettler *et al.* (2012) in the middle and by Zilli *et al.* (2008) on floodplain sections. Zilli *et al.* (2008) also used other grabs depending on the substrate (*e.g.* Tamura for fast flowing water and Eckman for silt-clay sediments) to sample benthic invertebrate assemblages in the Paraná River.

In Europe, Schletterer *et al.* (2010) used a bottom grab sampler to sample macroinvertebrates in the upper Volga river. Three replicates were collected at each site from mud, cobble and sand substrates, and/or deposits of coarse particulate organic matter, and these were then pooled and sieved through a 500 µm net to provide a composite sample. However, a comparison of habitats within a particular site indicated that significant variation existed between the sampled substrates. Also in Europe, Móra *et al.* (2008) employed a Peterson grab to sample for benthic macroinvertebrates in the Tiza River (Hungary), while Bournaud *et al.* (1998) used a Rallier du Baty-style dredge (Figure 3e) to sample macroinvertebrates at different points across an asymmetric channel cross section of the River Rhône. Highest abundances of macroinvertebrates were collected from areas of less hydraulic stress while deeper habitats in the centre and to one side of the main channel were observed to be less favourable for macroinvertebrates. Abundances were linked to periphyton levels, sediment size and channel depth which reflect natural and anthropogenic impacts on the hydrogeomorphology of the river (Bournaud *et al.* 1998).

Airlift samplers, penetrating 20-25 cm into the substrate, have been demonstrated to yield higher abundances of macroinvertebrates than other methods available for sampling fast flowing and deep rivers (*e.g.* grabs and freeze corers), although fine sediments can quickly cause blocking of the collection bag and subsequent rupturing if the mesh size is small (Pehofer 1998). Airlift samplers are also likely to be less susceptible to differences between operators, and therefore yield better comparisons between sites as within site variability is reduced compared to other deep water sampling techniques (Neale *et al.* 2006). Schönbauer (1999) used a modified Freshwater Biological Association airlift sampler to collect invertebrates in fast-flowing sections of the River Danube. Samples collected from centre and near-shore habitats

and from groyne fields were sieved through a 100 µm mesh. While insects were the most diverse taxonomic group, Crustacea and Oligochaeta were the most abundant (Schönbauer 1999). Although spatial distribution can be patchy, the sampled macroinvertebrate fauna was most diverse and abundant in areas protected from the current, while highest abundances of macroinvertebrates were collected during spring/summer (Schönbauer 1999).

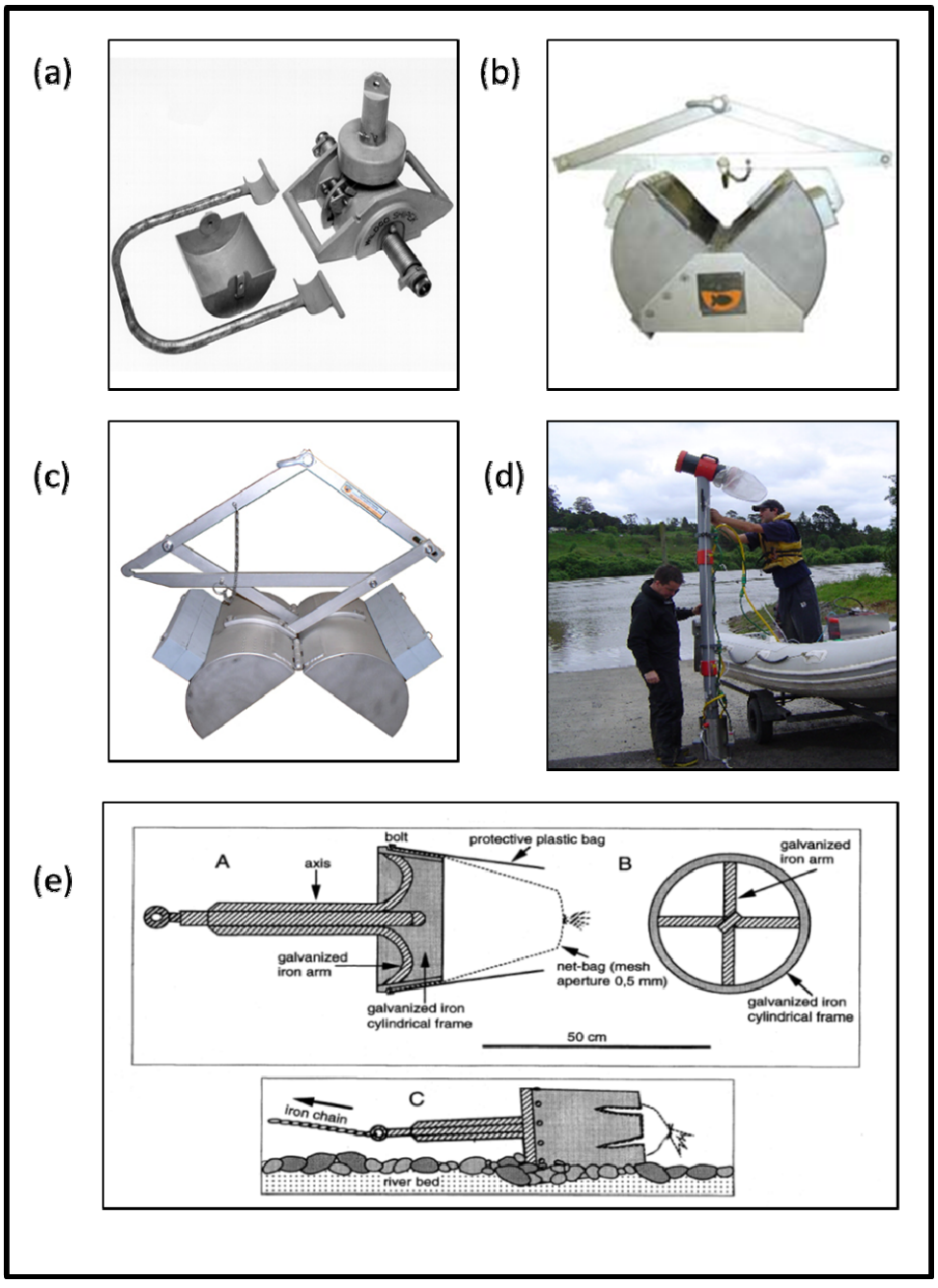

Figure 3. (a) Shipek grab http://www.wildco.com/Shipek-Grab-Includes-crate-SS-134lbs.html, (b) Ponar grab http://www.wildco.com/Ponar-Grab-Standard-9-x-9All-316-Stainless-Steel.html, (c) Peterson grab http://www.wildco.com/Peterson-Grab-Includes-shipping-crate-Zinc-plated-steel-75lbs.html, (d) An airlift sampler powered by compressed air, preparing to be deployed using a boat (e) Diagram of Rallier du Baty-style dredge (reproduced from Bournaud *et al.* 1998).

3.2. Non-wadeable river sampling in New Zealand

Most published studies of macroinvertebrates in non-wadeable New Zealand rivers have been conducted for a variety of purposes in the North Island. The following analysis excludes studies that sample single wadeable habitats in larger rivers, such as studies restricted to riffles or shallow runs, unless they provide comparisons among substrate types or compare sampling methods. A range of approaches have been employed to sample macroinvertebrates in non-wadeable New Zealand rivers, including:

- Diving to record taxa occurrence or measure abundance on riverbeds (*e.g.* Jowett *et al.* 1991; Coffey *et al.* 1992; Coffey *et al.* 1998)
- Using a dredge net to recover macroinvertebrates in deep inorganic substrates (*e.g.* Boubée 1977)
- Sweeping or brushing accessible near-shore littoral substrates into a net (*e.g.* Carter 2000; Collier & Lill 2008)
- Drift netting to catch invertebrates carried in the water column (*e.g.* Bayly 1990)
- Airlift sampling to access substrates in deep water (*e.g.* Carter 2000; Collier *et al.* 2012)
- Deploying artificial substrates for colonisation by invertebrates (*e.g.* Boothroyd & Dickie 1989; Davenport 1992; Collier *et al.* 2011).

3.2.1. Artificial substrates

Artificial substrates, used to provide consistent surfaces for comparison among sites, have included multiplate (Hester-Dendy) samplers made from wood (Davenport 1992) or inert perspex (Boothroyd & Dickie 1989), and samplers made from bunched coir to mimic habitat provided by fibrous root mats such as those of willow (Collier *et al.* 2009b). Davenport (1992) deployed hardboard multiplate samplers at six sites along the Waikato River for eight weeks and detected spatial variations downriver at sites influenced by flow variation, organic effluent and a thermal discharge. Collier *et al.* (2011) repeated the study of Davenport 25 years later, using both hardboard and perspex samplers, and noted some major differences in the dominance of macroinvertebrate groups between studies, potentially attributable to small differences in methodology, changes to the river over time, and long-term climatic variations between studies. Boothroyd & Dickie (1989) studied colonisation of perspex multiplate substrates in the upper Ohinemuri River, Waikato. Although conducted in shallow water (*c.* 0.6 m deep), their study suggested that most colonisation had occurred by 28-35 days, although new taxa continued to colonise throughout the 70-day study period due to changes on plates from accumulation of fine particulate matter, and changes in the composition of the drifting fauna affecting the pool of potential colonisers.

3.2.2. Drift sampling

Bayly (1990) collected the microcaddisfly *Oxyethira albiceps* while sampling seston entering the Waikato River from Lake Taupo by suspending a net attached to a 5 m long pole in the water for 5 minutes. He found *Oxyethira* densities peaked after sunset and were lowest in winter, when filamentous algae on which *Oxyethira* feed would also be expected to be lowest. Irvine *et al.* (1984) reported that drift densities of Chironomidae, Trichoptera and Oligochaeta in the regulated Hawea River responded positively to experimental flow releases which increased depth up to 2.6 m at some sites, although sampling appears to have been done in shallow water, as is the case for most other drift studies in larger rivers (*e.g.* Sagar & Glova 1992; Shearer *et al.* 2002; Shearer *et al.* 2003). It appeared that most invertebrates caught in the Hawea and Waikato rivers were associated with dislodged periphyton, although density-dependent drift may also occur (Collier & Wakelin 1992). Generally, correspondence of macroinvertebrate densities and composition in the drift and benthos is weak, and drift samples do not provide a good representation of river macroinvertebrate communities, due in part to the factors mentioned above (Shearer *et al.* 2003).

3.2.3. Near-shore littoral sampling

Sweep netting has been conducted by wading from shore (Collier & Lill 2008), when snorkelling (Taylor 2001) or from a boat (KJC unpublished data) to access near-shore littoral substrates exposed to flowing water. Littoral substrates have included wood, macrophytes, inorganic sediments and root mats, which have been sampled separately to determine invertebrate-habitat associations (*e.g.* Collier *et al.* 2012) or as a composite sample in proportion to substrate occurrence to characterise littoral communities at particular locations (Collier & Lill 2008). The latter authors reported littoral macroinvertebrate communities in the Waikato River were influenced by the proportion of wood sampled and the proximity of sites to tributary junctions. Water level variations brought about by tidal cycles or hydropeaking below dams can influence near-shore littoral macroinvertebrate composition and mask their response to human pressure (KJC, unpublished data).

Johnston (2011) compared macroinvertebrate communities from riprap, willow, combined riprap and willow, and sandy beach near-shore habitats in the Waikato River at three sites through Hamilton, using a pivoting net and stiff bristled broom to sample riprap boulders. Macroinvertebrate responses to habitat type were variable over time and across sites; riprap appeared to have relatively high sample diversity but supported a limited range of taxa compared to other habitat types, with the combination of riprap, beach and willow samples yielding the greatest near-shore macroinvertebrate community diversity in terms of species accumulation.

3.2.4. Deepwater sampling

Diving has been used to record taxa occurrence across river bottom transects (*e.g.* Coffey *et al.* 1992; Coffey *et al.* 1998) or has been applied more quantitatively using Surber samplers to access water up to 1.5 m deep in coarse substrate rivers where the aim was to assess macroinvertebrate flow preferences (Jowett *et al.* 1991; Collier 1993; Collier 1994; Collier *et al.* 1995). Densities were not correlated with depth in two North Island rivers, although no samples collected at >0.8 m deep supported high densities (Collier *et al.* 1995). Low densities in deep water appears to be a general feature typical of invertebrate taxa across other New Zealand rivers, although some mayfly, caddisfly and worm taxa were recorded with high densities in some deepwater samples (Jowett *et al.* 1991; Collier 1993). Jowett *et al.* (1991) reported that water depth up to about 1.6 m was negatively correlated with the mean size of the common mayfly, *Deleatidium*, Diptera and beetles, and positively correlated with the size of other mayflies and uncased and cased caddisflies, although there can also be differences in depth preferences among *Deleatidium lillii* and *myzobranchia* groups (Collier 1994).

Boubée (1977) used a corer or Wisconsin dredge net deployed by a diver to sample the edges and deeper benthos in the Waikato River through Hamilton. There was a high degree of spatial variation in community composition along this section of river, although edges supported higher densities than the central channel (Boubée 1977). Later, Carter (2000) used a small airlift sampler powered by a compressor to collect invertebrates from water <1 m deep along the Waikato River. A much larger airlift sampler powered by compressed air from dive bottles (Figure 3d) was used by Collier *et al.* (2012) to access benthic substrates in water 1.5-5 m deep across 11 boatable rivers throughout New Zealand.

3.3. Comparison of substrates/methods

3.3.1. Comparison of methods

Blocksom & Flotemersch (2005) compared six field sampling methodologies (Hester-Dendy substrates, drift nets and four littoral sampling approaches) for non-wadeable waterways employed by three major bioassessment programmes in the USA. Methods were compared in run of the river locations and at restricted flow sites across 60 locations on four rivers. Due to inadequate flow for deployment at a large proportion of sites and low collection rates compared to other methods (mean 74 organisms), drift nets were excluded from statistical analyses. On average the remaining methods collected similar numbers of organisms to each other in each of the two flow areas (*i.e.* all methods detected differences between flow sites). Metrics calculated from Hester-Dendy samples differed from the littoral net metrics, but represented abiotic conditions in both flow conditions well. Additional comparisons showed that, although calculated metrics could be similar between some collection methods, relationships with abiotic variables differed between methods. The authors

emphasised that sampling methods are unlikely to be interchangeable, that different stressors are better detected by certain methods, and that a set of complementary sampling approaches may provide for better results.

In a survey and review of methods used by agencies undertaking bioassessment in the United Kingdom, Ireland, and Europe, Jones *et al.* (2005) identified that marginal sweep netting, including disturbance of the substrate with a hand net, was the most commonly employed method of sampling deep rivers, while dredges and grabs were also widely used. Respondents were asked to rate the ease of use, collection efficiency, and the time required in the field and in the laboratory of the methods that they used. In general respondents indicated that marginal sweep netting was viewed as an efficient and simple sampling method, as was using a long handled pond net to disturb the channel substrate although pond nets were considered less efficient. Dredges were also considered moderately easy to use in the field; however their efficiency at collecting fauna and the time required to process samples in the laboratory varied widely and could be poor and/or lengthy, respectively. The authors recommended that any future comparisons of methods include replicates of both deep water and marginal habitats, as differences between these habitats can often be greater than that between methods (Jones *et al.* 2005). Subsequent testing supported the targeting of both marginal and deep water habitats to characterise the macroinvertebrate community diversity and responses to pressure in deep rivers of the United Kingdom and Ireland, and that deep water habitats were most effectively sampled using an airlift sampler (Neale *et al.* 2006).

Neale *et al.* (2006) set out to test the efficiency, effectiveness, precision and cost effectiveness of methods available for sampling deep rivers in the United Kingdom and the Republic of Ireland. At each site replicate samples were collected using a light dredge, airlift sampler and long handled pond net to sample benthic habitats and sweeps with a standard pond net to sample marginal habitats. While the dredge and pond net techniques may have been more efficient in terms of the amount of collection time and effort required, the airlift sampler performed better in terms of collecting adequate and precise faunal samples from the river channel. Samples collected from river margins differed from those collected in the river channels and also appeared to be responding to different environmental pressures (Neale *et al.* 2006). The authors recommended that an airlift sampler be used for routine monitoring of benthic macroinvertebrates at sites with extensive deep water habitats and that this should be complemented with samples from marginal habitats. Although the airlift sampler requires a boat to be used, this also allows effective sweep netting of marginal habitats that are unsafe or inaccessible from the bank. An advantage of this approach is that deep-water habitats may be able to be incorporated into existing classifications for shallow rivers (Neale *et al.* 2006).

Battle *et al.* (2007) used rock baskets to sample rocky substrates complemented by the use of a petite Ponar grab for fine sediment sampling to investigate spatial variation of benthic macroinvertebrate communities in the Upper Mississippi River.

Samples from both collection methods were processed using a 355 µm sieve. Both habitats were dominated by collector gatherers, however only 27 of 118 taxa were identified in both habitats (Battle *et al.* 2007). In fine sediment samples taxa richness was dominated by insects (55/68 taxa), although oligochaetes accounted for 77-95 % of the density of macroinvertebrates, while in rock baskets 42/50 of the identified taxa were insects and hydropsychid caddisflies dominated abundance. The authors concluded that skewness towards dominant taxa may be a feature of large river macroinvertebrate communities (Battle *et al.* 2007).

3.3.2. Comparison of artificial substrates

In New Zealand, Boothroyd & Dickie (1989) found that the macroinvertebrate fauna on perspex multiplate substrates was generally similar to that in benthic samples, although artificial substrates had greater macroinvertebrate density and total taxa number. *Austrosimulium australense* larvae dominated the fauna colonising the artificial substrates, while in the natural benthos Chironomidae and *Pycnocentrodes* sp. were also very abundant (Boothroyd & Dickie 1989). Comparisons of wood *vs.* perspex multiplate substrates indicated that the type of material did not have a significant effect on macroinvertebrate community composition, taxa richness, Margalef diversity, Pielou evenness or Shannon diversity (Collier *et al.* 2011). However, significant effects of substrate type (wood *vs* perspex) were evident for densities of *Paracalliope*, *Potamopyrgus*, and total invertebrates, with densities of these taxa consistently higher on wooden plates across all sites. It was concluded that *Paracalliope* and *Potamopyrgus* may have been deriving some nutritive value from the biologically active surfaces provided on wood, possibly by feeding on epixylic biofilms or fine particles trapped in biofilm exudates.

Collier *et al.* (2009b) compared multiplate substrates with bundles of coir, and found that coir samplers supported different macroinvertebrate communities dominated by *Potamopyrgus* and with higher densities of worms. They suggested that coir samplers may provide a useful supplement to multiplate samplers for documenting biodiversity in deep river sampling by creating a different habitat type than multiplate samplers. Most recently, comparisons have be made between perspex multiplate samplers, coir samplers and samples collected from natural littoral substrates in 10 Waikato non-wadeable rivers. This study found that community composition mostly reflected site characteristics rather than the type of substrate sampled (Figure 4), suggesting that the pool of potential colonists was the over-riding factor influenced community composition (KJC, unpublished data). Littoral sampling at the 10 sites retrieved 63 % of total taxa represented across all sampling methods.

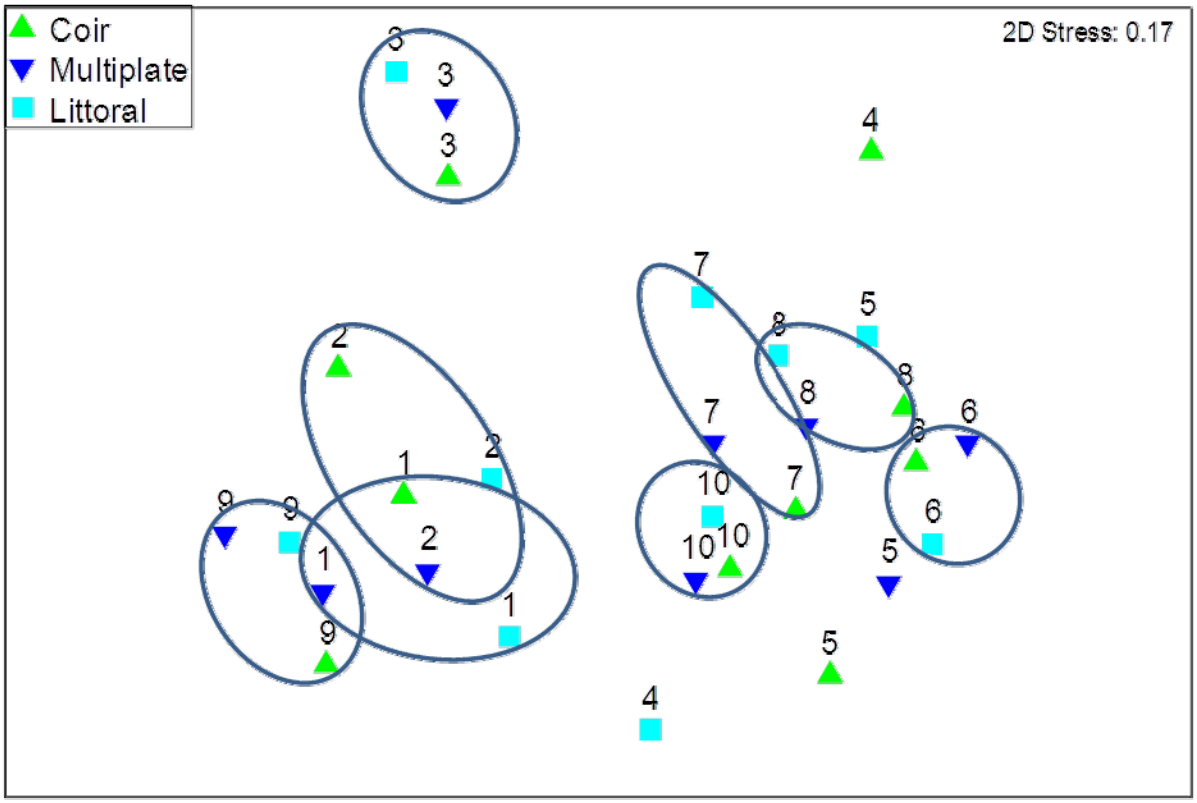

Figure 4. Non-metric multidimensional scaling plot of macroinvertebrate percent community composition at ten Waikato non-wadeable river sites sampled by sweeping near-shore littoral substrates, and from artificial multiplate substrates or coir samplers (Collier *et al.* 2009).

3.3.3. Comparison of natural substrates

Angradi *et al*. (2009b) found that wood samples generally yielded more filterer taxa and higher abundances overall than near-shore littoral sampling which provided a greater number of gatherer taxa. Collier *et al*. (2012) compared large wood with sampling of near-shore littoral habitat and deep-water benthic habitats that ranged from sand-silt to coarse gravels and cobbles in 10 boatable rivers throughout New Zealand. They recorded low numbers of taxa on wood which may have reflected lower sampling effort, and after rarefaction there was no significant difference in taxa richness among substrates types even when compared with riffles (Figure 5). Around 70 % of the total taxa were recorded from littoral habitats <1 m deep, and Collier *et al*. (2012) noted that the combination of littoral and deep samples yielded the greatest number of taxa. Sites grouped according to degree of human pressure rather than substrate type, except for riffle faunas which generally grouped together suggesting that riffles may provide less sensitive habitats for detecting human pressure responses than littoral, wood or deep water habitats in boatable rivers (Figure 6).

Collier *et al.* (1998) sampled macrophytes, wood and inorganic substrates in lowland Waikato waterways, several of which were non-wadeable with mean depths up to 1.7

m. They reported that densities of some common taxa on inorganic substrates and macrophytes were correlated with reach water depth, percentage of bed covered by macrophytes or sand/silt, and water quality factors. *Potamopyrgus* and *Austrosimulium* densities decreased with water depth whereas *Paracalliope* densities increased; mean values of biotic indices were similar for all substrate types, except for % EPT which was significantly lower on macrophytes than on wood.

Carter (2000) compared the macroinvertebrate fauna collected by a small airlift sampler in water <1 m deep at sites along the Waikato River and found that the airlift collected 47 benthic taxa compared to 38 on macrophytes obtained by sweep netting, with 31 taxa collected by both methods.

Poulton *et al.* (2003) also employed complementary methods to sample different habitats in a non-wadeable channelised section of the lower Missouri River. In faster flowing habitats rock baskets were deployed for six weeks. In addition, river margins were sampled using a 500-µm kick net and soft sediments in low flow areas were sampled using a petite Ponar grab at deployment and retrieval times. Rock substrates yielded fewer taxa than both marginal habitat and soft sediment samples with unique taxa ranging from 15-22 species for each method. While influences of substrate type could not be differentiated from sampling method or flow habitat, the results indicated that rock substrates had 75 % and 73 % taxa similarity between marginal habitat and soft sediment samples, respectively, while marginal habitat and soft sediment samples only shared 39 % similarity.

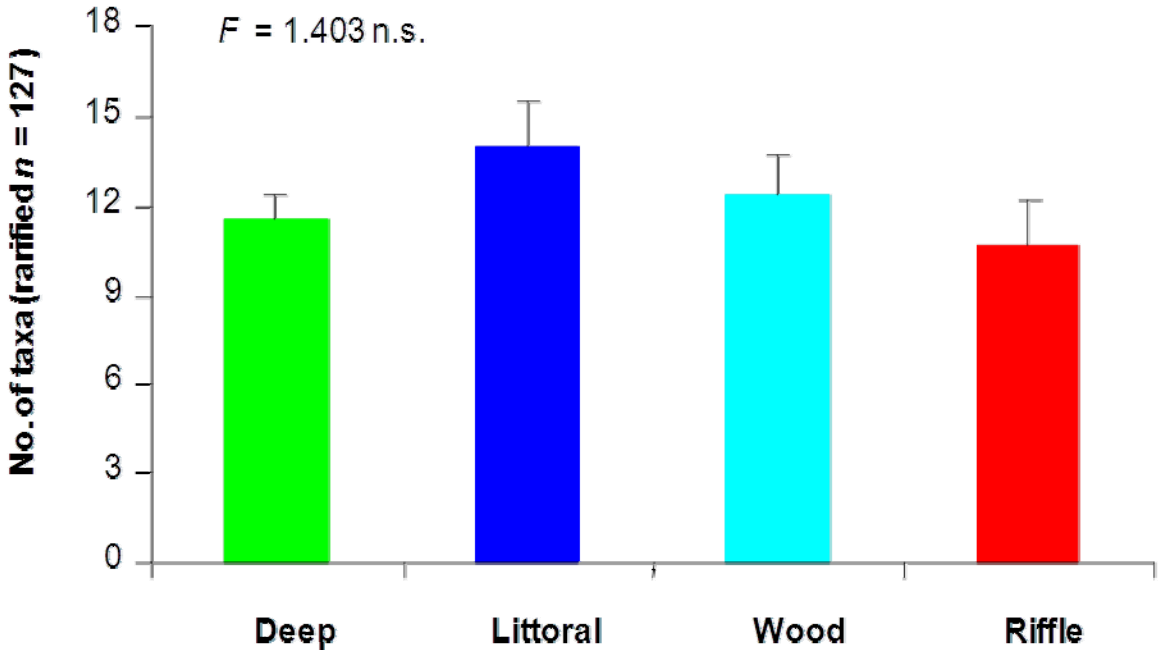

Figure 5. Mean (SE) rarified taxa richness for four habitat types sampled in seven moderate-gradient boatable New Zealand rivers.

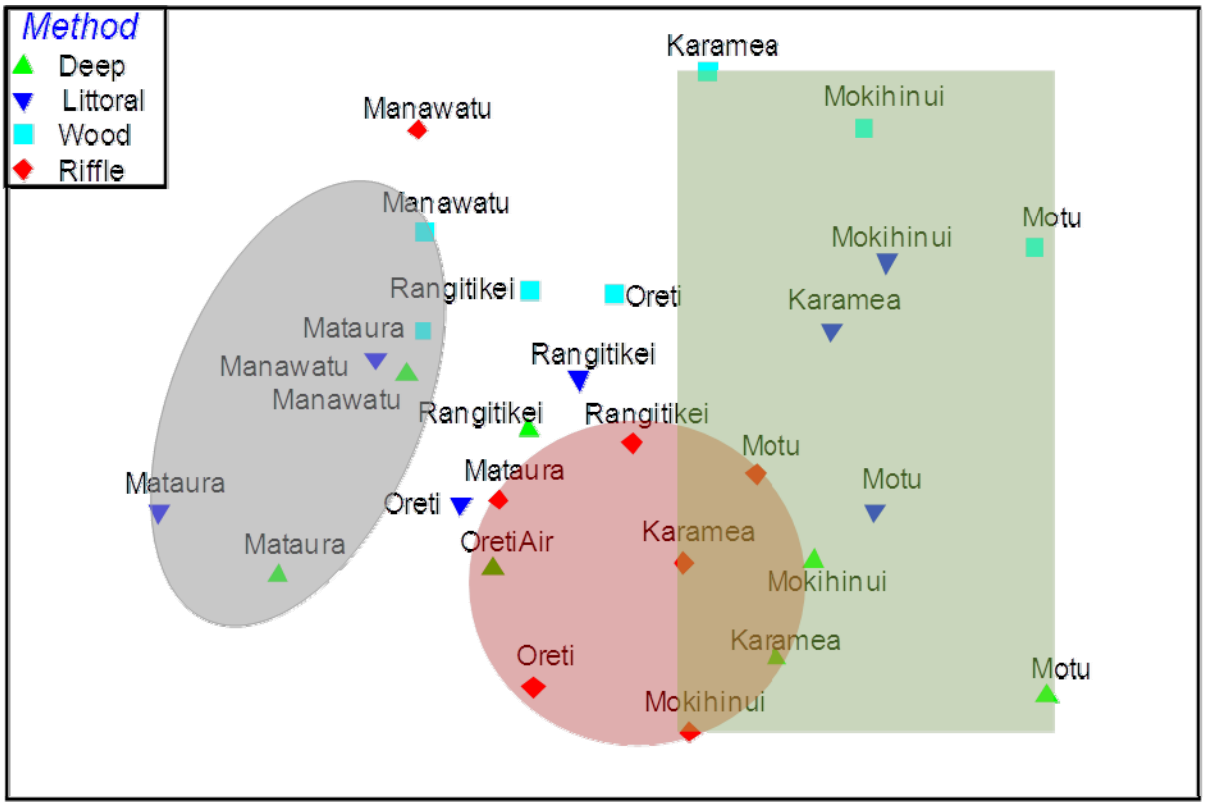

Figure 6. Non-metric multidimensional scaling plot of percent macroinvertebrate composition at seven moderate gradient non-wadeable river sites sampled in December 2007 (see Collier *et al.* 2012 for further details). Square encompasses low pressure sites draining catchments predominantly (≥ 75 %) in native forest, ellipse encompasses deep, littoral and wood samples from two high pressure sites (Mataura and Manawatu), and circle encompasses riffle samples from all sites excluding Manawatu River.

3.4. Development of sampling protocols

3.4.1. Sampling protocols

Several protocols for sampling reaches of large and non-wadeable waterways are currently in use by bioassessment agencies, particularly in the USA (Table 2). The most recent of these is the Large Rivers Bioassessment Protocol (LR-BP) which uses a standardised reach length and sweep/kick netting at points along both banks out to the centre of the river or until water depth reaches 1 m (Johnson *et al.* 2006). The utility of this protocol was tested by Blocksom & Flotemersch (2008), who concluded that overall the macroinvertebrate LR-BP was suitable for the bioassessment and monitoring of non-wadeable rivers in the USA, and that metrics calculated from samples using its field and laboratory protocols responded well to gradients of urban and agricultural land cover. Whilst developing this protocol, Flotemersch *et al.* (2006b) concluded that sufficient numbers of organisms and robust, comparable metrics could be provided by 6 transects spaced at 100 m intervals over a 500 m fixed reach length with a sample consisting of (i) 24 20-second kick net samples from both banks, and

(ii) 12 timed D-frame net samples from habitats complementing the kick net habitats collected.

Other non-wadeable river sampling protocols target potentially invertebrate rich habitat, such as large woody debris ensnared out in the main channel which require a boat (*e.g.* Angradi 2006) and the near-shore littoral zone which can be sampled on foot (*e.g.* Moulton *et al.* 2002).

Table 2. Summary of current international protocols used to sample macroinvertebrates in non-wadeable waterways for bioassessment purposes.

Protocol	Sample reach selection	Collection method/s
Large River Bioassessment Program (LR-BP) (Flotemersch *et al.* 2006a; Johnson *et al.* 2006)	The LR-BP uses a sample reach length of 500 m and at each site, there are a total of six transects. Transect A is located at the downstream end of the reach with the remaining five transects spaced at 100 m intervals. At each transect macroinvertebrates are collected from a 10-m sample zone (5 m either side of transect). This sampling zone extends from the edge of water to the mid-point of the river or maximum depth of 1 m. Sampling is therefore largely bank-oriented except in shallow reaches.	Each transect has two zones (one on each bank) and samples from the entire reach are composited into a single sample. This results in a reach-wide sample containing debris and organisms from 12 separate zones (total of ~12 m^2) that represent the 500-m reach. Six sweeps, each 0.5 m in length, are collected within each sampling zone using a 500-µm mesh D-frame net. The six sweeps are proportionately allocated based on habitats present within each sampling zone (*e.g.* snags, macrophytes, cobbles). This method negates the need for separate collection nets in the field and helps standardise the area sampled. Where a site is more than 1 m deep at the water's edge, the sweeps should be collected from a boat if possible.
NAWQA Program (Moulton *et al.* 2002)	The NAWQA programme collects two types of macroinvertebrate samples: firstly, a semi-quantitative sample is collected using a richest targeted habitat (RTH) approach, usually this is riffle habitat or woody snags. Secondly, a proportional multi-habitat sample is collected along the study reach.	Firstly, a 0.25 m^2 area is sampled using a slack (modified Surber) sampler (500 µm mesh) in riffles. Two snags are sampled by disturbing snags upstream and the area sampled is estimated for that habitat. Secondly, qualitative samples are taken with a D-frame kick net, visual collections and a grab. Water depth and substrate type are recorded. Large organic debris is removed from the sample and large invertebrates (*e.g.* crayfish and mussels) are recorded and returned to the river.
Great Rivers (Angradi 2006; Angradi *et al.* 2006)	In EMAP-GRE, benthic macroinvertebrates are collected by a sampling crew from two habitats: (i) Shallow (< 1 m), near-shore littoral areas, and (ii) from the surface of large woody debris (LWD) or 'snags' in the main channel.	In near-shore littoral areas, samples are collected using a using a standard rectangular-frame kick net (335 x 508 mm frame; 500 µm mesh) at 11 evenly spaced points along a 500-m reach. Samples are standardised using two 30-second, 0.26-m^2 kick samples and the 22 kick samples at each site are combined into a single composite sample representing a total bottom area of 5.7 m^2.

Protocol	Sample reach selection	Collection method/s
Great Rivers (Angradi 2006; Angradi *et al.* 2006)…*continued*		Where sampling points have vertical shorelines that prohibit safe kick sampling, a suitable site within 5 m up- and down-river from the station can be sampled. If no safe alternative is available then no sample is collected. A 1-m long sample from the up-current side of snags is collected from a boat using a 'snag' net (modified kick net with a 500-µm mesh). Snags considered to be suitable for sampling are those that are at least 0.6 m deep in water, are exposed to some current, and are at least 5 m long and at least 15 cm in diameter where the snag breaks or comes within 30 cm of the water surface. If no natural snags are present surrogate 'snag' samples may be collected from man-made structures (*e.g.* pilings and navigation markers). Characteristics of each snag, including depth, snag diameter at the water surface, snag surface features, water velocity, and the distance of the snag from the shore, are recorded, and a picture of the sampled snag is taken if possible.
Michigan Department of Environmental Quality (MIDEQ) (Merritt *et al.* 2003)	This protocol uses a 2000 m reach, divided equally into 11 transects. The river bank to sample is chosen by the toss of a coin. Provided they are in water less than 1 m in depth within the littoral zone and able to collect a large enough timed sample, all habitats within 10 m up- and downstream of each transect are counted and sampled.	A 15-second sample is taken from every habitat that is present with a D-frame net, with a mesh size of 500 µm. The net is emptied into a bucket or pan filled with water. Detritus is removed before placing the sample in a 500-µm sieve to remove excess water. Where at least eight transects contain large woody debris, these may be sampled instead of other habitats to reduce collection and processing time, and provide a habitat-independent macroinvertebrate sample.

3.4.2. Defining an appropriate reach length

Locating and determining an appropriate reach and reach length can be more difficult in non-wadeable waterways as river units tend to be large and repeating units further apart (Johnson *et al.* 2006). A detailed examination of methods for determining reach site length can be found in Flotemersch *et al.*'s (2011) review of major biological assessment design alternatives for boatable rivers. In their review, Flotemersch *et al.* (2011) concluded that sample site length is ultimately determined by the quality and quantity of information required to meet sampling objectives, and the funds available to achieve these. Sampled taxa richness is dependent on the effort applied (Hughes *et al.* 2012), and the required spatial extent and purpose of collection are important for setting the length of the site sampled and the coverage of habitats within it (Flotemersch *et al.* 2011). Although precision will likely increase with sampling effort, cost of sampling will also increase (Flotemersch *et al.* 2006a). However, while long sample reaches may allow broad scale patterns to be quantified they may obscure smaller scale (*e.g*. habitat-specific) patterns or points of interest (such as pollution sources or river modifications), making linkages between river conditions and their drivers more difficult for resource managers to resolve (Flotemersch *et al.* 2006a).

While some studies employ a set reach length approach which can range from ≤ 500 m (*e.g.* Angradi *et al.* 2009b) to ≥ 2000 m (*e.g.* Wessell 2008), other studies have employed a more formulaic approach to setting a sample reach length based on multiples of the channel width to set the length of the study reach *e.g.*, up to 50 times Hughes *et al.* 2012). The advantages and disadvantages of these approaches are outlined in more detail in Table 3. Compromises between these two approaches have also been employed, such as a multiple of the wetted width used with minimum and maximum limits being set, to ensure representativeness of the biological data collected and avoid personnel fatigue (Fitzpatrick *et al.* 1998). Some authors have criticised this approach as potentially leading to inconsistent sampling effort with implications for data interpretation (Flotemersch *et al.* 2006a). Several existing protocols from the USA employ a fixed length approach, for example, the Large River Bioassessment Program (LR-BP) in the USA uses a set 500 m reach length (Johnson *et al.* 2006). This length was chosen because it can provide representative samples where both banks are sampled and is a manageable length in that the entire sample reach is usually observable from a single point (Blocksom & Flotemersch 2005).

Table 3. Examples of methods used for determining the length of a sampling reach and their advantages and disadvantages.

Reach length method	Advantages	Disadvantages
Fixed length	• Easy to apply and plan • Standard sampling effort between sites • Can often see entire sample reach from a single point.	• May lead to unequal sampling effort relative to river size • May not encompass sufficient habitat units and therefore detected biological differences may reflect differences between habitat units present at different sites
Multiples of wetted width	• Sampling area/effort increases proportionally with river size • Better chance of including wider range of habitat units.	• By definition sampling effort will be different between sites • Potentially significant and costly sample effort required, which will increase with river size
Meander cycles	• Based on wetted width, but set so that reach length encompasses one complete meander, theoretically covering all major habitat types within a geomorphic reach.	• In altered rivers, meanders have often been straightened, so that identifying their location or extent and boundaries of habitat units are unclear.

4. METHODS FOR SAMPLING THE ECOLOGICAL FUNCTION OF NON-WADEABLE WATERWAYS

4.1. What are functional indicators?

Functional indicators quantify the rates of key ecosystem processes and can be used to indicate the ecological health of an ecosystem, in this case rivers. In contrast to structural indicators, such as those based on invertebrate assemblages, functional indicators provide an assessment of the rate of biologically-mediated processes, *e.g.* growth rates, nutrient cycling, ecosystem metabolism[2]. An advantage of functional indicators is that they are not necessarily limited by available habitat, *i.e.* similar methodology can be applied in wadeable and non-wadeable rivers. Secondly, functional indicators can reflect processes that occur across a range of compositional levels (*e.g.* populations to ecosystems), spatial scales (*e.g.* microhabitat to river reach), and temporal scales (*e.g.* hours to months). Hence functional indicators provide an integrative measure of river health, integrating multiple biotic and abiotic effects across time and space.

It is widely acknowledged that an assessment of ecological health requires knowledge of both ecosystem structure and function (Meyer 1997; Bunn & Davies 2000; Gessner & Chauvet 2002). Structural indicators do not necessarily respond to anthropogenic pressures in the same way as functional indicators (Matthews *et al.* 1982; Clapcott *et al.* 2010): change to ecosystem structure can occur without changes in function and vice versa, and structural and functional components can be sensitive to different pressures. However, apart from the Ecosystem Health Monitoring Program in southeast Queensland, Australia, to our knowledge there are currently no monitoring programs that regularly measure functional indicators, in wadeable or non-wadeable rivers. Yet key ecosystem processes are widely measured in flowing waters, including:

- Ecosystem metabolism
- Organic matter processing
- Primary productivity
- Heterotrophic activity
- Nitrogen processing.

The application of functional measurements as indicators of river ecological health is subject to the same principals as structural indicators – indicators should be sensitive to human impact, objective, transparent and reproducible (*i.e.* calculated from quality assured data collected using standardised methodology). So whilst methodology

[2] In this review, functional indicators do not include processes that are not primarily driven by biological structure, *e.g.* hydrology, geomorphology.

exists to measure ecological functions, the application of that data as indicators of river health is still under development in both wadeable and non-wadeable rivers. The level of development is discussed in the following sections. Here we focus on functional measurements that have been recommended as functional indicators because they have standardised methods, are relatively cost effective to implement, and are sensitive to human impact.

4.2. Ecosystem metabolism

Ecosystem metabolism is the rate at which energy in the form of organic carbon is produced (productivity) and consumed (respiration). River metabolism has been measured as early as the 1950s when Howard Odum (1956) reported oxygen metabolism rates in a range of flowing waters. Standardised methods exist to collect the oxygen data used to calculate ecosystem metabolism: single-station whole system technique (Young & Huryn 1996), two-station whole system technique (Marzolf *et al.* 1994), recirculating benthic chamber technique (Fellows *et al.* 2006). The single-station technique is most commonly applied in non-wadeable rivers.

Because metabolic rates are sensitive to changes in light, temperature, flow, nutrients and pollutants they can be used to indicate the effects of anthropogenic pressures on river health. Numerous studies have demonstrated an increase in ecosystem metabolism associated with land-use intensification (Young & Huryn 1999; Gücker *et al.* 2008; Von Schiller *et al.* 2008; Tank *et al.* 2010); catchment disturbance from urbanisation (Fellows *et al.* 2006; Clapcott *et al.* 2010), forestry (Clapcott & Barmuta 2010a) or even military operations (Houser *et al.* 2005); and flow pulses in regulated rivers (Vink *et al.* 2005; Chester & Norris 2006; Tuttle *et al.* 2008). In New Zealand, a study focussed on non-wadeable rivers demonstrated a linear increase in GPP and a non-monotonic change in ecosystem respiration (ER) in response to catchment vegetation clearance (Collier *et al*. 2009). The highest rate of gross primary production (GPP) ever reported was observed in the Manawatu River by Collier *et al.* (2009) and subsequent sampling showed consistently high metabolic rates for this non-wadeble waterway (Young & Clapcott 2010).

Ecosystem metabolism also varies seasonally in response to changes in light and temperature, flows and the type and amount of dissolved and particulate organic matter entering a river (Young & Huryn 1996; Roberts *et al.* 2007; Clapcott & Barmuta 2010b). As such, for monitoring purposes it is recommended to stratify sampling by season, with the primary focus on summer low flows when a river is likely to be most stressed from human impacts (Young *et al.* 2008).

Benthic metabolism is currently used in ecosystem health monitoring of streams in southeast Queensland (Bunn *et al.* 2010), although no non-wadeable rivers are part of that monitoring network. Benthic chambers have been applied in the littoral habitat of large rivers (*e.g.* Fellows *et al.* 2009), but it has been suggested that the amount of

replication required to obtain a representative sample and detect a meaningful change, as well as criticism of resultant artefacts of using chambers, make this method non-feasible for regular monitoring of larger rivers (Grace & Imberger 2006).

The balance between the amount of energy consumed (ecosystem respiration) and energy produced (gross primary productivity) is the PR ratio and reflects whether river metabolism is driven by internal (autotrophic) or external (heterotrophic) energy sources. Conceptually, the PR ratio naturally changes along the length of a river with large river systems expected to be driven by dissolved and fine particulate organic matter delivered from upstream (Vannote *et al.* 1980) or flushed in from floodplains during above-bank flows (Junk *et al.* 1989) (*e.g.* PR <1). However, non-wadeable rivers may have highly productive near-shore littoral zones or seasonally low flows may support large seston abundance and productivity (Thorp & Delong 1994; Bunn *et al.* 2003) (*e.g.* PR >1). This natural variability makes it preferable to compare metabolic rates to relative reference conditions when using metabolism measurements as functional indicators (Young *et al.* 2008), although this is problematic for non-wadeable rivers where reference sites generally cannot be found. Young *et al.* (2008) reviewed data from a range of international studies and used the distribution of metabolism values from 'reference' sites to recommend criteria to assess river health. This study provides valuable reference data for future assessments of ecosystem metabolism when relative reference conditions are unavailable.

We have collated data from a range of New Zealand studies (Young & Huryn 1996; 1999; Young 2006b; Young *et al.* 2006; Clapcott & Young 2009; Young & Collier 2009; Clapcott *et al.* 2010; Doehring & Young 2010; Young & Clapcott 2010; Young *et al.* 2010; Collier *et al.* 2012) to compare wadeable and non-wadeable rivers to the guideline criteria recommended by Young et al. (2008) (Figure 7). Data from 209 sites were analysed, with the average summer value reported for sites with replicate data. Results showed no statistical difference between GPP or ER values recorded in wadeable *vs.* non-wadeable rivers with similar levels of pressure. However, there was a trend for higher values of productivity (GPP) to be seen in non-wadeable rivers (mean = 6.54 $gO_2/m^2/day$) compared to wadeable rivers (mean = 4.09 $gO_2/m^2/day$). There was also significantly greater PR ratios observed in non-wadeable rivers (mean = 0.82) compared to wadeable rivers (mean = 0.51) (Pooled Variance t = 2.817, df = 207, P = 0.005). This data compilation provides a reference data set for the future comparison for ecosystem metabolism measures. Site information could be further investigated to determine whether any environmental factors contribute to observed differences between wadeable and non-wadeable rivers.

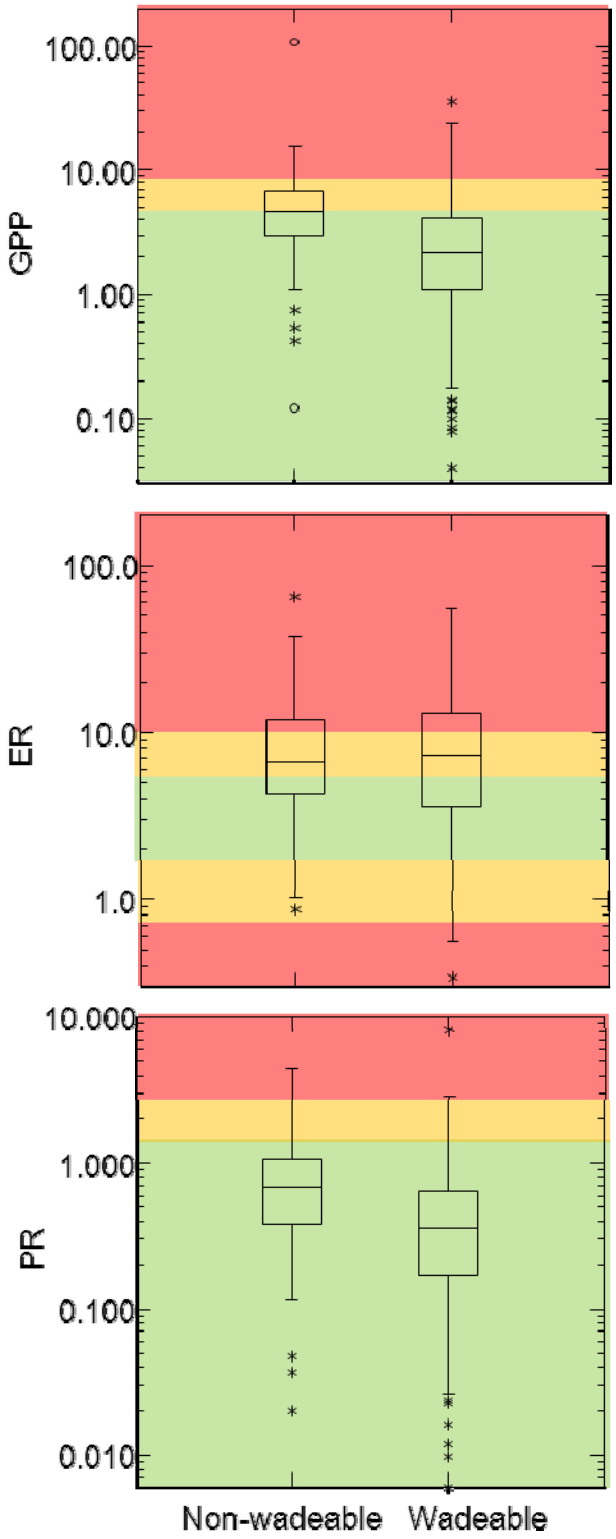

Figure 7. Range in GPP ($gO_2/m^2/d$), ER ($gO_2/m^2/d$) and PR ratios measured at non-wadeable (n = 70) and wadeable (n = 139) rivers in New Zealand collated from a variety of studies (see text for references). Colour bands represent healthy (green), satisfactory (orange) and poor (red) river health according to the recommended criteria of Young *et al.* (2008). Note the logarithmic scale on the y-axes.

Table 4. Suitability of ecosystem metabolism as a functional indicator in non-wadeable rivers.

Standardised methods	Yes – methods available for collection, calculation and interpretation of data all applicable in non-wadeable rivers
Sensitive to human impact	Yes – illustrated response to a range of human impacts
Objective	Yes – quantitative measure
Transparent	Yes – but can be conceptually difficult to communicate
Potential limitations	Requires continuous dissolved oxygen and temperature data.

4.3. Organic matter processing

Rivers are predominantly net heterotrophic, which means they consume more energy than they produce. Energy enters rivers in the form of dissolved and particulate organic matter. The rate of particulate organic matter processing provides an indication of river health and provides a direct measure of a valuable ecosystem service (breakdown of organic matter). Methods are equally applicable in wadeble and non-wadeable rivers and involve organic matter breakdown experiments using leaf litter, or wood or cotton substrates. Typically, organic matter substrates are submerged for 7-90 days after which time mass loss is used to calculate the rate of decay. Assays can be conducted in stratified habitats (*e.g.* near-shore littoral) to limit the effects of environmental variation and improve comparability between rivers.

4.3.1. Leaf packs

Litter decomposition has a demonstrated sensitivity to human impacts and activities including agriculture (Young *et al.* 1994), nutrient pollution (Woodward *et al.* 2012), urbanization (Chadwick *et al.* 2006; Imberger *et al.* 2008), coal mining (Maltby & Booth 1991; Fritz *et al.* 2010), and acidification (Hildrew *et al.* 1984). In New Zealand, litter bags were trialled as a potential functional indicator in wadeable rivers and leaf mass loss showed a broad response to land use (Young 2006a). However, this broad response made it difficult to identify potentially confounding effects of multiple stressors. Similarly, Collier *et al.* (2006) observed a wide range in leaf breakdown rates at undisturbed sites from different regions in New Zealand, suggesting universal baseline conditions would not be suitable for leaf assays. Furthermore, litter-bag assays lack standardisation. Leaf litter decomposition varies with species type, within species across regions and even among leaves from the same tree over time; this natural variation can swamp any human effects (for review see Boulton & Boon 1991).

4.3.2. Cotton-strip assays

In attempt to standardise organic matter decomposition experiments, wood and cotton substrates have been suggested as potential assays. The cotton-strip assay was first developed by the textile industry as a routine test to evaluate the effectiveness of

fungicide treatments. Loss of tensile strength of the material was measured rather than mass loss to indicate degree of decay. Applied in flowing waters, cotton-strip assays have been shown to be sensitive to an array of human impacts including urbanisation and agriculture (Clapcott *et al*. 2010), pH (Hildrew *et al*. 1984) and metal contamination (Chew *et al.* 2001). The assay can also be applied in river sediments (Boulton & Quinn 2000; Clapcott & Barmuta 2010a). Furthermore, cotton strips have been shown to be sensitive to point-source nutrient and temperature impacts in the Waikato River, suggesting this assay is sensitive to localised impacts in non-wadeable rivers (Clapcott & Young 2008). A further study of non-wadeable rivers in the Waikato region showed greater rates of cotton decomposition at sites with less native vegetation in their catchments and faster decay rates at far-shore than near-shore habitats where water velocities were different (Clapcott & Young 2009).

We collated cotton strip data from a range of New Zealand studies (Young 2006a; Clapcott & Young 2009; Clapcott *et al.* 2009; Collier *et al.* 2009a; Young & Collier 2009; JEC unpublished data; KJC unpublished data) to compare wadeable and non-wadeable rivers (Figure 8). Significantly higher rates of cotton tensile strength loss (CTSL) per day were observed in non-wadeable river sites (mean = 4.78 % CTSL/day) compared to wadeable rivers (mean = 3.29 % CTSL/day) (Pooled Variance t = 3.355, df =169, P = 0.001). The average cotton decay coefficient was significantly different between non-wadeable (mean = 0.078 k/day) and wadeable rivers (mean = 0.044 k/day) (Pooled Variance t = 3.403, df = 169, P = 0.001). A significant proportion of the non-wadeable sites were located in the Waikato River (28 of 62), however there was no significant difference between Waikato River and non-Waikato river sites.

For a selection of sites where temperature was readily available (n = 148), non-wadeable river sites were warmer (mean = 18.6 °C) than wadeable sites (mean = 15.2 °C). We compared temperature corrected rates of cotton decay coefficients (k/degree day) at these sites and results suggest temperature was a major factor contributing to the difference in cotton decomposition, *i.e*. there was no significant difference between non-wadeable and wadeable sites. It would be interesting to look at other environmental variables associated with the sites to determine whether differences were explained by other factors associated with river size or the cumulative human impacts observed in larger rivers. Meanwhile, the lack of difference between temperature-corrected rates suggests data from both non-wadeable and wadeable rivers could be combined in a valuable reference data set for the future comparison of cotton strip measures in New Zealand.

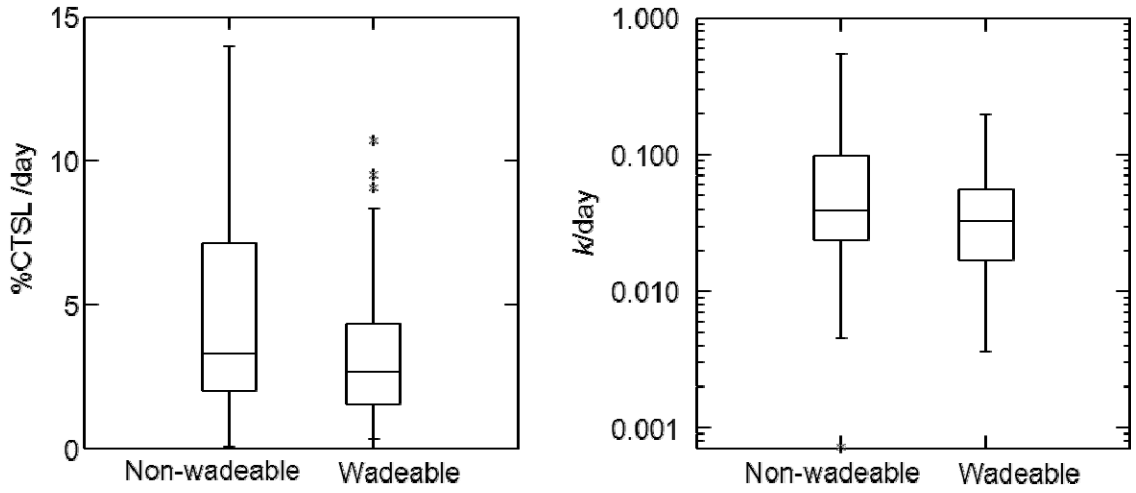

Figure 8. Range in cotton decomposition reported as cotton tensile strength loss (% CTSL/day) and decay coefficient (k/day) measured at non-wadeable (n = 62) and wadeable (n = 109) rivers in New Zealand collated from a variety of studies (see text for references). Note the logarithmic scale on the y-axes in the second graph.

Table 5. Suitability of cotton assays as a functional indicator in non-wadeable rivers.

Standardised methods	Yes – methods available for collection, calculation and interpretation of data all applicable in non-wadeable rivers
Sensitive to human impact	Yes – illustrated response to a range of human impacts, but multiple stressor responses can be confounding
Objective	Yes – quantitative measure
Transparent	Yes – easily understood
Potential limitations	Reference data sets are limited

4.4. Nitrogen processing

In rivers, key nitrogen transformations are mediated by microbes and primary producers and include assimilation (NO_3 into plant material), denitrification (NO_3 to N_2), and nitrogen uptake (ammonification: N_2 to NH_4 and nitrification: NH_4 to NO_3 and NO_2). The rate of nitrogen transformations can be indicative of the health of microbial and primary producer communities; it can also indicate the ability of the ecosystem to process excess nutrients due to human impacts. Nitrogen processes can be measured *in situ* and in laboratory assays.

4.4.1. Nitrogen uptake and denitrification

Nitrogen uptake is most often estimated in wadeable river using N addition (Newbold *et al.* 1981) or enriched N^{15} isotope addition (Mulholland *et al.* 2000). Such whole-system manipulations are generally difficult and present many challenges in non-

wadeable rivers (McCutchan *et al.* 2003, but see Tank *et al.* 2008), instead habitat-scale measures have been used to estimate whole system rates of nutrient uptake and denitrification (*e.g.* Seitzinger *et al.* 1993). The main criticisms of habitat-scale measures for monitoring purposes are artefact effects associated with core/chamber methods, high replication required, and potentially important habitats are not readily accessible for applying standardised methods.

4.4.2. Stable isotope analysis

Recently, $\delta^{15}N$ (the ratio of ^{15}N:^{14}N natural isotopes) of dissolved organic nitrogen (Kendall *et al.* 2010) sediments (Udy *et al.* 2006) and biological tissues (Diebel & Zanden 2009; Clapcott *et al.* 2010) have been trialled as indicators of the effects of human impacts on nitrogen processes in flowing water. Consistently, the $\delta^{15}N$ of biota is recommended as an indicator because it provides a temporally and spatially integrated measure of source nutrients and the degree of nutrient processing in the catchment (Lake *et al.* 2002; Fry & Allen 2003; Moore & Suthers 2005; Anderson & Cabana 2006; Udy *et al.* 2006; Diebel & Zanden 2009). $\delta^{15}N$ of biota is sensitive to land-use effects of agriculture (Anderson & Cabana 2006; Diebel & Zanden 2009), land clearance and urban development (Clapcott *et al.* 2010). The collection of biotic samples from non-wadeable rivers may be limited by availability and/or the need to stratify sampling by common taxa *e.g*. mayflies or periphyton. Several studies of $\delta^{15}N$ values of macroinvertebrates from wadeable rivers in New Zealand present a valuable dataset for future non-wadeable comparisons (*e.g.* Harding *et al.* 2004; Clapcott *et al.* 2010; JEC unpublished data; Simon Stewart, University of Canterbury unpublished data). A meta-analysis of existing data, along with data collection from non-wadable rivers, could be conducted to determine whether there is any regional variation in reference values. A recent analysis, developing a national indicator of nitrogen-loading for coastal New Zealand, reported a narrow baseline for $\delta^{15}N$ of *Ulva* spp., and significant divergence from this baseline in relation to a variety of terrestrial land-use impacts (Barr *et al.* In press).

Table 6. Suitability of $\delta^{15}N$ of biota as a functional indicator in non-wadeable rivers.

Standardised methods	Yes – further information on regional variation in reference condition is required
Sensitive to human impact	Yes
Objective	Yes – quantitative measure
Transparent	Can be confounded by multiple stressors in specific situation, *e.g.* when point source impacts are greater than diffuse pollutants
Potential limitations	Requires laboratory analysis, which is readily available in New Zealand

5. RECOMMENDATIONS AND INFORMATION GAPS

5.1. Interim recommendations

Based on the international literature review and analysis of the limited sampling conducted in New Zealand, the following interim recommendations were developed for non-wadeable rivers.

5.1.1. Macroinverebrates

1. Sampling of near-shore littoral zones in runs of unregulated gravel-bed rivers is recommended over riffle sampling to discriminate macroinvertebrate responses to land-use pressure in upstream catchments.

2. Near-shore littoral sampling for macroinvertebrates is not recommended for detecting responses to water quality at sites influenced by water level variability (*e.g.* due to flow regulation or tidal influence) or where habitat quality is constrained (*e.g.* by excessive growth by willow or riverbank engineering).

3. Habitat-specific sampling of macroinvertebrates only on large wood is not recommended for detecting responses to human pressure in non-wadeable rivers because of its patchy distribution and the influence of water level variations on colonisation potentially limiting the number of taxa collected.

4. Based on overseas studies and supported by New Zealand work, airlift sampling of deepwater habitats provides more species than dredge or Ponar sampling, and provides a cost-effective method for characterising deepwater macroinvertebrate faunas across a range of river types.

5. Based on limited comparative habitat sampling of New Zealand boatable rivers and work conducted in non-wadeable rivers of the United Kingdom, the combination of deepwater airlift sampling and near-shore littoral sampling (notwithstanding recommendation #2) is recommended to provide the greatest representation of macroinvertebrate species and the most consistent responses to human pressure.

6. Near-shore littoral and deepwater samples should be processed separately to enable habitat-specific responses to human pressure to be evaluated.

7. Sampling from a boat is recommended where access to suitable habitats in flowing water is restricted to ensure near-shore littoral and deepwater samples

can be collected from a range of lotic sites (*cf* lentic marginal habitats) along study reaches.

8. The use of artificial substrates deployed from riverbanks may provide an alternative approach for characterising near-shore macroinvertebrate communities, in particular to compare water quality responses among sites with contrasting natural substrate composition or direct comparison of point source discharges, but require two site visits over an extended period (6-8 weeks apart) and the risk of loss of substrates.

5.1.2. Functional indicators

1. Sampling of ecosystem metabolism using the single-station dissolved oxygen method is recommended to discriminate land-use pressure in upstream catchments. Dissolved oxygen loggers should be deployed in flowing water, often established hydrology sites are suitable.

2. Two-station dissolved oxygen method is recommended when the objective is to assess ecosystem metabolism in relation to localised land-use change, e.g. in response to riparian rehabilitation at a reach scale or point source impacts.

3. Benthic metabolism is not recommended as a routine functional indicator in non-wadeable rivers.

4. Cotton strips assays are recommended as a functional indicator of organic matter decomposition potential. Deployment time (between 7 and 28 days) should be optimised to ensure approximately 50 % tensile strength loss. Strips should be deployed in the near-shore littoral zone and weighed down to be in vicinity of the river bed, but not buried in sediment.

5. There are currently no developed indicators of nitrogen processes for non-wadeable rivers. $\delta^{15}N$ of primary consumers, Deletidium, mayfly in particular, shows great promise (and could be a simple add on to macroinvertebrate monitoring), but requires further research on baseline variability.

5.2. Gap analysis

The following information gaps have been identified as being required to progress the development of macroinvertebrate and functional indicator monitoring protocols for New Zealand non-wadeable rivers.

- What intensity of sampling is required to characterise macroinvertebrate communities in deepwater habitats for rivers with different substrate size?
- How many replicate samples and what sampling effort are required to provide an acceptable level of precision for non-wadeable river biomonitoring purposes?
- What macroinvertebrate metrics reflect different types of human pressure and how do these vary among target sampling habitats?
- What is the role of natural environmental variability, including latitude and source of flow (*e.g.* spring-fed), on non-wadeable river macroinvertebrate metric responses?
- How can macroinvertebrate metrics be benchmarked (*e.g.* against reference sites) to determine the magnitude of impacts?
- Are there natural environmental factors that can explain the difference between ecosystem metabolism of wadeable and non-wadeable rivers, and if so, can factors be used as covariates in assessments?
- What is the reference baseline for cotton decomposition in non-wadeable rivers and does it vary across a natural environmental gradient, if so, are regional reference conditions required?
- What is the reference baseline for $\delta^{15}N$ of primary consumers in non-wadeable rivers and does it vary across a natural environmental gradient, if so, are regional reference conditions required?

5.3. Implications for Marlborough District Council

Based on the above analysis, the following responses were developed to questions posed by Marlborough District Council (MDC):

1. **How useful is the current sampling regime and does it need adjusting?**

- MDC non-wadeable rivers include gravel-bed braided rivers that are too deep and fast-flowing to cross, and spring-fed streams that are too deep to wade and with steep banks. In braided rivers, near-shore littoral sampling of gravel substrates in the main channel is preferable to riffle sampling. No information is currently available on non-wadeable spring-fed rivers (see Section 5.2, Gap analysis).
- MDC does not currently monitor ecosystem function. To date, the National Environmental Monitoring and Reporting (NEMaR) process does not recommend the inclusion of functional indicators for SOE reporting, however, ecosystem metabolism is listed as a secondary variable that can be retrospectively added when continuously dissolved oxygen data are available (Hudson *et al.* 2012). As noted in this review, a complete picture of ecosystem health/integrity is not obtained without knowledge of ecosystem function. Functional indicators are currently being tested for ease of application and maintenance in non-wadeable

rivers in three regions in New Zealand. We recommend the deployment of continuous dissolved oxygen loggers in MDC. Data from spring-fed streams will be directly comparable to existing datasets.

2. **How can we incorporate biological monitoring at non-monitored sites?**

- In the absence of standardised non-wadeable river macroinvertebrate sampling protocols, no recommendations can be provided regarding the development of specific non-wadeable river monitoring methods. However, as an interim measure based around the LR-BP method, near-shore littoral samples could be collected at 5-6 sites along 500 m long reaches (not affected by flow regulation or tidal cycles) to provide data for future analysis, assuming that similar sampling would be a component of future protocols. To inform this process, standardised sampling could be conducted at a range of sites with contrasting levels of pressure and accompanying water quality and hydrological data.
- Dissolved oxygen loggers can be deployed at flow gauging sites. Organic matter decomposition studies (*e.g.* cotton strip assays) are a relatively cost-effective method for monitoring the effect of both diffuse and point source impacts on ecosystem function in non-wadeable rivers.

3. **How do we know if it is nationally comparable?**

- The only nationally comparable macroinvertebrate dataset for non-wadeable rivers is that of Collier *et al*. (2012). The NEMaR process has highlighted the national need for non-wadeable river monitoring protocols, and any national standard will not be known until this work has been done.
- Initial meta-analysis provided in this report suggests cotton strip assay results are directly comparable between wadeable and non-wadeable rivers; for metabolism, potential natural variability warrants further investigation; for $\delta^{15}N$ of primary consumers, national baseline (and potential regional variability) is unknown. Frameworks for assigning benchmark criteria exist for all methods, but require validation.

4. **Are we using the best/most robust approach available?**

- The above analysis indicates that for single-channel, low-gradient non-wadeable rivers sampling of both marginal and deepwater habitats is required to adequately characterise macroinvertebrate community composition. Addition of airlift sampling would enhance the quality of the macroinvertebrate data obtained from non-wadeable rivers but would require purchase of specialised equipment and greater investment of effort and money.

5. **Do non-wadeable rivers differ enough to suggest that different environment types require different monitoring tools?**

- There is some evidence that different monitoring tools are required for sites influenced by flow variability (*e.g.* through tidal influence or dam operations), and that macroinvertebrate metrics in different habitat types may respond differently to prevailing pressures. While sampling of near-shore littoral areas in runs of moderate-gradient, gravel-bed rivers may be sufficient to characterise macroinvertebrate community responses, more complex influences affect responses in deep, single-channel, low-gradient rivers, including littoral substrate type and benthic substrate size composition. The work by Collier *et al.* (2012) also highlighted a potential influence of underlying natural environmental variation, including latitude, on macroinvertebrate community composition, suggesting that regional benchmark levels may need to be derived for non-wadeable river macroinvertebrate metrics once they are developed.
- The advantage of functional indicators is that they are not habitat limited and can be applied in both wadeable and non-wadeable rivers. However, calculation of ecosystem metabolism metrics can be difficult in large braided rivers where a lot of hyporheic exchange occurs or in very shallow reaches where estimates of re-aeration are difficult. Clapcott *et al.* (2010) demonstrated how patterns in ecosystem metabolism (GPP and ER) and cotton strip decomposition responded more to land-use pressure than regional variation in wadeable rivers. Similarly, Collier *et al.* (2009a) demonstrated a predictive relationship between land use and GPP in non-wadable rivers. These studies suggest that functional indicators could be applied confidently across different environment types in New Zealand.

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APPENDICES

Appendix 1. Regional council responses to a survey of current monitoring practices in non-wadeable rivers

Below are the collated responses from contacts who responded to a short questionnaire, using the following definition of a non-wadeable waterway:

"A waterway where water depth, velocity, and/or clarity restrict or prevent the sampling of biota from representative habitats using standard protocols."

Question 1. Do you have an alternative written definition or criteria which you use to identify a non-wadeable waterway in your region? If so, please quote.

Respondent	Response
1	No
2	No
3	No we don't.
4	No
5	No, but should it include a proportion of the waterway? Many of our rivers and streams have non-wadeable portions and in some of the lower reaches of our larger rivers large sections can be non-wadeable – however, given with inverts (and these are typically hard-bottomed rivers) we are targeting riffle type habitat we can normally find somewhere to sample…although admittedly at times, in the lower reaches of our largest river (the Ruamahanga) these riffles aren't always the best riffles…And is clarity a separate issue from 'non-wadeable'?
6	Sounds good. But is substrate not a part of it? Some large rivers do have somewhere where macros can be sampled *i.e.* the Grey River has big cobbly riffles ideal for macro sampling. Though it may not always be easy to find a suitable spot in a reach that you want to sample from. Comparing the Grey to the lower reaches of the Whanganui River (North Island), where there are less cobbly riffles and there is more boulder/sediment substrate: the problem in this case is more one of suitable sampling habitat, rather than access. Boulders and mud can be sampled but if sampling is for water quality assessment, it provides less than ideal substrate to sample.
7	No, as stated above – restricted by depth/velocity mostly.
8	No
9	No
10	The above adequately describes the term. In Marlborough it would cover braided rivers which are too deep and fast flowing to cross, however sampling of shallow riffles is possible using the standard protocols for wadeable streams, it would also cover spring fed streams which are too deep to wade and where the banks are steep, however sampling is sometimes possible in these streams using a 'sweep net' approach.
11	No we do not have any alternative definition
12	

Question 2. Approximately, what proportion of the total number of biological state of the environment (SOE) monitoring sites in your region would be considered non-wadeable according to the definition provided above (*e.g.* 2 of 50)?

Respondent	Response
1	None – all of our biological SOE sites are wadeable. This is one of the criteria used in selecting sites. Some of the SOE sites where water quality and/or functional measures (Ecosystem Metabolism) are assessed would be considered non-wadeable.
2	Zero. All of our SOE monitoring sites for macroinvertebrates are wadeable under base flow conditions. We don't have any sites in non-wadeable streams because we can't do them physically. If these rivers did drop low enough during droughts they would get sampled for drought monitoring.
3	'Council' has no such sites in its SOE pgm but some artificial substrates were used 30-40 years ago in the deeper reaches of the Patea R now under the waters of Lake Rotorangi
4	If the site is a biological SOE site then it is sampled. We don't sample non-wadeable sites although of course we do sample a large number of sites for water physico-chemistry but these tend to be independent of the biological sites
5	We undertake biological sampling at all 55 of our SOE sites. However, approximately 7 out of the 55 could be considered borderline non-wadeable. These seven sites are where I would feel uncomfortable about attempting to try and wade the entire width of the river/stream. Six of these seven 'non-wadeable' sites are on relatively small, soft-sediment streams with deep, incised channels and five are quite 'coastal' in location. We sample these streams using protocol C2 and by moving carefully in and along the margins of the streams. We can generally sample the types of habitat mentioned in the protocol if these are present *e.g.* macrophytes, bank margins and woody debris.
6	We can wade all our SOE sites, even the Grey and Buller (210 and 245 cumecs median flow). This is not a boast but I suspect what constitutes non-wadeable depends to some extent on individual approaches. Is non-wadeable something you can't wade across from one side to the other, or a river that you can't get any reasonable access to at all? We certainly could NOT wade right across the Buller or Grey but we can wade enough to get an appropriate macro sample. Approx. 0 of 50
7	Approximately 7 out of 75 sites
8	Zero
9	0 of 56
10	9 of 51
11	We do not have any biological SOE monitoring sites
12	

Question 3. Do you conduct macroinvertebrate or functional indicator monitoring in non-wadeable rivers for reasons other than SOE monitoring, and if yes, at how many sites (*e.g.* compliance monitoring of eight sites – invertebrates (5), metabolism (3))?

Respondent	Response
1	No
2	No.
3	No
4	Yes – as part of a periphyton model development programme (nutrients-flow and periphyton relationships) we have also sampled macroinvertebrates in some "Alpine-source of flow" sites. We have a total of 24 sites of which about seven are Alpine. Of the Alpine sites we only sample up to 60 cm depth and in high flows this tends to mean that only the edge of the site is sampled.
5	Functional indicator – no, we don't do any type of functional indicator monitoring…although we might need your definition of functional indicator to be sure. We are collecting continuous measurements of dissolved oxygen at a few sites each year, although as yet we have not used them in a 'functional indicator' approach (that I am aware of). Invertebrates – not really, compliance monitoring is undertaken by consent holder and none springs to mind. I could see that we might sample these types of non-wadeable environments on occasion through investigations *etc.*
6	No, Cawthron trailed functional indicator monitoring in the lower Hokitika using temperature.
7	No
8	Yes, to test the suitability of ecological indicators for non-wadeable river health. Over the last three years we have investigated 30 non-wadeable sites for river metabolism, habitat quality and macroinvertebrates, and in addition have sampled macroinvertebrates from 21 sites on the Waikato River
9	No
10	No
11	No
12	

Question 4. When, and how often, do you collect macroinvertebrate or functional indicator monitoring data from non-wadeable waterways and are locations comparable between years (*e.g.* annually in February)?

Respondent	Response
1	Macroinvertebrates – never. Continuous DO measurements are collected from 13 permanent sites, of which five would be considered non-wadeable.
2	Yearly. In summer when conditions allow.
3	
4	As stated above, we don't
5	Only inverts, no functional indicators. Annually, our sites are constant between years (except we did shift one of our potentially non-wadeable sites upstream to get away from the odd tidal influence). Generally attempt to sample in late summer (Jan, Feb and March), although these days we are struggling to get enough periods of stable river flows and sampling can occur in April.
6	Not applicable
7	Annually in summer, usually in the same month plus or minus one month.
8	Annually (2009-11) in December at different (randomly selected) locations in each year
9	Never
10	Samples collected annually, usually in spring/summer but the sampling season can run into autumn
11	Not applicable
12	

Question 5. When undertaking macroinvertebrate sampling of non-wadeable waterways for SOE monitoring purposes, what habitats do you collect samples from and what methods do you use to collect them (*e.g.* sweep netting; please also include details of area and/or time sampled, net mesh size used)?

Respondent	Run	Riffle	Pool	Macrophytes	Edge	Littoral vegetation	Wood	Other? Please specify.
1								
2								Not applicable as we don't sample unwadeable streams
3								
4								As stated above, we don't
5	Yes			Yes	Yes	Yes	Yes	
	I would generally describe the bulk of the habitat at these six of these seven sites as being 'run'. And we follow protocol C2, although we are limited to the habitat that we can safely access which is generally along the edges of the channel. However, in most cases I feel we are typically getting a pretty representative sample from the habitat types identified in Protocol C2 (macrophytes, bank margins and woody debris) and that not too much would be gained by access to the middle of the channel… (Of course this not my area of expertise). Mesh size is 0.5 mm.			Sweep/jab netting	Sweep/jab netting	Sweep/jab netting	Use hand to wipe off or scrub off anything from the wood into the net	

Respondent	Run	Riffle	Pool	Macrophytes	Edge	Littoral vegetation	Wood	Other? Please specify.
6								
7	Yes	Yes	Yes	Yes	Yes	Yes	Yes	
	Kick net (1 mm mesh) margins of river until it gets too deep/swift to sample	Kick net (1 mm mesh) margins of river until it gets too deep/swift to sample	If present, kick net	If present, 3 x 1 m long sweeps	If present ,3 x 1 m long sweeps	If present, 3 x 1 m long sweeps	If present, 3 x 1 m long sweeps	Our non-wadeable rivers are mostly gravel/cobble substrate riffle/runs so we kick net the edges until if become unsafe to do so.
8								
9								
10	Yes	Yes	No	No	Yes	Yes	No	
	3 min kick net, 3 min sweep net	3 min kick net			3 min sweep net	3 min sweep net		
11								
12								

Question 6. When undertaking macroinvertebrate sampling of non-wadeable waterways for reasons other than SOE monitoring, what habitats do you collect samples from and what methods do you use to collect them (e.g. sweep netting; please also include details of area and/or time sampled, net mesh size used)?

Respondent	Run	Riffle	Pool	Macrophytes	Edge	Littoral vegetation	Wood	Other? Please specify.
1								
2								Not applicable.
3				Yes	Yes			
				Sweep from bank	Artificial substrate			
4								As stated above, we don't
5								None taken – if we did, would be as above.
6								
7								N/A
8	Yes			Yes	Yes	Yes	Yes	
	Airlift			Sweep	Sweep	Sweep	Sweep	
9								
10								
11								
12								

Question 7. What functional monitoring methods do you currently employ in non-wadeable rivers?

Respondent	Reason	Ecosystem metabolism	Cotton strip assay	Organic matter decomposition	Biological Oxygen Demand	Organic matter retention	Other? Please specify.
1	SOE Other	Yes No	No No	No No	No No	No No	
2							
3	SOE Other				No Yes		
4							
5	SOE Other						As mentioned above, we are collecting continuous measurements of dissolved oxygen at a few sites each year, although as yet we have not used them in a 'functional indicator' approach (that I am aware of).
6							
7	SOE Other	No	No	No	Yes At some sites monthly (7 out of 72 WQ sites)	No	
8	SOE Other	 Yes	 Yes				
9							
10							
11							
12							

Question 8. What habitat information do you collect concurrently with macroinvertebrate or functional indicator sampling at non-wadeable sites (*e.g.* depth at five points across five transects over 500 m for metabolism)?

Respondent	Response
1	Depth, land use and riparian characteristics (from Stream Ecological Valuation)
2	Not applicable.
3	Not applicable
4	Not applicable
5	Estimates of different proportions habitat sampled: macrophyte cover (species), bank vegetation, woody debris *etc.* General estimate of macrophyte cover. Water clarity.
6	A technique not mentioned for potential use in non-wadeable rivers (or rivers with unsuitable habitat for sampling via conventional methods) is the use of artificial substrates. These provide standardized area and surfaces, are stable (if anchored properly), and depth, velocity can also be standardised with careful site selection. I've found them highly effective for water quality monitoring in large rivers with sub-optimal substrate for sampling.
7	Riffle depth, max depth, channel width, macrophyte % cover, cyanobacteria presence and % cover, habitat sampled (pool, riffle, run, woody debris, macrophytes *etc.*), water level, shaded/open site.
8	Functional indicators – depth at five points across five transects over 500 m. Macroinvertebrates – depth and substrate type. Occasionally dissolved oxygen, temperature, conductivity and turbidity.
9	
10	Rivers are non-wadeable so no depth measurements are taken. Habitat assessments using a mix of SHAP protocols 1 and 2 are carried out. Visual assessments only made from the bank.
11	Not applicable
12	

Question 9. Other feedback

Respondent 11

Please note we don't undertake biological monitoring in the Region 11.

Respondent 12

Questionnaire not completed, see below.

I've had a quick look at the questionnaire and I can't really answer any of the question - we do not currently monitor non-wadeable reaches of river *e.g.* the lower Whanganui *etc.* (we obviously can monitor the headwaters *etc.* as they are wadeable) because we are not able to be representative of the habitat that is present and therefore we would be collecting data that would not be representative of what was going on in the system.
We would love to start sampling or be involved in trialling monitoring techniques for some of these non-wadeable sections of rivers if there were to be developed national protocols.