Title	Establishing the Citizen Science Stream Index (CSSI) to monitor water quality in freshwaters
Authors	McSorley, Brendan
Publication date	2022-03-01
Original Citation	McSorley, B. 2022. Establishing the Citizen Science Stream Index (CSSI) to monitor water quality in freshwaters. MSc Thesis, University College Cork.
Type of publication	Masters thesis (Research)
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Download date	2025-07-05 07:51:49
Item downloaded from	https://hdl.handle.net/10468/14061

Establishing The Citizen Science Stream Index (CSSI) to Monitor Water Quality in Freshwaters

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A thesis submitted to the University College Cork in fulfilment of the requirements for the degree of Master of Science

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March 2022

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Declaration

This is to certify that the work I am submitting is my own and has not been submitted for another degree, either at University College Cork or elsewhere. All external references and sources are clearly acknowledged and identified within the contents. I have read and understood the regulations of University College Cork concerning plagiarism.

Acknowledgements

Abstract

Streams and rivers are amongst the most endangered ecosystems in the world. Water quality is an important measure for maintaining ecosystem function. Despite several decades of the EU Nitrates and Water Framework Directives, inputs of nutrient-rich organic matter of both agricultural and municipal origin continue to pollute many waterways in Ireland, most of which are not routinely monitored in terms of water quality. This lack of data hampers efforts to improve water quality. Citizen science projects involve non-experts contributing to scientific data voluntarily and have been identified by the EU as a growing field of practice that is likely to yield significant outcomes for water quality and data capture.

In this thesis a biotic index called the Citizen Science Stream Index (CSSI) was established using a principal component analysis of an EPA data set of macroinvertebrates. A further analysis was made using the provided Q-Values in this data set to find the most indicative macroinvertebrates for a citizen science index. The CSSI uses six easily identifiable and common benthic macroinvertebrates with narrow pollution tolerances that indicate water quality, to give a rapid indication of the ecological status of a stream in a sampled area. The CSSI is an easily taught and simple to use biomonitoring index that enables non-experts to identify where pollution has affected the macroinvertebrate community. The protocol involves taking a thirty second kick sample and checking it for the presence or absence of the six taxa, giving a score from -3 to +3. This is repeated three times and the resulting three scores are summed to give a CSSI score between -9 and +9. From this score the sampler can band the water quality of the stream into three water quality bands, red (poor), orange (moderate) and green (good).

This thesis validates the CSSI's indicator taxa, protocol and scoring system by using multiple data sets with varying spatial distribution, water quality and seasonality, comparing the CSSI with contemporary metrics such as the EPA Quality-Values (Q-Values), the Biological Monitoring working Party's (BMWP) Average Score Per Taxon (ASPT) and the Small Stream Risk Score (SSRS). A pilot study to further test the quality, accuracy and feasibility of the index in the field was carried out on the Nore River catchment with volunteers from the NoreVision project.

It was found that the CSSI compared sufficiently with the contemporary metrics tested and provided accurate results in the field study. Therefore, it is fit for purpose as a rapid biomonitoring citizen science index. The CSSI is currently being rolled out in volunteer initiatives around Ireland. The CSSI has received a positive response from participants and provided consistently reliable data capture when compared to existing data points thus far.

1.Introduction

Streams and rivers are amongst the most endangered ecosystems in the world. Water quality is an important measure for maintaining ecosystem function (O'Boyle et al., 2019). Despite several decades of the EU Nitrates and Water Framework Directives, inputs of nutrient-rich organic matter of both agricultural and municipal origin continue to pollute many waterways in Ireland, most of which are not routinely monitored in terms of water quality. This lack of data hampers efforts to improve water quality.

1.1 Water quality

In general water quality can be described as the suitability of water for a particular use such as drinking, recreation or irrigation. In regard to river water, the term 'water quality' can be used to refer to different aspects based on selected physical, chemical and biological characteristics. Turbidity, temperature, total solids, conductivity, dissolved oxygen, levels of phosphorus, nitrates, pH, total alkalinity, faecal bacteria, biochemical oxygen demand (BOD) and macroinvertebrate biodiversity for example. By using these types of measurements, standards can be set and changes in water quality can be monitored to maintain healthy and safe water (Rolston and Linnane, 2020).

In Ireland there are over 84,000km of waterways but only 13,200km (16%) of these are surveyed over a three-year cycle (Toner et al., 2006, Clabby et al., 2008, O'Boyle et al., 2019, Feeley et al., 2020). Stream order is a measure of the relative size of streams. The smallest tributaries are referred to as first-order streams, when two streams with the same order join, the resulting stream is categorised at the next highest order. First, second and third order streams are usually called headwater streams. The majority of the rivers surveyed in Ireland are third order rivers, yet 77% of Ireland's

waterways are first and second order headwaters (McGarrigle, 2014). This lack of monitoring could result in polluting inputs going unnoticed and not amended.

From the monitoring of Ireland's waterways that is being done it is clear that urbanisation, arable farming and extent of pasturelands are the principal pressures, at the catchment scale, that impact on the ecological quality of streams and rivers throughout Ireland and Europe (Trodd and O'Boyle, 2020). As a result, the European Union introduced directives for the improvement of water quality in all European waterways and the surrounding catchments such as the Water Framework Directive (WFD) (Teodosiu et al., 2003, the Environmental Quality Standards Directive (EQSD), (Directive 2008/105/EC), the Groundwater Directive (GWD), (Directive 2006/118/EC), the Floods Directive (FD), (Directive 2007/60/EC) and the Nitrates Directive (ND), (Council Directive of 12 December 1991 (91/676/EEC)). These ambitious directives aim to bring in a new era for European water management, focusing on understanding and integrating all aspects of the water environment, to be effective and sustainable. Despite the fact that many of these initiatives have been in place for many years, it is clear that the water quality of Europe's rivers has been declining for a number of years (Trodd and O'Boyle, 2020, Yoshimura et al., 2001, Malmqvist and Rundle, 2002, Saunders et al., 2002, Wolfram et al., 2021), suggesting that a change is necessary. In order to improve water quality, the identification of pollution must be improved. One non-traditional source of data collection shown to be useful at complimenting official data sources is citizen science (König et al., 2020).

1.2 Pollution

Water pollution is the release of substances into waterways to the point where the substances interfere with the beneficial use of the water or with the functioning of natural ecosystems (Nathanson, 2021). Water can be polluted by many different sources including domestic wastes, insecticides and herbicides, food processing waste, livestock operation outputs, volatile organic compounds (VOCs), heavy metals, chemical waste and others (Iyyanki et al., 2017). These substances are described as pollutants. The prevention and amending of anthropogenic pollution are the main purposes of most river monitoring initiatives and directives.

Water pollution is described as coming from a point or a non-point source (Schultz and Edwin, 2000). Point sources are associated with pollutants that can be traced to a single source, such as an outflow pipe or runoff coming from a farmyard entering at one point (Nathanson, 2021). Once a point source has been identified, managing the effluent from the point source requires a commitment of time and money but can be tended to by government officials or the owner of the land. If identified these sources of pollution can be reported to government officials in Ireland such as the Local Authority Waters Program (LAWPRO) or Agricultural Sustainability Support and Advisory Programme (ASSAP). Non- point sources are more diffuse and associated with the landscape and its response to water movement and land use (Nathanson, 2021). These types of pollution are more difficult to pinpoint but can still be identified and rectified in a number of different ways such as instituting natural wetlands and changing farming practices (Scholz, 2016).

1.3 Biomonitoring

Physical and chemical analysis can detect pollutants in the water, but this type of analysis is not always possible as it is often time-consuming, cost-intensive and dependent on specialist instruments (Aazami et al., 2015). Petchy and Gaston described biomonitoring as the quickest and most cost-effective way of accurately identifying pollution (Petchey and Gaston, 2006), although this is not necessarily the case in all situations.

Biomonitoring is the act of monitoring and assessing ongoing changes in ecosystems, components of biodiversity and landscape including the types of natural habitats, populations and species (Bondaruk et al., 2015). Biomonitoring allows monitoring of an environment without the need for chemical and physical analysis and can often provide an accurate and rapid view of a river's ecosystem (Moog et al., 2018, Polatera et al., 2001, Cairns and Der Schalie, 1980, Cairns and Der Schalie, 1998, Matthews et al., 1982, Herricks and Cairns, 1982, Buikema et al., 1982, Cherry and Cairns, 1982).

Biomonitoring is routinely used in many different fields of science. For example, monitoring of lichens and bryophytes indicate air pollution (Pescott et al., 2015). Indicator species are also used to identify ancient woodlands requiring protection which are a priority for conservation (Abe et al., 2021). Multiple different fungal communities are monitored to detect nitrogen compounds (Trundell and Edmonds, 2004, Trundell et al., 2004), and indicator fungi also predict declines in forests (Mair et al., 2017). The migration of indicator birds help to monitor climate change (Sullivan et al., 2009). These projects use the presence or absence of indicator organisms with narrow tolerances for environmental stressors, to deduce the ecological state of an ecosystem in question without sampling and quantifying the whole ecosystem.

River biomonitoring on the other hand usually involves investigating the benthic community on the riverbeds such as fish, algae, plants, and other periphyton (Li et al., 2010). River biomonitoring is a well-established practice to detect pollutants such as: organic pollution (Armitage et al., 1983, Rae, 1989, Zamora- Muñoz and Alba-Tercedor, 1986), heavy metals (Winner et al., 1980, Smolders et al., 2003, Poulton et al.,1995), nutrient enrichment (Hellawell, 1986, Hynes, 1960, Hellawell, 1978, Hering et al., 2006, Johnson et al., 2006), hydromorphological degradation (Lorenz et al., 2004, Friberg et al., 2009) and acidification (Sandin and Johnson, 2000, Braukmann, 2001, Sandin et al., 2004, Davy-Bowker, 2005). It should be noted that different indices are used to test for different pollutants. For example, very different indices are used to test for heavy metals than for organic/nutrient pollution.

Using biotic organisms to detect the above stressors has been demonstrated in many studies. Three of the most common organisms used to detect these stressors are periphyton (Coste et al., 2008, Whitton, 2013, Vis et al., 1988, Whitton and Rott, 1996), benthic macroinvertebrates (Statzner et al., 2001, Rosenberg and Resh, 1993, Buffagni et al., 2004, Bhadrecha and Khatri, 2016) and fish (Joy and Death, 2002, Pont et al., 2006, Oberdorff et al., 2002). Of these three, macroinvertebrates are the easiest and most reliable organisms to use in a citizen science initiative.

1.4 Macroinvertebrates

Macroinvertebrates are defined as any animal lacking a backbone and large enough to be seen without the aid of a microscope (Michaluk, 2019). Macroinvertebrates are part of almost every freshwater ecosystem in the world, even those that are seemingly inhospitable to life. They form the base of the aquatic food chain serving as a source of food for other animals (Michaluk, 2019). By participating in the breakdown of both living and dead plant material in freshwater ecosystems, they transfer plant material into forms of energy that can be consumed by other aquatic animals (Callisto et al., 2001). Alone they are the most common and widely used biomonitoring organisms for rivers. Using macroinvertebrates to indicate water quality is a well-established technique that was first documented in 350 BC by Aristotle (Moog et al., 2018). Many biotic indices have been established based on macroinvertebrate sampling as they are nonmigratory, spending all their lives in a small area, are easy to collect using a kick net and differ in their tolerance to polluted environments where pollutants have changed percentage dissolved oxygen, pH or increased harmful chemical levels for example (Tampo et al., 2021).

1.5 Historical river biomonitoring

The first published paper noting the relationship between organic pollution and river fauna came from two European researchers A. H. Hassal, London in 1850 and F. Cohn, Breslau around the mid-1800s as a result of a severe cholera outbreak (Moog et al., 2018). Around 1900, two German scientists R. Kolkwitz and M. Marsson developed the saprobic system to determine the state of a river using macroinvertebrates where saprobic organisms are found in waste waters and katharobic organisms are found in clean rivers (Kolkwitz and Marsson, 1902). This saprobic system was later adopted by

Liebman after World War II who listed indicative species and "colour branded" the quality and ecological status of the river (Liebmann, 1951). The Trent Biotic Index (TBI) did not follow the saprobic system but derived water quality from a mix of the presence or absence of certain indicator species and the number or diversity of taxa (or groups) of organisms present (Woodiwiss, 1964). The TBI made findings more understandable to non-biologists by presenting them in numerical form as an index value or score. Although some biologists were sceptical about expressing complex biological communities as a single numerical value (Hawkes, 1956; Hynes,1960), this proved accurate and was followed by others like the Indice Biotique (IB) (Verneaux and Tuffery, 1967), Chandler Biotic Score (Chandler, 1970) and the Department of the Environment (DOE) classification (Department of the Environment and Welsh Office, 1971).

Today, there are many different biomonitoring protocols and indices in use that classify and score the hundreds of macroinvertebrates and other organisms to indicate water quality. Commonly used biomonitoring approaches include biotic indices, multi-metric approaches and multivariate approaches, functional feeding groups (FFG) analysis and multiple biological traits analysis. Among these techniques, biotic indices, multivariate approaches and multi-metric approaches are most frequently used to evaluate the environmental state of streams and rivers (Li et al., 2010). FFG analysis and multiple biological trait analysis which can detect more subtle changes in the aquatic community structure than would be apparent from biotic indices are used less often.

1.6 Biotic indices

A biotic index is a scale for showing the quality of an environment by using the sensitivity or tolerance of organism collections within that environment (Marques, 2008). This results in a numerical expression referred to as a score. Within freshwater river biomonitoring, the relationship between water quality and the macroinvertebrate community is usually described by one numerical value or score. Although such a compression of biological information, inevitably results in a loss of information this compression is generally regarded as a necessary method to indicate the water quality of a river in simple terms to non-professionals (Hawkes, 1998, Toner et al., 2006).

Many macroinvertebrates show a narrow tolerance range of water quality that they can live in, so their presence or absence is a particularly good indicator of water quality (Krabbenhoft and Kashian 2020). Different biotic indices use different methods of achieving a score to classify the populations of macroinvertebrates at that sampling point. A score can be generated, for example, as an average of scores of several indicators to reflect an index (Lamberti, 2017). The principle of biotic indices is to assign different types of taxa to different levels of anthropogenic disturbance. Sensitive taxa decrease or disappear, and tolerant taxa emerge or increase under stress (Moog et al., 2018).

1.6.1 The Biological Monitoring Working Party (BMWP)

The Biological Monitoring Working Party (BMWP) is a biotic index that forms the basis for most of the currently used biotic indices (Moog et al., 2018). In 1976 The Biological Monitoring Working Party, the namesake of the index, was set up by the

British Department of the Environment in response to criticism of the 1970 National River Pollution Survey which used biomonitoring but found that the type of river also affected the benthic community not just the pollution levels. To overcome this, the working group of 11 members set up a standard scoring system that successfully classified relationships between the macroinvertebrate communities and the environmental factors of the river (Biological Monitoring Working Party, 1978; Hawkes, 1998; Armitage et al., 1983). This index proved accurate and useful in many regions although it required certain adaptions depending on the region (Barton and Metcalfe-Smith, 1992, Zamora-Munoz and Alba-Tercedor, 1996, Capitulo et al., 2001, Mustow, 2002, Czerniawska, 2005). The BMWP system considers the sensitivity of invertebrates to pollution; families are assigned a score between 1 and 10 with 1 being the most tolerant of pollution and families assigned 10 the least tolerant to pollution. The BMWP score is the sum of the values for all families present in the sample. Scores greater than 100 are associated with clean streams, while the scores of heavily polluted streams are less than 10 (Mason, 2002). The BMWP system requires the ability to identify all the families of macroinvertebrates in a river system (Biological Monitoring Working Party, 1978, Department of the Environment, 1976; Barbour et al., 1999).

Index scores are largely affected by the number of scoring taxa in a sample, as this is usually dependent on the sample size, sampling technique and sample processing efficiency. To overcome this variation, it was proposed that the index score should be divided by the number of contributing taxa providing an average pollution tolerance of all the families of organisms present in a sample. This is known as the Average Score Per Taxon (ASPT) and can be determined by dividing the index score by the number of scoring taxa present (Hawkes, 1998). A high ASPT score is considered indicative of a clean site containing large numbers of taxa indicationg good water quality. The ASPT

has been shown to diminish the possible impact of natural seasonal differences in the levels of organisms (Roche et al., 2010, Armitage et al., 1983).

1.7 Multivariate approach

The measurement of different biomonitoring organisms can be combined with each other, and with chemical, physical and bacteriological tests. This is called multivariate approach and gives a very thorough analysis of river water quality. Since this is an intensive investigation involving high levels of training, specialist equipment and prior knowledge of the river ecosystem, which is costly in terms of economy and workforce, it is often focused at one sample point, that is usually assigned a single numerical score. This compression of information makes such a thorough analysis inefficient and unlikely to be used to test a large number of first and second order headwaters. In other words, intensive small-scale sampling is useful but spatially extensive large-scale sampling would provide more data on where currently unmonitored streams are being polluted (Toner et al., 2006, Metcalfe, 1989).

1.7.1 The Quality rating (Q-Value)

The Environmental Protection Agency in Ireland use a Quality rating or Q-Value system to classify Irish streams. A Q-value is a multivariate approach used to standardize different water quality attributes so that they can be combined to find an overall water quality value for the river. The Q-Value investigates benthic macroinvertebrates by dividing them into five arbitrary 'Indicator Groups' as follows: Group A, the sensitive forms, Group B, the less sensitive forms, Group C, the tolerant forms, Group D, the very tolerant forms and Group E, the most tolerant forms as shown in Figure 1B For the assessment of organic pollution, the more commonly measured

physio-chemical parameters include dissolved oxygen (DO), biological oxygen demand (BOD), ammonia, oxidised nitrogen (nitrites plus nitrates) and phosphates. In practice this is impossible for financial, technical and logistical reasons (Toner et al., 2005). The Q value instead evaluates five, more discrete, physio-chemical "additional qualifying criteria", namely: Cladophora spp. abundance, macrophytes (typical abundance), lime growths (sewage fungus), dissolved oxygen saturation and substratum siltation. These five criteria have allowances for each Q-Value band Q1-Q5. An overall assessment of all these criteria makes up the final score of the stream from Q1 to Q5 as shown in Figures 1A and 1B (Toner et al., 2005). This is a water quality index used by governmental agencies in Ireland and so can be used as a reference standard for water quality monitoring but requires a high level of training and a detailed knowledge of macroinvertebrate identification to carry it out.

Biological Assessment of Water Quality in Eroding Reaches (Riffles & Glides) of Rivers and Streams*						
Biotic Indices (Q Values) and typical associated macroinvertebrate community structure. See overleaf for details of the Faunal Groups.						
Macroinvertebrate Faunal Groups**	Q5	Q4	Q3-4	Q3	Q2	Q1
Group A	At least 3 taxa well represented	At least 1 taxon in reasonable numbers	At least 1 taxon Few - Common	Absent	Absent	Absent
Group B	Few to Numerous	Few to Numerous	Few/Absent to Numerous	Few/Absent	Absent	Absent
Group C	Few	Common to Numerous *Baetis rhodani* often Abundant Others: never Excessive	Common to Excessive (usually Dominant or Excessive)	Dominant to Excessive	Few or Absent	Absent
Group D	Few or Absent	Few or Absent	Few/Absent to Common	Few/Absent to Common	Dominant to Excessive	Few or Absent
Group E	Few or Absent	Few or Absent	Few or Absent	Few or Absent	Few / Absent to Common	Dominant
Additional Qualifying Criteria						
Cladophora spp. Abundance	Trace only or None	Moderate growths (if present)	May be Abundant to Excessive growths	May be Excessive growths	Few or Absent	None
Macrophytes (Typical abundance)	Normal growths or absent	Enhanced growths	May be Luxuriant growths	May be Excessive growths	Absent to Abundant	Present/Absent
Slime Growths (Sewage Fungus)	Never	Never	Trace or None	May be Abundant	May be Abundant	None
Dissolved Oxygen Saturation	Close to 100% at all times	80% - 120%	Fluctuates from < 80% to >120%	Very unstable. Potential fish-kills	Low (but > 20%)	Very low, sometimes zero
Substratum Siltation	None	May be light	May be light	May be considerable	Usually heavy	Usually very heavy and anaerobic

Note occurrence/abundance of groups in above table refers to some but not necessarily all of the constituents of the group. The Additional Qualifying Criteria apply in virtually all circumstances. Single specimens may be ignored. Seasonal and other relevant factors (i.e., drought, floods) must be taken into account.
* Macroinvertebrate criteria do not apply to rivers with mud, bedrock or sand substrata, very sluggish or torrential flow, head-water or high altitude streams and those affected by significant ground water input, excessive calcification, drainage, canalisation, culverting, marked shading etc.
** See Further Observations overleaf.

Figure 1A. Q-value scoring system (Toner et al., 2005)

Macroinvertebrates grouped according to their sensitivity to organic pollution					
TAXA	Group A	Group B	Group C	Group D	Group E
	Sensitive	*Less Sensitive*	*Tolerant*	*Very Tolerant*	*Most Tolerant*
Plecoptera	All except *Leuctra* spp.	*Leuctra* spp.			
Ephemeroptera	Heptageniidae Siphlonuriidae *Ephemera danica*	Baetidae (excl. *Baetis rhodani*) Leptophlebidae	*Baetis rhodani* Caenidae Ephemerellidae		
Trichoptera		Cased spp.	Uncased spp.		
Odonata		All taxa			
Megaloptera				Sialidae	
Hemiptera		*Aphelocheirus aestivalis*	All except *A. aestivalis*		
Coleoptera			Coleoptera		
Diptera			Chironomidae (excl. *Chironomus* spp.) Simuliidae, Tipulidae		*Chironomus* spp. *Eristalis* sp.
Hydracarina			Hydracarina		
Crustacea			*Gammarus* spp. *Austropotamobius pallipes*	*Asellus* spp. *Crangonyx* spp.	
Gastropoda			Gastropoda (excl. *Lymnaea peregra* & *Physa* sp.)	*Lymnaea peregra Physa* sp.	
Lamellibranchiata	*Margaritifera margaritifera*		*Anodonta* spp.	Sphaeriidae	
Hirudinea			*Piscicola* sp.	All except *Piscicola* sp.	
Oligochaeta					Tubificidae
Platyhelminthes			All		

Figure 1B. Q-value scoring system for macroinvertebrates (Toner et al., 2005)

1.8 Multi-metric approach - The AQEM

A multi-metric approach uses multiple different indices to produce one score for a river biomonitoring index. This was found to provide an accurate and full evaluation of the ecological status of water bodies (Hering et al., 2006). An example of a multi-metric approach is "The Development and Testing of an Integrated Assessment System for the Ecological Quality of Streams and Rivers throughout Europe using Benthic Macroinvertebrates" otherwise known as the "AQEM". This is a stressor-specific approach developed in 2002 by the European Union that uses different metrics depending on the stressors on the river to produce a water quality score ranging between 1 and 5. By combining multiple different metrics depending on which stressors are thought to be affecting a river, the AQEM can accurately define how badly the suspected stressor is polluting the river as well as the overall water quality. This is used to give information about possible degradation and direct future management practices (Buffagni et al., 2004).

Although all of the above can give a good view of the ecosystem's ecological state, the cost (economic and manpower), need for expert knowledge of river organisms and need for specialist equipment pose challenges to monitoring a large number of first and second order headwaters (Rae et al., 2019, Lewandowski and Specht, 2015, Pinto et al., 2020). As there are a large number of small headwaters across Ireland that are unmonitored, many pollution inputs may go unnoticed adding to the declining state of Ireland's streams and rivers.

1.9 Citizen science

Despite years of freshwater improvement initiatives, water quality in Ireland is still deteriorating with 230 water bodies having declined in water quality in a 2020 report (Trodd and O'Boyle, 2021). To change this trend, it is important to utilize every resource as efficiently as possible. Citizen science is a resource that has shown to be underutilized and has significant potential to be improved in Ireland by focusing on the management of the programmes, communication between programmes and attraction and retention of participants (Donnelly et al., 2014, Krabbenhoft and Kashian, 2020). Biomonitoring citizen science projects present a wide range of advantages over environmental monitoring due to its effectiveness in monitoring the ongoing state of the environment, not just a current attribute (Toner et al., 2005). Citizen science projects involve non-experts called citizen scientists who contribute to scientific data voluntarily (Cavalier and Kennedy, 2016). The European Union has identified citizen science as a growing field of practice that is likely to yield significant outcomes for water quality and data capture (Introduction to the EU Water Framework Directive - Environment - European Commission, 2021). It was agreed in a study of professionals that citizen

science is not used to its full potential in regard to data capture and citizen engagement (Golumbic et al., 2017).

In recent years, there has been a steady rise in nature-based citizen science projects that aim to build knowledge and awareness whilst also collecting useful data that help with conservation and maintenance. This has been aided by the availability of online submission of scientific data. Innovative technology, new handheld devices and widespread digital platforms that help to classify and collect species occurrence have also been a large factor in this growth (Feldman et al., 2021). However, numerous operational and strategic challenges exist (Lee et al., 2020).

Citizen science biomonitoring initiatives are useful but can be biased or inaccurate if not managed correctly (Feldman et al., 2021). There could be disparity in the quality of information gathered and where it is gathered, due to bias and/or lack of knowledge amongst volunteers. This disparity can happen for several different reasons. For example, a bias towards human population centres, areas that are easy to access, protected areas, or regions frequented by active observers (Reddy and Davalos, 2003, Botts et al., 2011, Martin et al., 2012). There may be geographically biased coverage toward well financed areas and more industrialised areas (Schmeller et al., 2009, Martin et al., 2012, El-Gabbas and Dormann, 2018). Observations and recordings could also be taxonomically biased as volunteers are attracted to large, common and brightly coloured species (Ward, 2014, Amano et al., 2016, Boakes et al., 2016, Newbold, 2010). A variation in expertise amongst a wide range of volunteers especially for species that are harder to identify could also lead to a disparity in results (Fitzpatrick, 2009, Cox et al., 2012, Kamp et al., 2016, Kelling et al., 2015). Bias from each of these four sources

can decrease the credibility and accuracy of a citizen science initiative and must be considered when developing a citizen science index (Feldman et al., 2021).

Parsons highlighted the need for simple citizen science projects that collect less complicated data to prevent a reduction in participation and/or an increase in misidentification of species that could lead to poor data quality and poor engagement with the project. This trade-off between participation and data accuracy can be avoided by simplifying the data that are collected (Parsons et al., 2011). Current river biomonitoring protocols, even those targeted at non-experts, tend to require advanced levels of taxonomic expertise, which are likely to discourage citizens from engaging in the important issue of local stream water quality.

A citizen science index should aim to reduce the variation between volunteers of all skill levels by only looking at indicator species that are easily found and identified. This reduces the need for intensive training and makes a citizen science index much more practical for volunteers. The literature suggests that a good indicator species should have characteristics such as taxonomic soundness meaning they are easily recognised by non-specialists, wide or cosmopolitan distribution, low mobility indicating local habitation, well-known ecological characteristics, numerical abundance, suitability for laboratory experiments, high sensitivity to environmental stressor(s) and the potential for quantification and standardisation (Füreder and Reynolds, 2003, Hilty and Merenlender, 2000, Rosenberg and Resh, 1993).

Another effective way of engaging participants and collecting more general data, is the use of open-ended questions and comments sections. This has been shown in successful online citizen science initiatives like 'EBird' where participants from around the world describe the type of bird they see, rather than try to identify the species (Sullivan et al.,

2009). On the other hand, bias could play a larger role in initiatives like EBird (Genet, 2003). Several studies have emphasised the importance of avoiding estimates of subjective data such as group size, relative abundance or cover, when designing surveys for volunteers (Genet, 2003, Galloway et al., 2006, Lovell et al., 2009, Gollan et al., 2012). A citizen science index for water quality should encourage users to record additional evidence such as excessive algae or fine sediment, farm animal access, surface foam, presence of trout/salmon… etc. This could possibly be done by the addition of an observations section along with the recording sheet.

Krabbenhoft and Kashian carried out a study on the difference in volunteers' accuracy at identifying benthic macroinvertebrates compared to that of experts. They found that with half a day's training in macroinvertebrate identification and collection, the main differences in the data collected by experts and volunteers were largely due to rare taxa identification (Krabbenhoft and Kashian, 2020). Krabbenhoft and Kashian also found that volunteers found it hard to differentiate between species that are in extremely low abundance and are missed due to random chance in sampling (false 'zero' abundance), and taxa which are truly absent (true 'zero' abundance) (Krabbenhoft and Kashian, 2020). For this reason, a citizen science macroinvertebrate protocol should take multiple samples. Thus, if a volunteer misses a species in one sample, they could likely identify it in the next sample. Multiple samples would also allow a volunteer to sample a few different areas in a stream giving a better indication of the benthic macroinvertebrate community.

There have been studies reviewing the most efficient ways to overcome these challenges in order to increase participant engagement in science whilst still providing meaningful data (Roche et al., 2021, Lee et al., 2020, Brossard et al., 2005). Additionally, international bodies such as the European Citizen Science Association and the EU-

Citizen Science platform have been set up to advise and govern new programmes (Roche et al., 2021). Krabbenhoft and Kashian have shown that citizen science can have a dual purpose of collecting reliable data to supplement existing data whilst increasing public engagement (Krabbenhoft and Kashian, 2020).

The reliability of citizen science initiatives is often questioned. A study by Lewandowski and Specht in 2015 carried out a systematic review of the literature on the quality of data that were collected by volunteers. They studied 71 papers, 63% of which examined systematic monitoring schemes, 31% considered opportunistic schemes and 6% examined both types. More than one third of the papers examined directly whether volunteers can collect data that are as good or comparable to that of professionals. They found that volunteer data was no less precise than professional data finding that on average only 4 in 7 professionally collected data sets were more accurate than that of volunteers, when using the same accuracy standard. Furthermore, no studies conclusively showed that professional data were less variable (Lewandowski and Specht, 2015).

In the Republic of Ireland, there are many organisations that provide citizen science programmes but few of them look at waterways specifically e.g., National Biodiversity Data Centre coordinates programmes such as: Nature Watch, Leaf Watch Phenology Trail, Butterfly Monitoring Scheme and Bumblebee Monitoring Scheme and Bioblitz and Countryside Bird Survey conduct independent citizen science programmes. The few initiatives that use citizen science to look at waterways monitor species distribution but not as an indication of ecological state, e.g., Waterways for Wildlife, Seashore Spotter, Rocky Shore Safari, National Frog Survey, Dragonfly Ireland etc… (Roche et al., 2021). With a simplified method of indicator macroinvertebrate identification and classification that could be used by experts and non-experts, more rivers could be

sampled. There is a clear need for such an index in Ireland to allow monitoring of more of Ireland's streams and headwaters (Feeley et al., 2020).

1.10 Citizen science freshwater biomonitoring initiatives

Some existing citizen science biomonitoring initiatives in operation abroad include the Riverfly Partnership (UK), Virginia Save Our Streams Protocol (VASOS)(USA) and The Open-Air Laboratories (OPAL) water surveys (UK). The only index that can be used by non-experts currently in use in Ireland for water quality monitoring is the Small Stream Risk Score (SSRS) which requires a training course and a significant amount of time to carry out in the field (Ryan et al., 2015).

1.10.1 The Riverfly Partnership

The Riverfly partnership is a citizen science initiative set up in 2004 to assess river water quality and monitor macroinvertebrate populations in the UK. Since the beginning of the initiative, it has had huge success and as of 2019 has more than 2,000 volunteers monitoring more than 1,600 rivers. It has been described as an exemplary citizen science initiative that enables people to protect and reconnect with their local rivers whilst also contributing to scientific research (Brooks et al., 2019). The Riverfly Partnership uses a recording method called the Anglers Riverfly Monitoring Initiative (ARMI) which estimates the $\log_{10}$ abundance of 8 macroinvertebrate species with varying pollution tolerances in order to monitor changes in the population densities and water quality (www.riverflies.org). The ARMI protocol uses a three-minute kick sample and one minute collecting large stones and wiping them into the mouth of the net to collect macroinvertebrates. These taxa (cased Trichoptera (cased caddis), caseless Trichoptera (caseless caddis), Ephemeridae (mayfly), Ephemerellidae (blue-winged

olive), Baetidae (olives), Heptageniidae (flat-bodied mayfly), Plecoptera (stonefly), and Gammaridae (shrimp)) were chosen because they are easy to identify at this taxonomic resolution, cover a range of sensitivities to pollution, have national applicability, are present year-round (with the exception of Ephemerellidae), and are familiar to most anglers (Moolna et al., 2020). The Riverfly partnership asks volunteers to upload their findings to a portal on their website which automatically alerts the user if their if the report is below "trigger level" at which point the volunteer should reassess the river and if there is no change take further reporting actions(www.riverfly.org).

Studies have been carried out on the effectiveness of this initiative and have found the ARMI scores are reliable and consistent throughout different water qualities or inter-sampler difference (Cahill, 2019, Di Fiore, 2017). Overall, this initiative is a great model for freshwater citizen science initiatives and should be followed by citizen science initiatives in Ireland.

1.10.2 Virginia Save Our Streams Protocol (VASOS)

A multi-metric biotic index commonly used in the USA by a volunteer biological monitoring programme was developed in Virginia and called the Virginia Save Our Streams (VASOS) protocol. The VASOS protocol requires identification and counting of all macroinvertebrates caught in a flat net after rubbing cobbles over the net for twenty seconds. The macroinvertebrates caught are then used to calculate six different metrics, they were:

- Percentage Mayflies + Stoneflies + Most Caddisflies
- Percentage Common Netspinners
- Percentage Lunged Snails
- Percentage Beetles

- Percentage Tolerant Species

- Percentage Non-Insects.

Using these metrics, a final Save Our Streams Multi-Metric Index Score is calculated. A score between 7 and 12 is an indication that the stream is of acceptable ecological condition and a score between 0 and 6 is an indication that the stream is of an unacceptable ecological condition.

In a study done by Steven Christopher Haas, 127 people who discontinued participation in the VASOS were surveyed (Haas, 2000). When participants were asked to rate a number of possible reasons for discontinuing participation. The second highest answer was that the volunteers "did not have enough time" and the fourth highest answer was that "stream monitoring took too much time" (Haas, 2000). Although this programme was successful, participation could have been boosted by making the index less time consuming.

1.10.3 The Open-Air Laboratories (OPAL) water surveys

Another similar study in 2019, The Open-Air Laboratories (OPAL) water survey, was a citizen science project carried out by Imperial College London that involved over a million participants in the UK (Davies et al., 2011). This citizen science initiative was designed to study the ecological state of Sustainable Drainage Systems (SuDS) such as stormwater ponds, detention basins, swales, infiltration ditches and permeable pavements. The OPAL water survey used thirteen invertebrate taxa to determine the ecological state of SuDS (Rae et al., 2019). These taxa were collected using a number of different sampling techniques such as funnel trapping, egg searching, flash lighting/torching and dip netting. The total sampling time for each site was three-minute netting plus a further minute of shore visual searching following the UK National Pond

Survey protocol (Biggs et al., 1998, https://www.imperial.ac.uk/opal/). The thirteen freshwater invertebrate groups had different pollution or eutrophication tolerance ranges and were assigned the following values: Cased caddisfly larvae (10), Dragonfly larvae (10), Alderfly larvae (10), Damselfly larvae (10), Caseless caddisfly larvae (10), Mayfly/stonefly larvae (5), Water beetles and/or larvae (5), Water bugs (5), Pond skaters (5), Water shrimps (5), Water snails (1), Water slaters (1) and Worm-like animals (1). Their presence or absence were added up and resulted in a score between 0 and 78; 0 being the worst and 78 being the best (Rae et al., 2019).

The findings of Rae et al. 2019 show that the OPAL water survey was a robust tool for assessment of the ecological state of SuDS and could be used as a stand-alone method of assessment. Furthermore, this study showed that due to the elementary manner of sampling many participants intended to return to the sampling area to maintain and sample the SuDS again in the future. This project is a good demonstration of how a citizen science project can sustainably monitor a valuable resource for biodiversity and ecological state whilst still engaging the public. However, this resource is only for SuDS and takes significant training and time to carry out (Davies et al., 2011).

1.10.4 Small Stream Risk Score (SSRS)

The Small Stream Risk Score (SSRS) is a biotic index that requires the full analysis of a kick sample including species abundance in order to achieve a score. The SSRS is primarily used to detect if a river is at risk of pollution and can be used by experts and non-experts. The SSRS does this by splitting the taxa present in a kick sample into five groups. All Ephemeroptera, all Plecoptera and all Trichoptera are grouped as three intolerant groups, all Gastropoda, Oligochaeta and Diptera (GOlD) are grouped as one tolerant group and *Asellus aquaticus* is a stand-alone tolerant species that is treated as a

tolerant group and has its own calculations. The total number of taxa within each of the groups and their relative abundance are counted and applied to a flow chart to achieve an SSRS score. This score is then grouped into three categories: At Risk, Indeterminate or Not at Risk. The SSRS has been shown to give an accurate risk assessment on Irish streams and is already in use in some cases (Ryan et al., 2015). The aim of developing this score was to identify pollution inputs and the SSRS does so efficiently (McGarrigle, 2014). Although this assessment system can be used by non-specialists after a training course, anecdotally, it is difficult for volunteers with little to no experience in macroinvertebrate identification to learn the skills necessary to carry out the SSRS accurately and efficiently. As discussed above, the main differences in the data collected by experts and volunteers were largely due to rare taxa identification (Krabbenhoft and Kashian, 2020, Lewandowski and Specht, 2015) and simple citizen science projects that collect less complicated data tend to have more consistent participation (Parsons et al., 2011). These factors make the SSRS not ideal for a standalone citizen science index.

1.11 Aims

Using contemporary and historical biological metrics, and the above research it is a clear that there is a need for a simplified macroinvertebrate biomonitoring protocol, based on a small number of commonly occurring, easily identifiable and distinct taxa that are strong indicators of polluted or non-polluted waters.

In order to develop a simplified macroinvertebrate biomonitoring protocol this thesis aims to:

1. Establish a small number of easily identifiable and common macroinvertebrates with narrow pollution tolerance that can be used to indicate water quality.

2. Develop a Citizen Science Stream Index (CSSI) protocol using the presence or absence of these indicator macroinvertebrates.

3. Validate the CSSI using Q-values as a reference standard.

4. Compare the CSSI with contemporary metrics using multiple data sets with varying spatial distribution and water quality.

5. Carry out a pilot study to test the quality, accuracy and feasibility of using the index in the field by citizen scientists.

1.12 Flow chart showing the development of the CSSI

2. Establishment of indicator macroinvertebrates.

Two different methods of ranking data were used to establish which macroinvertebrates were most useful as indicator taxa for a citizen science index both of which used a data set collected by Feeley et al from 2007 to 2012 and published in 2020 (Feeley et al., 2020). The first was a principal component analysis of the data and the second was a Q-Value analysis.

2.1 Principal component analysis and ranking of data

To establish the indicator macroinvertebrates that would be most appropriate for a citizen science initiative, a data set collected by Feeley et al. from 1,277 rivers in Ireland was analysed using XLSTAT. In this data set macroinvertebrates were collected every year from June to September when the rivers were low enough to be waded. If the rivers were not low enough to be waded an extension pole and drag net were used but this was rare. The personnel collecting the samples also collected physical and chemical data at most sample points using various equipment (Feeley et al., 2020). This data set analysed ammonia, alkalinity, biological oxygen demand (BOD), conductivity, nitrate, pH, temperature, calcium, colour and turbidity of the river as well as the macroinvertebrates present and the Q-value rating (Feeley et al., 2020).

Although 11 types of physical and chemical data accompanied the data set only 7 types were complete enough to create a PCA. Within the 7 types of data there were still various gaps in 55 sample sites, so they were excluded from the PCA. In total, the PCA was developed using 7 types of data on 1,222 sites. The data types analysed were ammonia, alkalinity, biological oxygen demand (BOD), conductivity, nitrate, pH and temperature. From this data a PCA was carried out using XLSTAT software. This PCA produced five possible axes. The axis with the highest contribution to the variables, F1

(37.72%) was used as a reference standard for water quality. Using existing knowledge of water quality stressors and the correlation circle of the PCA (Clabby et al., 2008), it was established that water quality had an inverse relationship with the F1 axis so sample sites with negative values on the F1 axis had good water quality relative to the data types included in the PCA and vice versa.

Subsequently, using each sample site's value on the F1 axis of the PCA, the sample sites were ranked from lowest to highest (best water quality to worst water quality). The sample sites were then banded into bands of F1 values which represented water quality i.e., less than -2, greater than or equal to -2 and less than -1, greater than or equal to -1 and less than 0, greater than or equal to 0 less than 1, greater than or equal to 1 and less than 2 and finally greater than or equal to 2. These bands split the sample sites into 6 bands of water quality. Within these bands the percentage frequency of each macroinvertebrate taxa was calculated and ranked from those which were present most in the lowest band to those which were present least in the lowest band.

For the purposes of a citizen science index three families of easily identifiable and common macroinvertebrates with narrow pollution tolerances were grouped as follows. All snails (Gastropoda) were grouped as they all showed a strong negative correlation with water quality according to the PCA analysis, and they are particularly easy for non-experts to identify. All stoneflies (Plecoptera) were grouped as they all showed a strong positive relationship with water quality and again were easily identified by their two tails and crawling movement. Finally, all leeches (Hirudinea) were grouped as they had a strong negative relationship with water quality and were easily identified by their anchoring movement and suckers on either end of their bodies. Additionally, species that were easily confused with other species were discounted as this could lead to misidentification by volunteers. For example, Sphaeriidae showed a strong negative

correlation with water and Ancylidae showed a strong positive correlation with water quality, but both of these are hard to discern and identify in the field.

The most indicative and suitable macroinvertebrates were selected as the ones which had the strongest relationship with water quality/the F1 axis whilst still being easily identified by non- experts. These indicative taxa were compared to each other in a bar chart showing percentage frequency of each taxon in each band.

2.2 Macroinvertebrate analysis using Q-values

The Q-value that was provided in the data set of 1,277 sample sites (Feeley et al., 2020) was used as another reference standard for water quality. In a similar way to the PCA analysis, the sample sites were ranked and split into bands accordingly. Although Q-values range from Q1- Q5 it was found that very few sample sites had a Q1 or Q2 so having separate bands for very few sites would have led to an uneven distribution of sample sites across the analysis. To account for this, rivers with a Q-value of less than three (<Q3) were grouped together. The other bands were Q3, Q3-4, Q4, Q4-5 and Q5. The same taxa grouping, and discounting was applied as above and from the data of the percentage occurrence of macroinvertebrates within each band was made and ranked. The most indicative taxa were selected. A 100% stacked graph was developed to illustrate the likelihood of finding the each of these taxa in each band of Q-values.

Using these two exploratory statistical methods, six indicator macroinvertebrate groups were selected for use in developing a protocol for a citizen science stream index. A standard protocol and methodology for sampling rivers was developed and adjusted to suit a citizen science index in accordance with the literature review above and the study of contemporary biotic indices. The name decided as appropriate for the index was The Citizen Science Stream Index (CSSI).

2.3 Results

2.3.1 Principal component analysis and ranking of data

Figure 2. shows the correlation circle of a PCA of physical and chemical data collected from 1,277 river sample sites (Feeley et al., 2020). The F1 and F2 axis are shown along with the river variables and their correlation to the axes. Acute angles between variables depict closely linked variables, whereas right angles between variables depict unrelated variables. Vector length reflects representativeness in the selected plan.
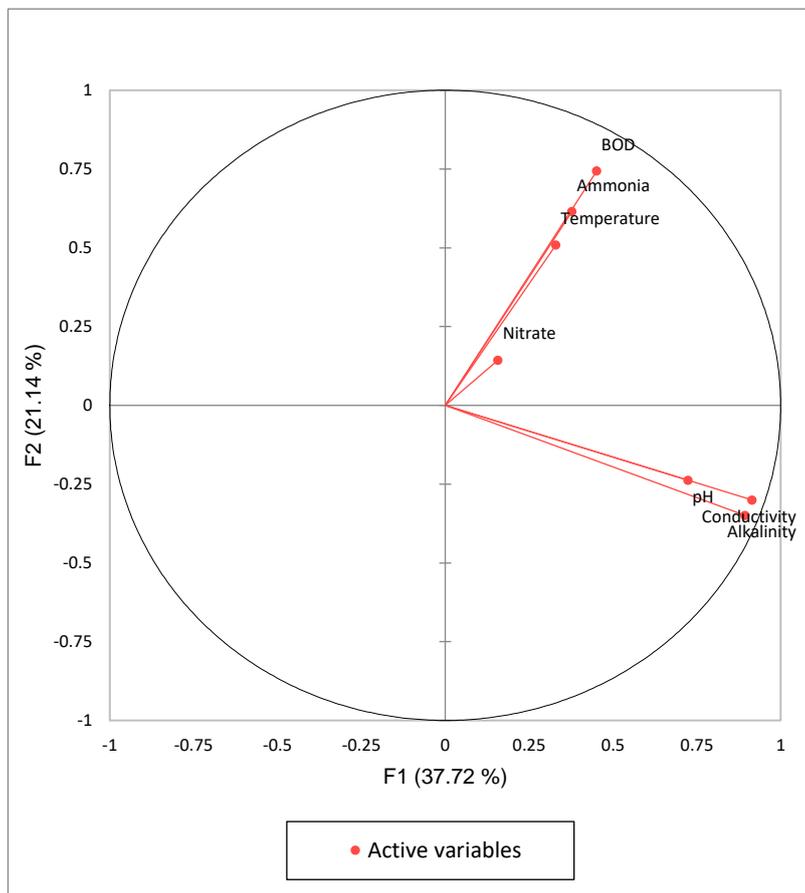

Figure 2. Correlation circle of variables in the PCA of the physical and chemical analysis of 1,222 river sample sites analysed by Feeley et al. 2012 plotted on the F1 and F2 axis (Feeley et al., 2020).

The F1 axis of this PCA was used as a standard for water quality as it had the largest eigenvalue and therefore represented the most attributes on one axis. Each sample site in the data set can be represented on this graph according to the PCA results. Using the position of each sample site on the F1 axis, the river sample sites were ranked from lowest to highest. 6 arbitrary boundaries were then used to split the sample sites into 6 bands. The macroinvertebrates were ranked according to their frequency of occurrence in the sample sites within each of the 6 bands of water quality. It was determined that values on the F1 axis were inversely related to water quality using existing data. This means the macroinvertebrates with high frequencies in the PCA band '<-2' and low frequencies in the '>2' are intolerant of polluted water and so indicate good water quality and vice versa. For example, *Asellus aquaticus* which is a documented indicator of poor water quality (Toner et al., 2005) should have a high frequency in the '>2' and low frequency in the '<-2' band. Macroinvertebrates with consistent relationships between the two extreme bands of PCA ('<-2, and '>2) are considered indicative of water quality. The 6 taxa that were most indicative according to the PCA data were selected and shown in Table 1.

Table 1. Table of frequency of 6 indicator macroinvertebrate taxa in 6 PCA bands of water quality (Feeley et al., 2020).

Frequencies per PCA band	<-2	>-2 <-1	>-1 <0	>0 <1	>1 <2	>2
Rhyacophilidae (Green caddisfly)	74.12	63.89	71.37	58.37	49.41	41.35
Heptageniidae (Flattened Mayflies)	72.35	83.33	82.57	74.29	62.06	40.60
Plecoptera (Stoneflies)	88.24	76.11	69.71	59.18	39.13	22.56
Asellus (Waterlouse)	8.24	17.22	37.34	48.57	56.92	80.45
Hirudinea (Leeches)	31.18	41.11	34.44	39.59	39.13	61.65
Gastropods (Snails)	27.06	48.33	55.60	62.04	70.75	62.41

This information was then graphed in Figure 3A. All other macroinvertebrates in the data set were analysed in the same way for their correlation to water quality. Some showed a relationship with water quality but were either too common (Baetidae, Gammaridae), so presence/absence would not provide meaningful data, or too uncommon (Odonticeridae and Polycentropodidae), so their presence or absence would not provide data often enough to provide an outcome. These examples are shown in Figure 3B along with other macroinvertebrates such as Hydropsychidae and Ephermerellidae that are commonly used in freshwater biomonitoring initiatives. None of these have a significant enough correlation to water quality to be useful in a presence or absence type index like the CSSI.

Figure 3A. Bar chart of frequency of 6 indicator macroinvertebrates over 6 PCA bands of water quality derived using Feeley et al. 2012 data set.

Figure 3B. Bar chart of frequency of 6 other macroinvertebrates over 6 bands of PCA water quality (Feeley et al., 2020).

2.3.2 Macroinvertebrate analysis using Q-values

A similar analysis was carried out using the Q-values of the Feeley 2012 data set as a reference standard. This analysis was used to determine which macroinvertebrates showed a consistent relationship to the Q-value ratings which is used by the EPA in Ireland to monitor water quality (Toner et al., 2006). Similar taxa to the PCA analysis were found to be most indicative of water quality as shown in the 100% stacked column graph (Figure 4).

Figure 4. 100% stacked column graph of frequency of 6 indicator macroinvertebrates in 6 Q-Value bands (Feeley et al., 2020).

2.3.2.1 CSSI optimal boundary selection

Three graphs were made to identify the most suitable boundaries of water quality that the CSSI is intended to indicate, poor, moderate and good. The Q-values that marked the boundaries are the same that was used in Trodd and Boyle, 2018 (Trodd and Bolyle, 2018). Using Feeley et al. 2020 Figures 5A, 5B and 5C show boundary variations of the equivalent CSSI score compared to three Q-value water quality bands.

Figure 5A. Bar chart showing the frequency (%) that three water quality bands of the CSSI appears in three water quality bands of Q-values. The equivalent CSSI score was banded so that -3 and -2 were poor, -1, 0 and +1 were moderate and +2 and +3 were good (variation 1) (Feeley et al., 2020).

Figure 5B. Bar chart showing the frequency (%) that three water quality bands of the CSSI appears in three water quality bands of Q-values. The equivalent CSSI scores were banded so that -3, -2 and -1 were

classified as poor water quality, 0 was classified as moderate water quality and +1, +2 and +3 were classified as good water quality (variation 2) (Feeley et al., 2020).

Figure 5C. Bar chart showing the frequency (%) that three water quality bands of the CSSI appears in three water quality bands of Q-values. The equivalent CSSI scores were banded so that -3 was classified as poor water quality, -2, -1, 0, +1 and +2 were moderate water quality and +3 was classified as good water quality (variation 3) (Feeley et al., 2020).

In variation 1 (Figure 5A) the score was banded so that -3 and -2 were poor -1, 0 and +1 were moderate and +2 and +3 were good. Using these boundaries, the Q-value bands show a good relationship to the equivalent CSSI bands with the majority of both index bands agreeing. However, there was still over 23% of poor CSSI sample sites incorrectly classified in the moderate Q-value band and 16% of good CSSI sample sites incorrectly classified in the moderate Q-value bands. There is also a high percentage of moderates in both good and poor water quality bands. Although neither are ideal, it is preferable that moderate water quality is over expressed rather than good or poor water quality, as over reporting a pollution area could lead to false claims being made against landowners, undermining the validity of the CSSI.

To test for the most suitable boundaries two more variations were assessed with narrower and broader boundaries for poor and good water quality. In variation 2 (Figure 5B) the moderate boundary became narrower, although this resulted in a higher frequency of CSSI sample sites agreeing with good and poor water quality in the appropriate Q-value bands, there was an increased expression of poor CSSI sample sites incorrectly classified in the moderate Q-value band (43%) and good CSSI sample sites incorrectly classified in the moderate Q-value bands (34%).

The opposite was true when the moderate boundary was broadened and the other two narrowed as in variation 3 (Figure 5C).

2.4 CSSI protocol

Two completely different methods of analysis to classify indicative macroinvertebrates were selected to be used in the CSSI. In the field, the CSSI protocol consists of gathering three individual kick net samples. A standard D-frame net 250mm wide on the flat side of the D, with a mesh size of 500µm was used, as described by Feeley et al. in the EPA water quality reports (Feeley et al., 2020). Three thirty second kick samples are taken. The use of three short kick samples was chosen for a number of reasons. As there are only six macroinvertebrates the minimum and maximum scores per sample are -3 and +3. This range of results is too small for statistical analysis so a larger range must be achieved by taking multiple samples. The standard professional sample takes one 120 second kick sample (Toner et al., 2021, Feeley et al. 2020). It was felt that the least amount of time spent by the citizen scientist taking the kick sample and analysing the tray of invertebrates would be preferable to increase engagement and accuracy. Three thirty second kick samples adding to 90 seconds in total was decided as acceptable for a coarser citizen science index. Lastly, three samples investigated separately should prevent false zero abundances i.e., missing species that are there in small abundances (Krabbenhoft and Kashian, 2020). In order to validate the efficacy of this method of sampling the author used the standard professional method in the field study for comparison of the volunteers CSSI scores that used the protocol.

The rest of the protocol is derived from the methods generally used in the literature (Toner et al., 2021, Feeley et al., 2020). Briefly, the net is held in a fast-flowing gravel bedded or rocky area of the stream by the sampler before they disturb (kick) the rocks and debris in the area upstream of the net. This is done for five to ten seconds before stepping upstream and repeating these actions for a total of thirty seconds. The contents

of the net are then emptied into a white tray containing around 1cm of water. If the sample contains too much heavy debris, the sample should be elutriated. Elutriation involves swirling the contents of the tray before pouring the water carefully into the net. This pours all the neutrally buoyant material in the sample (macroinvertebrates, other biotic fauna and leaves) into the net, leaving just the stones behind in the tray. The tray is then filled with water again and the process is repeated as many times as necessary (usually six to eight times) to leave only stones in the sample. The stones are then poured out before the tray is refilled with water and the contents of the net added as per the video shown in appendix 1 and leaves are removed by hand if necessary.

The CSSI protocol looks for the presence or absence of six indicator invertebrates. These six invertebrates were grouped into two groups, tolerant and intolerant species. As part of the protocol these two groups were simply labelled 'The Good Guys' and 'The Bad Guys'. Although this gives the impression that certain species are inherently 'good' or 'bad', the reality is that all species have a valuable role to play in any ecosystem. What is 'good' or 'bad' is the impact (or lack of impact) that humans have on the environment which creates an imbalance in how these organisms interact and, therefore, their dominance and occurrence in any given situation. However, in order for the index to be universally user friendly for non-experts of all levels of education, the over-simplified terms 'good guys' and 'bad guys' are used to maximise engagement and understanding (Parsons et al., 2011). The three 'Good Guys' which are associated with good water quality are: Heptageniidae (Flattened Mayfly), Plecoptera (Stonefly) and Rhyacophilidae (Green Caddisfly) and the three 'Bad Guys' which indicate bad water quality are: Hirudinea (Leeches), Gastropoda (Snails) and Asellus (Waterlouse). The citizen science volunteers are instructed on how to identify these 6 taxa by their common

name rather than their Latin scientific name. This training is ideally carried out in the field by an expert tutor and supported by an online video tutorial and leaflet.

A single kick sample score is calculated by adding 1 to the sample score if any Stonefly, Flattened Mayfly or Green Caddisfly (Good Guy(s)) is/are present and subtracting 1 from the sample score if any Snail, Leech or Waterlouse (Bad Guy(s)) is/are present. This produced a sample score with a maximum of 3 and minimum of -3 for each individual kick sample. Each of the three kick sample scores are added to give a total CSSI score between -9 and +9. This score indicates the water quality at that site. The boundaries for the water quality boundaries were initially arbitrarily assigned. In order to find the most appropriate boundary of water quality within the CSSI scores three variations of equivalent CSSI scores were considered, as discussed in the statistical analysis section. If the score is between -9 and -4 inclusive, it is an indication that the water quality is poor; if the score is between -3 and +3 inclusive, it is an indication that the water quality is moderate and if the score is between +4 and +9 inclusive, it is an indication that the water quality is good. This allows the CSSI to be colour coded into a traffic light water quality system that can be used as to identify river water quality easily.

The macroinvertebrates are identified using the CSSI leaflet provided (Figure 6A and Figure 6B). The leaflet and key were developed to be used in the field by a citizen scientist. After use of a few iterations by the author, and trials of similar leaflets, this design was decided as appropriate as seen in Figure 6A and 6B The initial iterations were either not simple enough, not fully explanatory or not aesthetically pleasing. The final leaflet allowed a citizen scientist to use the CSSI in a straightforward step wise manner without the need for expert advice. Ideally this two-page leaflet should be

printed on one double sided laminated sheet that can be taken to the sample site in any weather and filled in using a whiteboard marker before being uploaded online.

The front side (Figure 6A) of the leaflet is a form that the citizen scientist should fill out streamside and includes space for the recorder's name, the stream name, the date and the GPS/location. Under those is a small explanation of the CSSI that reads "*The Citizen Science Stream Index (CSSI) is based on the presence or absence of six key aquatic invertebrates. Three pollution-sensitive invertebrates ('good guys') are commonly found in clean streams and three pollution-tolerant invertebrates ('bad guys') are commonly found in polluted streams. Citizens use a pond net to take three 30-second kick-samples (the three samples should be a few metres apart) from a shallow (<20cm), gravelly, fast-flowing part of the stream. The invertebrates captured in each sample are examined in a white tray on the bankside. The six key invertebrates are easily spotted amongst the many other species in the tray, by their characteristic shape, colour or movement. The citizen will score each sample depending on which, if any, of the six key invertebrates occur in the tray. The three 'good guys' have a score of +1 each and the three 'bad guys' have a score of -1 each. The score for each kick-sample can range from +3 (all three good guys and no bad guys) to -3 (all three bad guys and no good guys). When the scores from all three samples are added together, the CSSI ranges from +9 to -9.*".

A user-friendly guide for calculating each sample's score and the total CSSI score is also included. Alongside this is a reminder to photograph each sample for review and an explanation of the traffic light system that helps explain the good, moderate or poor indication of water quality that the CSSI can show. Finally at the bottom of the leaflet is a box for any extraordinary observations that allows citizen scientists to record anything they think may be important.

On the back of the leaflet (Figure 6B) is a key of the six macroinvertebrates; these act as references if the sampler is unsure if the macroinvertebrate they are looking at is one of the six key macroinvertebrates. Along the bottom are some other common macroinvertebrates that are not scored in the CSSI.

Figure 6A. The CSSI leaflet side A. The form.

Figure 6B. The CSSI leaflet side B. The key.

3. Validation of the CSSI

3.1 Desktop validation

Two lines of validation for the CSSI protocol were planned: a desktop validation and a field study. The first line of validation was a desktop analysis of a large data set published by Feeley et al. in 2020 and two unpublished data sets, the 'Rylane' data set and the 'Implant' data set, collected by Harrison et al. in UCC. Three different biotic index scores, the BMWP, ASPT and SSRS, were calculated using these data sets and then correlated to an equivalent CSSI score, an actual CSSI score as calculated from a data set. This analysis outlines the CSSI's efficacy over varying sample sizes, longitudinal distances and seasons. The published data sets used one two-minute kick sample per site whereas the CSSI is meant to be calculated with three thirty second kick samples. This means that the maximum and minimum CSSI scores that can be calculated from the published data sets is 3 and -3 respectively rather than 9 and -9. Although this is not ideal, using a large data set is necessary in order to see how the 6 indicator macroinvertebrates indicate water quality over a wide range of Irish rivers and streams so an 'equivalent CSSI' between -3 and 3 was used as a proxy for the CSSI protocol. In data sets with triplicate readings per sample site, the BMWP, ASPT and SSRS were calculated using the average of the triplicate samples. As the ASPT is a function of the BMWP that is shown to be more accurate, the ASPT was compared rather than the BMWP (Roche et al., 2010, Armitage et al., 1983). The BMWP, the ASPT and the SSRS were derived for each kick sample in each data set using the following.

- The BMWP score was calculated using Uherek and Pinto Gouveia., 2014 and Paisley et al. 2014 as sources.

- The ASPT was calculated by taking the BMWP score and dividing it by the total number of scoring taxa present in the sample as per Uherek and Pinto Gouveia., 2014 and Paisley et al. 2014.

- The SSRS requires a relative abundance of each invertebrate between 1 and 5, the published data sets used a 5-category qualitative scoring system single; few, common, numerous and dominant. These were directly substituted for 1 to 5 in order to calculate the SSRS (Kelly-Quinn, 2015).

The Q-values were provided with the data set when used.

3.1.1 Feeley et al. 2020 analysis

The published data set used was Feeley et al. 2020. This data set collected chemical, physical and macroinvertebrate data at 10,995 sample sites. The data set was collected by the EPA Ireland who are responsible for monitoring and assessment of 37 hydrometric areas covering 46 river catchments and over 13,000 km of river channels nationwide (Feeley et al., 2020). In this data set macroinvertebrates were collected every year from June to September when the rivers were low enough to be waded. If the rivers were not low enough to be waded an extension pole and drag net were used but this was rare. The macroinvertebrates were collected via a semi-quantitative two-minute macroinvertebrate kick-sample from the riverbed, preferably from the faster flowing riffle habitats setting, similar to the 2012 data set (Feeley et al., 2020). The data collected at each site of the data set included chemical and physical data, the Q-values and the relative abundance of up to 100 different macroinvertebrates.

3.2 Unpublished data sets analysis

Further desktop analysis was carried out using two unpublished data sets. The Implant data set and the Rylane data set were collected by Harrison et al in UCC from a smaller number of Irish river systems. These data sets were in triplicate form and can be used to calculate a CSSI score between -9 and +9 as per the protocol.

3.2.1 Implant data set analysis

The Implant data set was collected on tributaries of the river Lee in County Cork over three seasons in 2012. Again, three 1-minute kick samples were taken to collect macroinvertebrates. The data set recorded 64 different macroinvertebrates in the benthic community at 33 sites in a number of rivers. The mean of the three samples was used to calculate the SSRS, BMWP and ASPT. This data set was used to validate the efficacy of the CSSI through different seasons (Harrison et al., unpublished 2012).

3.2.2 Rylane data set analysis

The Rylane data set was collected in 2018 on a tributary of the river Dripsey in County Cork. A few metres above the first sampling point there was an obvious input of pollution labelled "input" in Figure 7. This data set used three 1-minute kick samples to record 40 different macroinvertebrates at 18 sample sites from 100m downstream of the pollution input to 3300m downstream of the initial sample site. The mean of these three samples was then used to calculate the SSRS, BMWP and ASPT. This data set was used to validate the efficacy of the CSSI across an expected series of water quality in a small stream from poor water quality to progressively better water quality along the course of the river with distance from the point source pollution. (Harrison et al., unpublished 2018)

Figure 7. Satellite image of the tributary of Dripsey River used to make the Rylane data set with sample points labelled 1 to 18 and pollution input source labelled "input".

3.3 Field study

The second line of validation compared 40 CSSI scores, collected by 20 citizen science volunteers, to data that were collected by the author from the same sites and will be referred to as expert data set hereafter. The participants were given six months to sample the given sites and the author took a further 3 months to retest their sample sites. In total all sites were sampled within, at most, 9 months of initial testing, but some were retested in much less time. Three different biotic index scores, the BMWP, ASPT and SSRS, were calculated using the expert data set and correlated to CSSI scores submitted by the volunteers. The aim of this validation was to assess the quality, accuracy and feasibility of the CSSI when used in the field, independent of experts by citizen scientists with a small amount of initial training.

The 40 sampling sites were collected as a part of the NoreVision volunteer programme. The NoreVision initiative was set up to improve the water quality of the Nore river (http://www.norevision.ie/). The sampling sites are shown on Figure 8. Initially, the 20 volunteers returned 67 CSSI scores in total, but 27 of the sites sampled by the volunteers could not be used for comparison with the expert collected data, either because the sites were unreachable due to high water levels, or they had been repeatedly sampled by one or more volunteers. In the case of repeat samples averages of volunteer CSSI scores were taken.

The citizen scientists had received 30 minutes tutoring by an expert in freshwater science on how and where to kick sample and how to elutriate the samples as per the CSSI protocol. Additionally, they watched a ten-minute tutorial video on the CSSI as described below (https://youtu.be/HsDZ0siO6Ds). They were then provided with the

CSSI leaflet as shown in Figure 6A and 6B, a white tray and a D framed net as per the protocol.

The volunteers were given 50 sample sites between them. Initially, sites were selected based on their Q-values which indicated the water quality of the river (gis.epa.ie/EPAMaps/, Figure 8). The sites given to the volunteers for sampling were chosen to give an evenly distributed range of ecological conditions, from sites with little apparent human impact and best attainable ecological conditions, to sites where human activities were obviously causing changes in the streams and ecological conditions were likely to be impaired. Other criteria for sites were decided by visual observation using Google.com/maps followed by onsite visits where necessary. At all sites there had to be a mostly gravel or rocky substrate. This substrate type is necessary for sufficient macroinvertebrates to be collected. A description of ideal kick sampling conditions is included in the tutorial video and in the in-person tutorial provided to the participants. For safety purposes, the sites selected had to be shallow enough to be waded and for ease of access were usually near bridges. Each volunteer took the GPS location of the area that they sampled to allow the expert sampler to consistently sample the same area of the stream when reassessed. The sampling took place from September 2020 to April 2021.

The ASPT, SSRS and an equivalent expert CSSI score were calculated and compared to the CSSI scores collected from volunteers in the NoreVision initiative. The equivalent expert CSSI was used to validate the feasibility of the CSSI in the field by citizen scientists independent of experts with a small amount of initial training.

Figure 8. Q-Values from gis.epa.ie/EPAMaps/ used as a guide for selection of rivers for NoreVision volunteers. Where blue = Q5, green =Q4, yellow =Q3-4, orange = Q3

3.3.1 Expert protocol for sampling and data analysis.

After all volunteer samples were uploaded, samples were taken by the author in the same GPS location within 9 months of the volunteer sampling. The aim was to evaluate volunteer CSSI results compared to an expert macroinvertebrate analysis. The expert sample consisted of one two-minute kick sample similar to the standard expert sampling technique used in Feeley et al. 2020. In brief, the sampler held a net, of the same measurements as the volunteers, in a gravel bedded or rocky area of the stream. The net was placed on the bed of the river in a fast-flowing area of the stream by the sampler before they disturbed (kicked) the rocks and debris in the area upstream of the net. This is done for five to ten seconds before stepping upstream and repeating these action for a total two minutes. If the stretch of the river as defined by the GPS location was not

long enough for one continuous kick sample, multiple samples were taken nearby in other available ripples totalling two minutes of sampling. The contents of the net were then emptied into a white tray containing around 1cm of water and elutriated if necessary. After elutriation, the sample was preserved in a plastic bag containing 95% ethanol for laboratory analysis.

In order to make the process more efficient subsampling was used. Subsampling reduces the effort required for the sorting and identification aspects of macroinvertebrate surveys and provides a more accurate estimate of time expenditure (Barbour and Gerritsen, 1996). The preserved samples were unbagged into a sieve and washed under water. Debris was removed, if possible, by hand. The sample was then placed in a 15cm x 30cm gridded white tray containing roughly 1-2cm water. The tray was swirled to evenly distribute the macroinvertebrates present. A circular sub sampling device 10cm in diameter was randomly placed in the tray. The tray was 5.73 times the size of subsample. This is a modification on the subsampling used by Caton in 1991 (Caton, 1991). Inside this 10cm diameter all macroinvertebrates were sorted and counted. This provided a representative sample of the macroinvertebrates in the kick sample. When multiplied by 5.73 we could estimate the relative abundance for each macroinvertebrate in the total. Furthermore, the remainder of the tray was inspected for any outlying species that were not found in the subsample. These were given the lowest relative abundance score of 1 as they were outliers. The SSRS and the ASPT were calculated from the expert sample for comparison with volunteer CSSI scores. A map was developed using QGIS software to compare the NoreVision volunteer CSSI scores to the existing Q-values maps that were used to select sample sites (Figure 18A and 18B).

3.3.2 Tutorial video

The NoreVision volunteers were asked to view a ten-minute video tutorial on how to carry out the CSSI (https://youtu.be/HsDZ0siO6Ds). This tutorial was developed by the author and presented by Dr Simon Harrison for this project on how to perform a kick sample, elutriate a sample, identify the six indicator taxa and fill out the CSSI leaflet (Figure 6A and 6B). This resource was also available for continual to the volunteers for review on YouTube.com. Screenshots of the video at different sections are included in Appendix 1.

3.3.3 CSSI online survey

As part of the NoreVision initiative the volunteers used a link or QR code to take them to an online survey developed in conjunction with a software company (Veri.ie) to upload their findings online. This allowed the volunteers to upload their collected data that were recorded in the field using the leaflet (Figure 6A and 6B) online where it was accessible to the administrator for validation. The survey included text boxes for the name of the volunteer, GPS location, stream name and date. The survey also had tick box questions about the macroinvertebrates found in their sample. For example, "Was there a flattened mayfly in sample 1? Yes/No". These types of questions are repeated for all 6 taxa and all three samples and end with a text box asking, "What is the sum total CSSI score for all three samples?". Once filled in and submitted these data were available to the administrator as an excel sheet (Excel version, 2018). Appendix 2 shows some screenshots of the form used by the volunteers. The form is available at https://evaluation.veri.ie/submit/761. Screenshots of the online survey can be seen in Appendix 2.

3.4 Desktop statistical validation

3.4.1 CSSI boundary variations

Feeley et al. 2020 data set was statistically analysed to test the correlation of the CSSI to existing indices. The CSSI analyses water quality in three bands as per a traffic light system (red-poor, orange-moderate, green-good). The Q-values was put into three similar bands in order to compare its results to that of the CSSI. These bands were the same as the bands used by Trodd and Boyle, 2018 (Trodd and Boyle, 2018).

In order to find the most appropriate boundary for the bands of water quality within the CSSI scores, three variations of equivalent CSSI scores were considered. Initially the equivalent CSSI scores were banded so that scores of -3 and -2 were classified as poor water quality, -1, 0 and +1 were classified as moderate water quality and +2 and +3 were classified as good water quality. The equivalent CSSI scores were then banded so that -3, -2 and -1 were classified as poor water quality, 0 was classified as moderate water quality and +1, +2 and +3 were classified as good water quality. Finally, a third comparison as made where the equivalent CSSI scores were banded so that -3 was classified as poor water quality, -2, -1, 0, +1 and +2 were classified as moderate water quality and +3 was classified as good water quality. These three graphs were compared to find which boundary allocation found the optimal amount of correct water quality bands without over classifying any particular band of water quality. These variations were compared to the Q-value bands in a bar chart that showed the percentage frequency of each band of CSSI scores in the three bands of Q-values.

3.4.2 Existing metrics compared to Q-values

Similarly, the SSRS and ASPT were compared with the Q-values reference standard. Boundaries for the ASPT were the same that was used in Medupin, 2017 and Everall et al. 2017 and boundaries used for the SSRS was the same as is set out in the Kelly Quinn, 2015. These correlations were used to determine the CSSI's accuracy and congruence with existing indices to outline its potential use for the nationwide water quality biomonitoring.

3.4.3 Rylane data set analysis

The ASPT, SSRS and CSSI were calculated and plotted in a line graph of scores over longitudinal distance.

3.4.4 Implant data set analysis

In the Implant data set, the three indices were calculated using the macroinvertebrate data. The average score for each index over each season was calculated and compared to see how the scores changed over the seasons and if the scores were consistent between the indices. Averaging the scores allowed for simpler manipulation of a large number of data sets. Rounded up averages (standard deviation (SD)) for each index and the water quality (as per the appropriate traffic light bands as set out above) was calculated and shown.

3.4.5 Field study

The ASPT and SSRS were put into three bands per the traffic light system (red-poor, orange-moderate, green-good), as defined in Everall et al. 2017 and Kelly Quinn, 2017,

and compared to the volunteers CSSI scores (VCS) which were banded as per the appropriate traffic light bands as set out above. This shows the CSSI's usefulness when taken by a volunteer independent of an expert compared to the existing indices. Similarly, how often volunteers correctly identified the six indicator species was validated by comparing the volunteer CSSI to an equivalent expert CSSI sample. The equivalent expert CSSI score was banded so that -3 and -2 were poor -1, 0 and +1 were moderate and +2 and +3 were good. A frequency table was constructed to show how often the three bands of equivalent expert CSSI appear in the three bands of volunteer CSSI. This allowed the percentage frequency of agreement between the equivalent expert CSSI and volunteer CSSI to be calculated.

Finally, a map of the Nore River catchment with the volunteers CSSI readings was developed using QGIS 3.16 software, illustrating the data points submitted by the NoreVision volunteers as red for poor water quality, orange for moderate water quality and green for good water quality. This was compared to the map available at https://gis.epa.ie/EPAMaps/ that was initially used to help select rivers for volunteers to sample.

3.5 Results

3.5.1 Feeley et al. 2020 analysis

To validate the use of the selected six macroinvertebrates a large data set of 10,995 samples sites collected by the EPA Ireland was analysed (Feeley et al., 2020). The data set was collected from rivers and streams around Ireland and had physical, chemical and Q-value analysis data as well as macroinvertebrate data. The Q-values provided in the data set were used as reference standards for water quality. From these the sample sites were ranked and put into bands as above. The frequency of the six indicator macroinvertebrates identified in sample sites withing the six bands of Q values are shown in Table 2. This table is illustrated in Figure 9A.

Table 2. Table of frequency of 6 indicator macroinvertebrate taxa over 6 Q-value bands of water quality (Feeley et al., 2020).

Frequencies per Q value Band	Q<3	Q3	Q3-4	Q4	Q4-5	Q5
Rhyacophilidae (Green caddisfly)	13.94	37.93	58.31	67.48	75.11	71.77
Heptageniidae (Flattened Mayfly)	3.64	7.83	67.34	92.08	98.04	97.58
Plecoptera (Stoneflies)	11.52	20.54	51.38	75.17	95.86	98.39
Asellus aquaticus (Waterlouse)	58.18	51.77	46.35	26.63	12.08	8.06
Hirudinea (leeches)	53.64	45.02	36.62	28.93	18.79	16.94
Gastropod (Snails)	53.64	61.34	66.76	59.92	37.98	24.19

Figure 9A. Bar charts of frequency of 6 indicator macroinvertebrate in 6 Q-value bands of water quality (Feeley et al., 2020).

The 6 selected indicator macroinvertebrates were shown to be more consistently indicative to Q-value water quality bands than the original two analyses showed. This further validates their selection for the CSSI. All other macroinvertebrates in the data set were analysed for their correlation to Q-values as shown in Figure 9B.
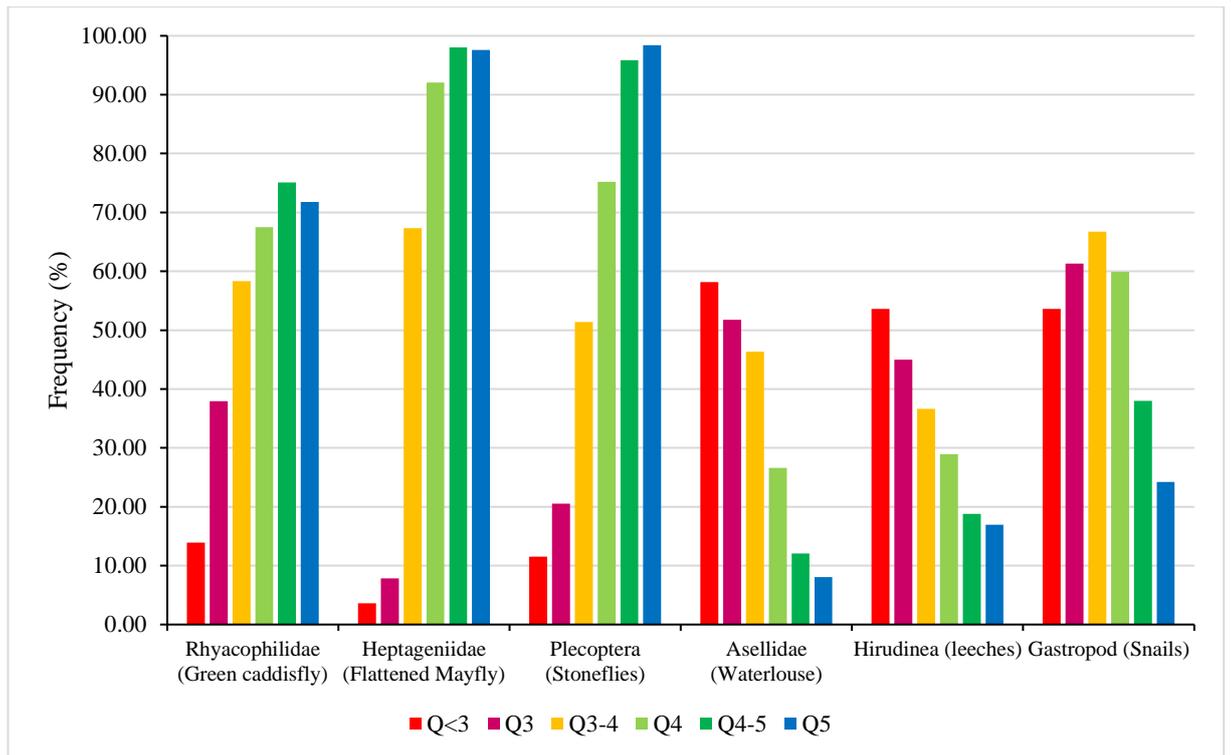
Figure 9B. Bar charts of frequency of 6 other macroinvertebrate in 6 Q-value bands of water quality (Feeley et al., 2020).

Figure 9B shows that some of these invertebrates showed a stronger relationship to the Q-values than to the water chemistry analysed in the PCA. However, similar issues still existed. Baetidae, Gammaridae were too common and so presence/absence would not provide meaningful data. Odonticeridae and Polycentropodidae were or too uncommon so their presence or absence would not provide data often enough to provide an outcome. Hydropsychidae and Ephermerellidae showed a strong relationship to water quality but not as strong as the six indicator taxa. None of these have a significant enough correlation to water quality to be used in the CSSI.

3.5.2 Existing metrics compared to Q-values

A similar comparison was made for the SSRS and ASPT, also using the Q-values for a reference standard. Boundaries for the ASPT were the same that was used in Medupin, 2017 and boundaries used for the SSRS was the same as is set out in the "Guidance on Application and Use of the SSRS in Enforcement of Urban Waste Water Discharge Authorisations in Ireland, 2017" (Medupin, 2017, "Guidance on Application and Use of the SSRS in Enforcement of Urban Waste Water Discharge Authorisations in Ireland, 2017"). The equivalent CSSI was considered poor water quality for -3 and -2, moderate for -1,0 and 1 and good for 2 and 3. Using Feeley et al. 2020 Figures 10 and 11 show three bands of the SSRS and ASPT respectively compared with the three bands of Q-values .

Figure 10. Bar chart showing the frequency (%) that each band of the SSRS appears in three simple bands of Q-value water quality (Feeley et al., 2020).

When compared to Q-values the SSRS identified poor water quality accurately, but over expressed poor rivers in the "indeterminate" and "probably not at risk" bands (Figure 10). Only 50% of good Q-values were classified as probably not at risk by the SSRS.

Figure 11.Bar chart showing the frequency (%) that each band of the ASPT appears in three simple bands of Q-value water quality (Feeley et al., 2020).

The ASPT comparison showed very similar results to the CSSI variation 1, but like variation 1, there was still poor ASPT sample sites incorrectly classified in the moderate Q-value band (22%) and good ASPT sample sites incorrectly classified in the moderate Q-value bands (23%) (Figure 11 and Figure 5A).

Figure 12. The frequency of the six possible CSSI scores in each band of Q values. (Feeley et al., 2020)

It was found that for the extreme low Q-values i.e., the rivers with extremely poor water quality, the CSSI did not accurately represent the poor water quality (Figure 12). In the rivers with Q-values less than Q3, -2 was the most common score. In rivers with a Q1 score the most common score was -1.

3.5.3 Implant data set analysis

Table 3 shows the average score and standard deviation (SD) of the three indices, SSRS, CSSI and ASPT, calculated using the Implant data set that collected macroinvertebrate data over three seasons.

Table 3. Table showing the average score (standard deviation) per season per index (Harrison et al., unpublished 2012).

Average score per season (SD)	Spring	Summer	Autumn
SSRS	8.13 (1.08)	7.11 (1.03)	6.84 (1.69)
CSSI	4 (3.06)	2 (2.94)	0 (2.75)
ASPT	6.05 (0.44)	5.32 (0.57)	5.30 (0.61)

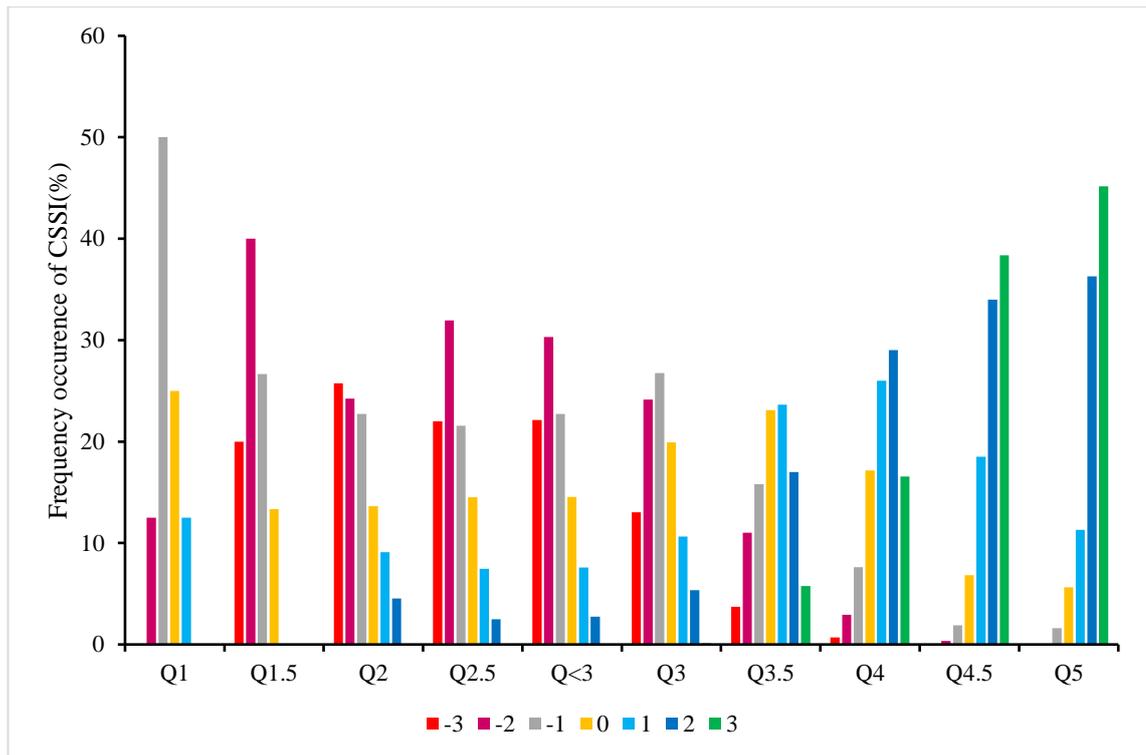As expected, the CSSI had a greater standard deviation due to a larger range of possible outputs, but deviation was relatively consistent throughout the seasons for all indices.

Table 4 Shows the average score data converted to three water quality bands of each index using the traffic light system.

Table 4. Table showing the average band per season per index (Harrison et al., unpublished 2012).

Average band per season (traffic light colour)	Spring	Summer	Autumn
SSRS	Good (green)	Moderate (orange)	Moderate (orange)
CSSI	Good (green)	Moderate (orange)	Moderate (orange)
ASPT	Good (green)	Moderate (orange)	Moderate (orange)

3.5.3 Rylane data set analysis

Using the Rylane data set, Figure 13 compares the ASPT, SSRS and CSSI over a longitudinal distance.

Figure 13. A line graph illustrating the changes in the scores attained from three metrics along the course of a river starting at the source (0m) and finishing at 3300m known as the Rylane data set.

The CSSI initially produced a score of -3 (moderate/orange in the traffic light system). This does not correlate with the ASPT, SSRS or the expected poor water quality conditions. After the initial anomaly, all three biotic indices generally correlate with each other and expected water qualities.

3.5.4 Field study analysis

Using the data collected by NoreVision volunteers in a field study and expertly collected Figure 14 compares the volunteer CSSI and expert ASPT and Figure 15 compares the volunteer CSSI and expert SSRS.

Table 5. Percentage of agreement between expertly taken ASPT bands of pollution as defined in (Medupin, 2017) and the volunteer taken CSSI score as per protocol of the CSSI. VCS stands for volunteer CSSI score.

	Poor ASPT	Moderate ASPT	Good ASPT
Poor VCS	66.67	21.05	13.33
Moderate VCS	33.33	63.16	40.00
Good VCS	0.00	15.79	46.67

Figure 14. The percentage frequency of each water quality band identified by volunteers using the CSSI compared to the bands of water quality set out by the ASPT (Medupin, 2017).

either at risk or probably not at risk and none being classified as indeterminate as defined by the SSRS.

These two graphs show the agreement of the volunteers CSSI scores compared to existing indices. By comparing the volunteer CSSI to an equivalent CSSI sample taken by an expert we can see get an indication of how often volunteers correct found the correct indicator species. Using the field study Figure 16 compares the equivalent expert CSSI to the volunteer CSSI.

Table 7. Percentage of correlation between bands of water quality on an equivalent CSSI taken expertly and the bands of water quality of the CSSI taken by a volunteer as per protocol of the CSSI.

	Poor Professional CSSI	Moderate Professional CSSI	Good Professional CSSI
Poor VCS	80.00	9.09	0.00
Moderate VCS	20.00	72.73	25.00
Good VCS	0.00	18.18	75.00

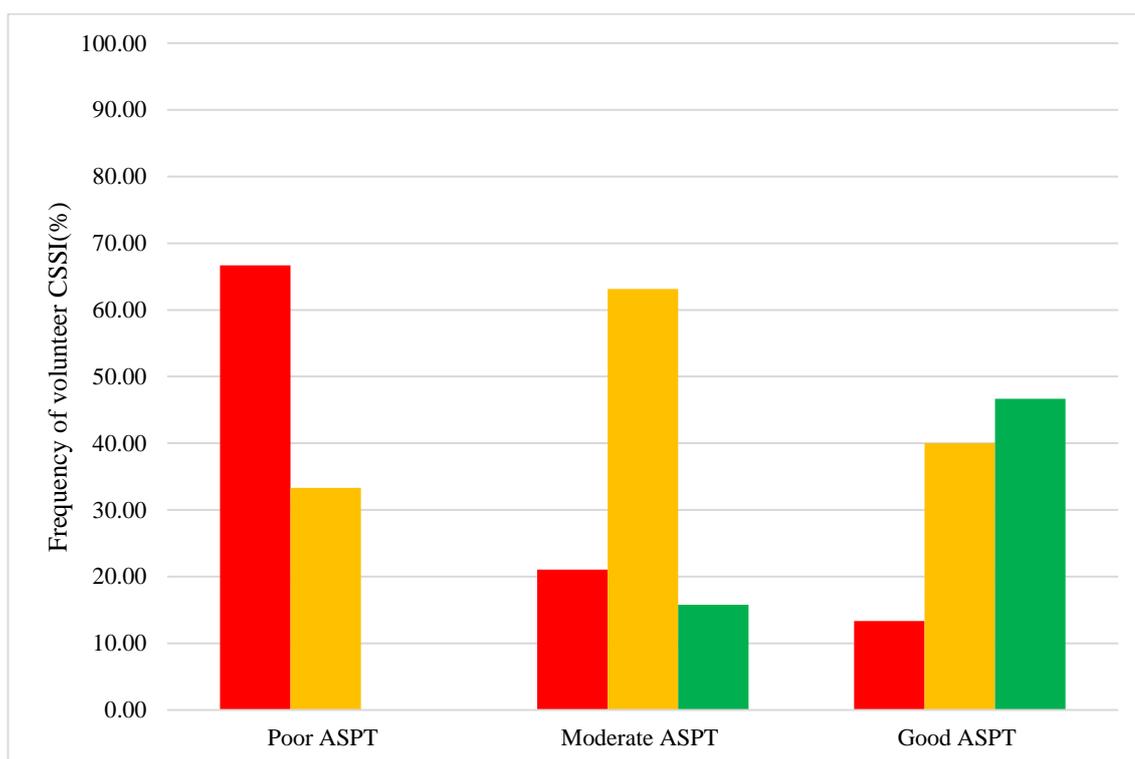

Figure 16. The percentage frequency of each water quality band identified by volunteers using the CSSI compared to the bands of water quality found by an expert using an equivalent CSSI

Table 8 shows how often the three bands of equivalent expert CSSI appear in the three bands of volunteer CSSI.

Table 8. Correlation values for the expert CSSI scores and volunteer CSSI scores. From this table it was found that 75% of the equivalent expert CSSI and volunteer CSSI samples agreed.

	Expert CSSI Poor	Expert CSSI Moderate	Expert CSSI Good
Volunteer CSSI Poor	8	2	0
Volunteer CSSI Moderate	2	16	2
Volunteer CSSI Good	0	4	6

Figure 16 shows the VCS compared to the equivalent expert CSSI. This test validates how often volunteers correctly identified the six indicator species. There is excellent agreement between the VCS and equivalent expert CSSI, with 75% total agreement of raw data as shown in Table 8. This validates the feasibility of the CSSI in the field by citizen scientists independent of experts with a small amount of initial training.

Figure 17A (left). A map of the Nore River catchment developed using QGIS 3.16, illustrating the data points submitted by the NoreVision volunteers. Green dots represent sampling points rivers with a CSSI score greater than 4, the orange dots represent sampling points with CSSI scores between -4 and 4 and the red dots represent sampling points with CSSI scores less than -4. Figure 17B (right). Q-Values from gis.epa.ie/EPAMaps/ used as a guide for selection of rivers for NoreVision volunteers. Where blue = Q5, green =Q4, yellow =Q3-4, orange = Q3

Figures 17A and 17B show a visual display of traffic light and colour coded water quality bands in the same area of the river Nore catchment based on the CSSI and Q-value respectively.

In Area 1, in the Southeast of catchment, there is a tributary of the Nore River (R. Arrigle) with multiple good water quality sample sites along the length of the river. This is shown by the Q-values on the right and confirmed by the VCS samples on the left.

In Area 2, Southwest of Kilkenny City near the town of Callan, there is an area of small tributaries with multiple poor water quality sample sites, as shown by the Q-values on the left and confirmed by the VCS on the right. This is an area of high intensity dairy farming which may be a factor in the river water quality. This is a common problem in Irish rivers (Hooda et al., 2000).

Area 3, North of Kilkenny, there is a tributary of the Nore River (R. Dinin) with multiple good water quality sample sites along the length of the river. This is shown by the Q-values on the right and confirmed by the VCS on the left.

4. Discussion

4.2 Establishment of the CSSI

Two independent methods were used in this study to identify macroinvertebrates that fit the criteria as ideal indicators (Füreder and Reynolds, 2003, Hilty and Merenlender, 2000, Rosenberg and Resh, 1993). The first method used was a PCA. In PCAs multiple variables are expressed mathematically as vectors. Numerous straight-line equations (principal components) can be calculated from the vectors such that projections of the original data on them have maximum variance. This allows the reduction of the dimensionality of large data sets, increasing interpretability but at the same time minimizing information loss. (Jolliffe and Cadima, 2016, Krzanowski, 2000, Scullion, 1989).

The second method of analysis used the Q-values as a reference standard for water quality. The Q-values have been used by the Irish Environmental Protection Agency (EPA) to assess river water quality since 1971 (Flanagan and Toner, 1972, Clabby et al., 1992, McGarrigle et al., 2002). The Q-value system has been shown to be a robust and sensitive measure of river water quality and has been linked with both chemical status and land-use pressures in catchments (Kelly et al., 2007). The Q-value is calculated primarily on the basis of macroinvertebrate communities in riffle areas, but also considers aquatic macrophytes, phytobenthos and hydromorphology (Flanagan and Toner, 1972; Clabby et al., 1992; McGarrigle et al., 2002).

These two independent methods were consistent in identifying six macroinvertebrates that could define water quality and be suitable for indicator taxa for citizen science. This compares favourably with the Riverfly Partnership and the OPAL water surveys methodologies which use 8 and 13 freshwater invertebrate groups to measure water quality respectively (Moolna. et al., 2020, Rae et al., 2019).

Of the six macroinvertebrates that were selected in this thesis, *Asellus aquaticus* and Plecoptera had the best relationship with poor water quality and good water quality respectively. *Asellus aquaticus* occurred in only 8.24% of the best water quality bands (<-2) and in 80.45% of the worst water quality bands (>2). Conversely, Plecoptera occurred in 88.24% of the best water quality bands and 22.56% of the worst water quality bands. Hirudinea and Gastropods, the other bad water quality indicators macroinvertebrates, although less indicative, were frequently present in poor streams (61.65% and 62.41% respectively) and infrequently present in good streams (31.18% and 27.06% respectively). These taxa were also selected for their characteristic shape and movement making them amongst the easiest macroinvertebrates for non- experts to identify. The remaining two indicator macroinvertebrates Heptageniidae and Rhyacophilidae were less indicative of good water quality than Plecoptera but were still present in 72.35% and 74.12% of the best water quality bands respectively and 40.60% and 41.35% of the poor water quality bands respectively. But they were included since Heptageniidae are easily identified with their flattened heads, three tails and lurching movement and Rhyacophilidae are easily identified with their green bodies, spikey abdomen gills and sigmoidal movement

In rivers with less than Q3 there is a 90% chance of finding the three bad water quality indicators and in the rivers with a Q5 there is an 85% chance of finding the three good water quality indicators. This is likely due to the taxa on the left being intolerant of pollution and the taxa on the right being tolerant of and or thriving in polluted rivers.

A protocol was developed that allowed identification and measurement of these invertebrates on the riverside by citizen scientists using a traffic light reporting system. The name of this index was chosen to be the Citizen Science Stream Index (CSSI).

4.2.1 CSSI boundary variations

The outcome of the data depends on the relative grading of the score within the traffic light system. In this thesis three variations of boundary limits were used to find the optimal boundary for the CSSI traffic light protocol. Simple bar charts were chosen as a form of validation as they clearly showed the information that the CSSI is designed to investigate i.e., is the water quality poor, moderate or good?

It was decided that somewhere between variation 1 and variation 3 would be ideal as this would minimise the frequency of poor and good CSSI water quality bands presenting in the moderate Q-value bands whilst still accurately reporting good and bad rivers sufficiently. As variation 1 would scale up in a triplicate CSSI score to a boundary of -6 and +6, and variation 3 would scale up to -3 and +3 it was decided that a triplicate score of -4 and +4 should be the boundaries of the traffic light system for a full reading of the CSSI protocol.

4.3 Validation of the CSSI

It is well recognised in the literature that the indices used to evaluate river water quality are very different and vary from country to country (Birk and Herring, 2006, Knoben et al., 1995, Tampo et al., 2021). Thus, compared to other scientific arenas there is a lack of gold standard or benchmark test that is recognised internationally to compare novel approaches to water quality testing (Nijboer et al., 2004). In the case of novel biotic indices, most are measured against the most acceptable local method when national studies are performed (Ghaini et al., 2018)

In 2003 the EPA and relevant local authorities were appointed under European community regulations as the competent authorities for the implementation of the EU

WFD (Barr et al., 2004). As such, the EPA's Q-Value system has local recognition as a measure with sufficient credibility to act as a good reference standard which was used in this study to evaluate the accuracy and validity of the CSSI.

Variation in the performance of metrics has been highlighted by other studies conducted within Europe (Dahl et al., 2004, Lorenz et al., 2004). However, the biotic metrics are routinely applied in summer with the view to determine the ecological quality when organic impacts may be highest.

4.3.1 Desktop validation

4.3.1.1 Feeley et al. 2020 analysis

When the protocol was checked using a much larger data set (Feeley et al., 2020) the distribution of the six invertebrate groups were found to be as expected. The first graph (Figure 9A) validated the findings in the larger Feeley et al. 2020 data set via a Q-value analysis. This analysis showed that the same six macroinvertebrates were capable of defining quality to an even greater extent as the relationship between the taxa and the water quality was more definite. Some variation in frequency was seen in the less indicative macroinvertebrates in the moderate water quality bands, this was similar to the variation found when the six macroinvertebrates were established as useful indicators using the Feeley et al 2012 data set. Overall, the tolerance of these macroinvertebrates agrees with the findings of other researchers (Uherek and Pinto Gouveia, 2014, Paisley et al., 2014). As such, this variation was considered acceptable.

Gastropods which are one of the poor water quality indicators had a particularly weak relationship with water quality in this analysis where it was shown that they tend to live

in moderate (Q3-4 66% frequency) as well poor water quality (<Q3- 53.64%). Although this is not ideal, Gastropods are very easily identifiable to any level of citizen scientist, and they are still rarely found in rivers with good water quality.

4.3.1.2 Existing metrics compared to Q-values

The SSRS identified poor water quality accurately when compared to the reference standard Q-value band, but over expressed poor rivers in the "indeterminate" and "probably not at risk" bands. This could be detrimental as nearly 80% of moderate Q-value sample sites are incorrectly classified as at risk and nearly 30% of rivers that are incorrectly classified as good Q-values will be reported as the opposite using the SSRS scoring system.

The ASPT comparison showed very similar results to the CSSI variation 1, a variation that was rejected. There was a 22% of poor ASPT sample sites incorrectly classified in the moderate Q-value band and over 23% good ASPT sample sites incorrectly classified in the moderate Q-value bands.

It was found that for the extreme low Q-values i.e., the rivers with extremely poor water quality, the CSSI did not accurately represent the poor water quality. In the rivers with Q-values less than Q3, -2 was the most common score. In rivers with a Q1 score the most common score was -1. This is likely due to the lack of poor indicator macroinvertebrates present in the sample. In other words, even the poor water quality indicators do not live in extremely poor streams. Furthermore, if the sampling in this data set was consistent and the CSSI protocol was carried out in triplicate a -2 would lead to a CSSI score of -6 over the three samples. Therefore, in Q-values less than Q3, the CSSI would still report a poor water quality band (red traffic light band) for the appropriate sample sites. Examination of the Feeley et al 2020 data set shows that sites

with water quality less than Q3 accounts for a small number of sites tested (338 of 10,995 (3.07%)) and minimal number of sites tested with water quality of Q1(23 of 10,995 (0.21%)). More work needs to be carried out on extremely poor water quality sites to clarify the relationships with macroinvertebrates.

4.3.1.3 Implant data set analysis

Many studies have been done reviewing the efficacy of biological monitoring indices over different seasons with varying results, but most studies have shown significant changes in water quality ratings between the spring and summer seasons. (Callanan et al., 2008, Outridge, 1988, Zhang et al., 2012). In a study of the effects of seasonal changes on the indices used in this thesis it was found that ASPT scores proved to be the most resilient between seasons, resulting in only 16% change between the spring and summer season. The various other metrics applied resulted in notable change in status assigned. The SSRS protocol was also relatively resilient with a 28% change in category during summer relative to spring. The other metrics were more variable between season, namely BMWP (63%) and Q-value (98%) (Callanan et al., 2008).

This data set was used to validate the CSSI's relative consistency through three seasons. In this data set there was no Q-values or water chemistry to compare with and the sampling was taken in triplicate. This closely resembles the data that citizen scientists will be able to produce themselves using the CSSI protocol. In this thesis the average scores and standard deviations of the SSRS, CSSI and ASPT were consistent with one another. It was expected that the CSSI's standard deviation would be higher than the other scores as the CSSI score have a wider range of possible outputs. Furthermore, the water quality bands based on the traffic light scoring system were in 100% agreement. This validates the use of the CSSI through different seasons.

4.3.1.4 Rylane data set analysis

This data set was selected as it can show how the CSSI reacts across a known set of water qualities i.e., initially polluted water and progressively less polluted samples. Using the macroinvertebrates that were collected the ASPT, SSRS and CSSI score were calculated. In this data set there was no Q-values or water chemistry to compare with and the sampling was taken in triplicate. This closely resembles the data that citizen scientists will be able to produce themselves using the CSSI protocol. Initially all three metrics showed low scores. The CSSI initially produced a score of -3 (moderate/orange in the traffic light system). This discrepancy could be explained by the lack of *Asellus aquaticus* in the entirety of the Rylane data set. *Asellus aquaticus* is one of the poor water quality indicator taxa in the CSSI. It has been shown that exposure of *Asellus aquaticus* to domestic and industrial effluents may cause sublethal biological effects and influence organism's ability to compete, grow, and reproduce (O'Neil, 2004). This could explain the lack of *Asellus aquaticus* in the river or this could simply not have a population of *Asellus aquaticus* present in this single river.

Despite this, the overall scores of all three metrics along the course of the stream validated the CSSI since it matched the ability of the ASPT and SSRS to define water quality in a longitudinal study.

4.4 Field study analysis

In this data set there are two CSSI scores. One gained from carrying out an equivalent CSSI on the expert samples named "equivalent expert CSSI" and one uploaded by the volunteers themselves that was named "Volunteer CSSI" (VCS). The VCS compares favourably with the ASPT, correctly classifying 67% of poor streams 63% of moderate streams and 46% of good sample sites defined by the ASPT.

The VCS agrees with the classification of the SSRS in 42% of the at risk (poor) and 56% of the probably not at risk (good) SSRS bands. However, there is a significant difference in the classification of moderate VCS to indeterminate SSRS, with all the moderate VCS being classified as either at risk or probably not at risk and none being classified as indeterminate as defined by the SSRS. This could be due to the small sample size of 40 sample sites, but it has already been indicated in Figure 12 that the SSRS often misclassifies moderates when compared to the reference standard Q-values. There is a lack of reviews of the SSRS in the literature making it hard to know if this is an anomaly with these results or is a common problem with the SSRS. As such, whereas the SSRS is a sensitive instrument that finds at risk waters easily the CSSI could be used as an economic, simple and rapid test in conjunction with the SSRS.

The VCS was compared to the equivalent expert CSSI. This test validates how often volunteers correctly identified the six indicator species. There is excellent agreement between the VCS and equivalent expert CSSI, with 75% total agreement of raw data as shown in Table 8. This validates the feasibility of the CSSI in the field by citizen scientists independent of experts with a small amount of initial training.

The NoreVision results were mapped to show a visual display of traffic light and colour coded water quality bands in the same area of the river Nore catchment based on the CSSI and Q-value respectively.

In Area 1, in the Southeast of catchment, there is a tributary of the Nore River (R. Arrigle) with multiple good water quality sample sites along the length of the river. This is shown by the Q-values on the right and confirmed by the VCS samples on the left.

In Area 2, Southwest of Kilkenny City near the town of Callan, there is an area of small tributaries with multiple poor water quality sample sites, as shown by the Q-values on

the left and confirmed by the VCS on the right. This is an area of high intensity dairy farming which may be a factor in the river water quality. This is a common problem in Irish rivers (Hooda et al., 2000)

Area 3, North of Kilkenny, there is a tributary of the Nore River (R. Dinin) with multiple good water quality sample sites along the length of the river. This is shown by the Q-values on the right and confirmed by the VCS on the left. This confirms the ability of the CSSI to identify and differentiate poor and good water quality areas.

Variations in biomonitoring are inevitable due to seasonal influences of macroinvertebrate breeding and flooding or by random chance and for this reason biomonitoring can only be used as an indication of water quality and should not be an outright assessment of the full ecological state of a water body. Which would require chemical, physical and biological assays (Moog et al., 2018), however, this is not feasible for a large volume of smaller headwaters.

In the field study, an online app was used by the volunteers to upload data collected, and by the administrator to analyse the data. Although the volunteers commented on the usefulness of the online app as a resource throughout the programme, especially during the Covid-19 pandemic when face to face meetings were not always possible, the online app did not allow additional observations to be made as was defined in the written protocol. This means that observations made by volunteers were potentially lost. In the future, an improved app would allow uploading of observations by the volunteers.

In the leaflet it was suggested that volunteers send a photograph of the sample in the white tray with their sample readings. Although initially this was thought to be a good method of macroinvertebrate validation, it was found that photographic quality, glare and debris in the sample made identifying the macroinvertebrates in the photograph

difficult. In the future, photographs of each indicator invertebrate could be sent with the sample readings for macroinvertebrate identification purposes.

The CSSI allows many sites to be assessed at a coarse resolution within a catchment avoids and expertise-intensive multi-taxa techniques. Citizen scientists can take periodic samples upstream from a polluted area and determine the exact geographical location of pollution inputs within a catchment as opposed to just defining the extent of the problem at one point.

To make a citizen science project operate smoothly this study found it was important to continue to motivate and communicate with the volunteers regularly through social media and group kick sampling session. Without this, volunteers can easily become disengaged.

After engaging with the CSSI, it was noted through communication with the volunteers via WhatsApp that they went on to participate in other river improvement schemes such as litter picks and nationwide biodiversity citizen science initiatives which shows a dual purpose for the CSSI as a method of data capture and engagement of volunteers with their local environment. For this reason, the CSSI could be used as an introduction to biomonitoring and water quality for non-experts of all skill levels.

This thesis shows that the CSSI tends to over express moderate water quality, in the future it may be sensible to rename the banding of the moderate to inconclusive.

5.Conclusions

In conclusion, in this study, six easily recognisable and common macroinvertebrates with narrow pollution tolerances, that can be used to indicate water quality were identified. These were incorporated in a Citizen Science Stream Index (CSSI) protocol which was validated using Q-values as a reference standard. The CSSI was found to be reasonably accurate and effective at allowing citizen scientists to differentiate good water quality and poor water quality independent of an expert.

In comparison with contemporary indices, the CSSI proved to be as robust as the ASPT and SSRS and therefore can work in conjunction with established metrics in nationwide data capture. This thesis shows that in a pilot study the CSSI protocol is feasible for citizen scientists to learn and implement in a reliable way. Furthermore, multiple volunteers in the pilot study commented on how easy the CSSI was to learn and use. The CSSI is an efficient index that allows a spatially extensive analysis to be carried out at a course resolution.

Defining water quality is a subjective undertaking as water quality can have different parameters for different uses. To accurately evaluate the overall health of a river in detail many different analyses must be carried out on the physical, chemical and biological aspects of the river. However, this is costly, time consuming and not practical for monitoring a large number of rivers especially smaller headwaters. Biomonitoring is a quick fix for water quality monitoring but is variable even when using established metrics like the ASPT, SSRS and Q-Values as shown in this study. As variability in scores exists among all biomonitoring indices regardless of the complexity of the methodology, a simple methodology such as the CSSI is preferable.

The CSSI has started to be rolled out all over Ireland with data now being captured in real time via LAWPRO website for Citizen Science https://lawaters.ie/citizen-science/.

Over the Covid 19 period over 700 volunteers have been trained in the use of the

Citizen Science Index. Approximately 20% also received kick nets and other

equipment. This training took the format of blended learning with an online session

and a field-based session to implement the training in Action. In the future the CSSI

will be rolled out around more of Ireland enabling volunteers to participate in the
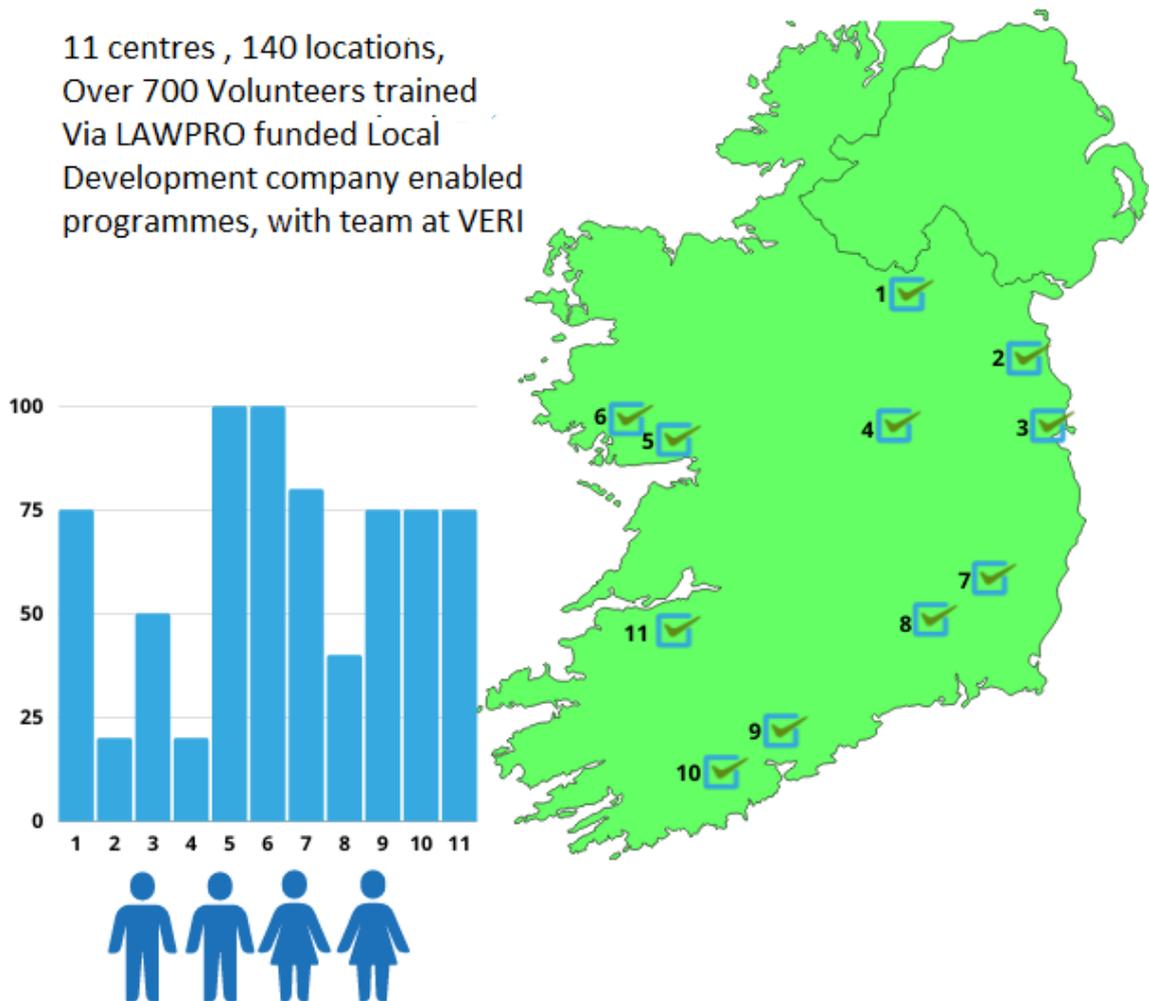
nationwide biomonitoring of Irelands streams.

Figure 18. Infographic on the CSSI's progress in training around Irelan

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APPENDIX 1

Picture 1. Opening picture of the CSSI tutorial

Picture 2. Dr.Simon Harrison demonstrating a kick sample on the CSSI tutorial video

Picture 3. Underwater shot of kick sampling, demonstrating how stones must be turned over with the foot
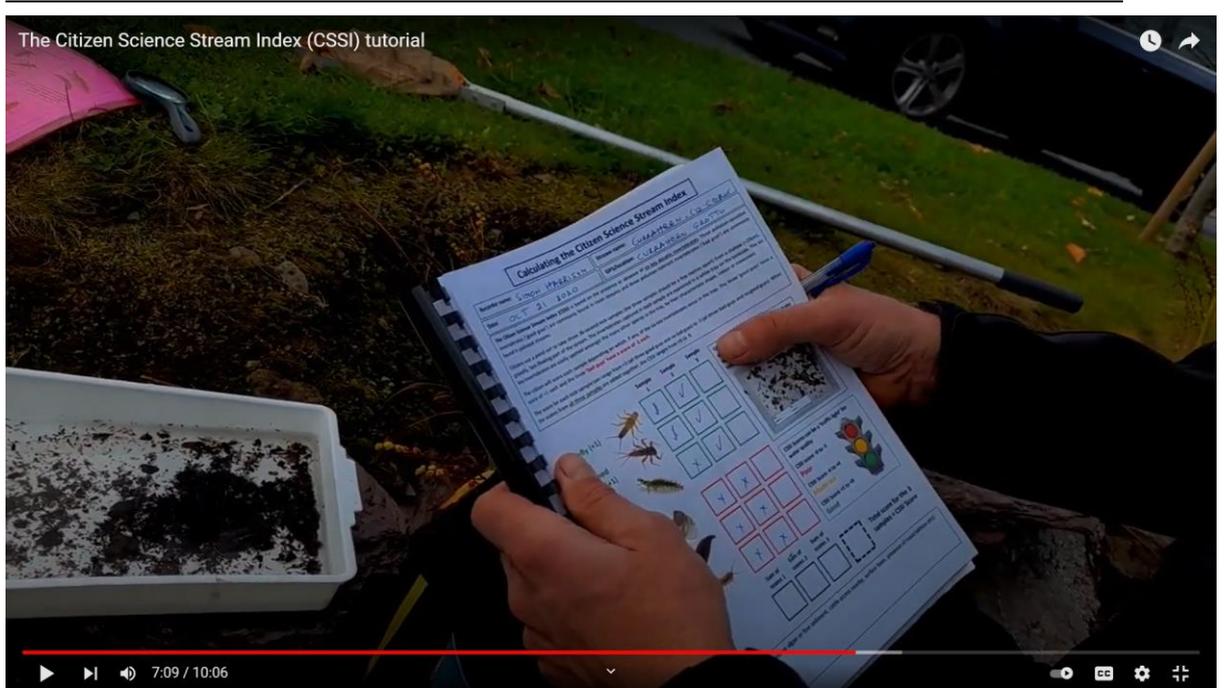
in the CSSI tutorial

Picture 4. Dr. Simon Harrison demonstrating elutriation on the CSSI tutorial video.

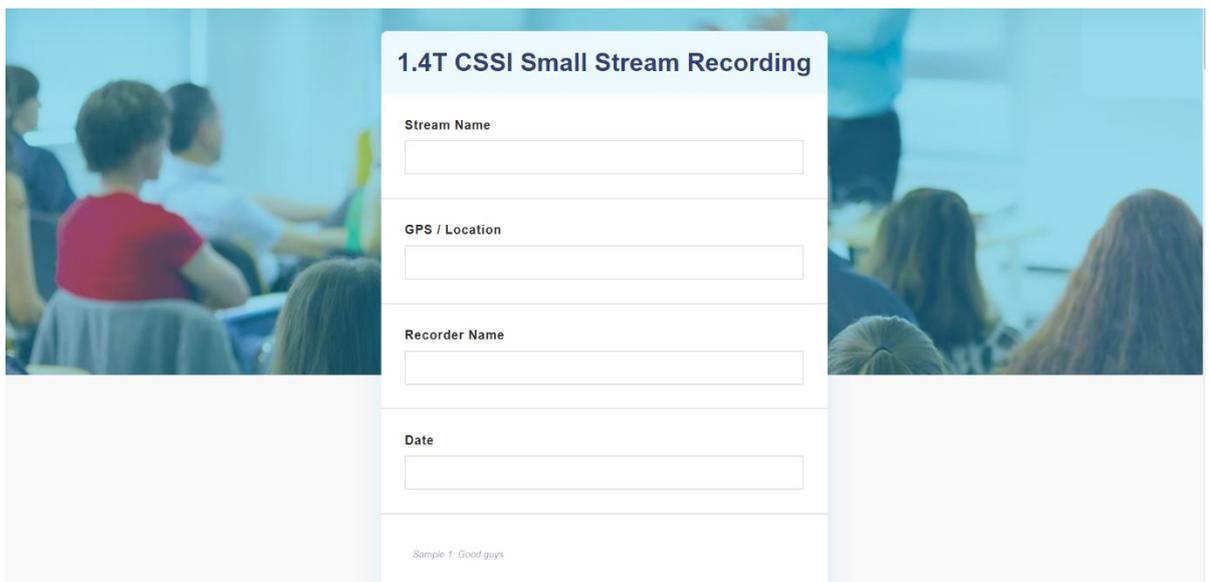Picture 5. An example of a stonefly, one of the six key indicators taxa shown and described in the identification section of the CSSI tutorial video.

Picture 6. Explanation of the key attached to the CSSI leaflet on the CSSI tutorial video

Picture 7. Explanation of the leaflet used to calculate the CSSI score on the CSSI tutorial video

APPENDIX 2

Picture 8. The top of the form that was used to upload the CSSI scores by the volunteers. The form contains the stream name, GPS/ Location, Recorder Name and Date.

Picture 9. Checklist on the form used by volunteers to upload their CSSI scores. This checklist asks which of the six key indicator taxa were present in kick sample 1.

Picture 10. Checklist on the form used by volunteers to upload their CSSI scores. This checklist asks what was the CSSI score of the first kick sample.

Picture 11. Checklist on the form used by volunteers to upload their CSSI scores. This checklist asks what was the sum total of the three kick samples taken otherwise known as the final CSSI score.