- **1** Evidence for humane stunning in the slaughter of
- ² wild-caught fish for food: A Systematic Map

3 Protocol

- ⁴ Katy L. James¹, Nilantha S. Jayasuriya¹, Tharangani K. Herath¹, Jeff
- ⁵ Lines², Lynne U. Sneddon³, Upali S. Amarasinghe⁴, Salvador Prats
- ⁶ Aparicio¹, Nicola P. Randall¹
- ⁷ ¹ Harper Adams University, Newport, Shropshire TF10 8NB, UK
- 8 ² Silsoe Livestock Systems Ltd, Wrest Park, Silsoe, Bedfordshire, MK45 4HS, UK
- ⁹ ³ University of Gothenburg, Department of Biological & Environmental Sciences, 40530
- 10 Göteborg, Sweden
- 11 ⁴ University of Kelaniya, Kelaniya, 11600, Sri Lanka

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23 Abstract

24 Background

25 An estimated 0.79 to 2.3 trillion finfish are caught from the wild globally each year. The vast majority of these fish are not humanely stunned before killing, and evidence suggests these 26 27 fish may experience significant suffering between the time they are captured and their death. Recommendations exist to improve the welfare of farmed fish at slaughter, through 28 the use of humane stunning methods/devices, to ensure immediate and irreversible loss of 29 consciousness which lasts until death. However, to date no specific guidelines exist for wild-30 caught fish. There is a growing interest in using humane stunning to improve the welfare of 31 32 wild-caught fish at slaughter but at present there is no systematic overview of the literature 33 on this topic.

34 Methods

35 This systematic map protocol addresses the following question: "What is the evidence for 36 humane stunning in the slaughter of wild-caught fish for food?" Searches will be performed 37 in 6 bibliographic databases, one search engine and 19 specialist websites. Searches will be performed in the English language. Coding and meta-data extraction will include 38 information on humane stunning device/method, fish species, study country and location, 39 and outcomes relevant to fish welfare, impact of stunning device/method on flesh quality 40 and any other economic, social, socio-economic, environmental, ethical or practical 41 considerations. All screening and coding will be done after initial consistency checking. The 42 43 outcomes of this systematic map will be a searchable database of coded studies. Findings 44 will be presented in a geo-informational system (i.e. an evidence atlas) and knowledge gaps and clusters will be visualised via heat maps. 45

46 Keywords

Animal welfare, Slaughter, Percussive stunning, Electrical stunning, Flesh quality, Finfish,
Fisheries

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51 Background

52 It has been estimated that between 0.79 and 2.3 trillion finfish were caught from the wild

53 globally each year for 2007-2016 [1]. Evidence that fish are sentient, and able to experience

54 fear, pain and suffering [2] has led to international recognition that there is a need to

improve the welfare of fish for consumption, including at point of slaughter [3].

56 Most commercially caught wild-fish that are alive when landed die either from asphyxiation,

57 (in air or ice), or evisceration [4]. These methods of slaughter (in addition to chilling with ice

in holding water, carbon dioxide (CO₂) in holding water; chilling with ice and CO₂ in holding

59 water; salt or ammonia baths; and live exsanguination), are not considered humane by

60 European Food Safety Authority EFSA [5,6,7,8,9,10,11] and the World Organisation for

Animal Health (OIE) [3], suggesting that wild-caught fish may experience significant suffering

62 between the time they are captured and their death.

To date, no specific guidelines or legislation exist to improve the welfare of wild-caught

64 finfish at slaughter. Guidelines and legislation do exist, however, for fish farmed for human

65 consumption. Based on available scientific evidence, the OIE Aquatic Animal Health Code

66 [3]) and EFSA [5,6,7,8,9,10,11,12] recommends as a general principal that fish should be

67 stunned before killing. Species specific guidelines, are however limited [3,

5,6,7,8,9,10,11,12]. The OIE code states that: "the stunning method should ensure

69 immediate and irreversible loss of consciousness. If the stunning is not irreversible, fish

should be killed before consciousness is recovered" [3].

71 Stunning can be defined as 'any intentionally induced process which causes loss of

72 consciousness and sensibility without pain, including any process resulting in instantaneous

73 death,' [13]. Stunning methods for finfish, regarded as humane and globally acceptable, fall

⁷⁴ into two main categories mechanical, including percussive stunning, spiking or coring and

75 free bullet methods', and electrical (in water, semi-dry and dry) [3, 6,7,8,9,10,11,12].

76 Depending on the species that the stunning is applied to, and the parameters used, the stun

77 may cause death (stun-killing method) or the stun may be reversible and require a follow-up

killing method before consciousness is recovered. In general, mechanical stunning if applied

correctly is irreversible, whereas unconsciousness following electrical stunning may be

80 reversible.

Electrical and percussive humane stunning technologies are widely used in some sectors of 81 82 the aquaculture industry, for example, salmon and trout farming [14]. The development of new commercial humane stunning technologies for aquaculture, is an active area of 83 84 research [15]. Knowledge and technology, from the aquaculture sector is likely to be highly 85 relevant, to the development and promotion of humane stunning in wild-capture fisheries. 86 Figure 1. illustrates some of the potential enabling processes for implementation of humane 87 stunning in wild-capture fisheries, including adaption of existing technologies from 88 aquaculture.

89 A wide range of challenges exist for the implementation of humane stunning in wild-capture fisheries. These include but are not limited to: the suitability of humane stunning methods 90 91 for different fish species [6,7,8,9,10,11,12,16], size of catch [3] and capture method [17]. 92 The method, efficacy and minimum stunning control parameters for the humane stunning of finfish have only been developed and validated for a limited number of species and their 93 94 environments [3]. Funding for research and development is required to determine 95 parameters for a greater range of fish and their environments, and to develop new technology, or refine and modify existing aquaculture technologies, for use in wild-capture 96 fisheries. 97

To ensure good welfare, loss of consciousness must be confirmed, for any new or modified 98 99 stunning methods. The European Food Safety Authority (EFSA) recommends that 100 confirmation in a controlled environment of an unconscious condition post stunning is 101 verified using neurological measures of brain electrical activity such as 102 electroencephalogram (EEG) [18]. Under field conditions, however, many researchers use 103 behavioural and reflex indications, which do not directly quantify neurological activity, and only provide an indication of the likely state of consciousness [18], as a more easily 104 105 obtainable alternative to EEG. Ideally, these indicators need to be correlated with EEG 106 findings demonstrated in controlled environment studies [18].

The commercial viability and practicality of stunning method/technology also needs to be
considered. For example, in a study by Nordgreen et al. [19], the authors stated, that
electrical stunning would promote the welfare of Atlantic herring but negatively affect fillet
quality. Sophisticated stunning equipment is typically expensive [20] and implementation on
board vessels may also require costly vessel modification.

A recent study by Anders et al. [17], illustrates some of the challenges associated with 112 research and implementation of humane stunning in wild-capture fisheries. The study 113 indicated that a commercially available dry electrical stunner was effective at slaughtering 114 115 mackerel in a manner consistent with good welfare, based on behavioural and reflex 116 indicators, and did not induce quality defects. However, the authors highlighted that further 117 research is required to verify unconsciousness by EEG, and that developing an efficient method for pumping large pelagic catches from nets in combination with 5 second electrical 118 stunning will be challenging. The authors stated that new technology may need to be 119 120 developed and modifications to dewatering units currently in use may be required to ensure 121 the fish are stunned in "dry" conditions.

122 Equally, there are potential economic gains for wild-capture fisheries to be made from 123 improving fish welfare, via humane stunning. Pre-slaughter stress has been shown to initiate behavioural (increased physical activity) and physiological (e.g. muscular pH) responses in 124 125 fish that negatively affect flesh quality [16]. Humane stunning methods that rendered fish unconscious to minimise pre-death struggling and potentially reduce stress between 126 capture and stunning (e.g. minimising handling or keeping fish in water) may improve flesh 127 quality and therefore bring about both animal welfare and economic benefits. It is also 128 129 possible that implementation of on board humane stunning technology, may also reduce 130 the cost of capture for fisheries. Furthermore, fisheries that implement humane stunning 131 may benefit economically from retailers and consumers requesting higher-welfare fish products which often command higher prices. 132

The aim of this review is to, identify and describe the evidence base surrounding humane stunning methods of relevance to the slaughter of wild-caught fish for food. The review will include evidence about the implications for fish welfare, and quality as well as practicality to implementation and sustainability, to provide a better understanding of the evidence base surrounding this topic.

To our knowledge no systematic collation of the evidence relating to this topic has beenconducted to date.

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- 141 Figure 1. Potential enabling processes for implementation of humane stunning in wild-
- 142 capture fisheries



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145 **Topic identification**

- 146 The topic was initially proposed by the research funder, the Humane Slaughter Association
- 147 (HSA), who called for a systematic evidence synthesis and feasibility analysis for the
- 148 development and use of humane stunning or stun/killing methods in wild-capture
- 149 commercial fisheries. The call specified that the research should also consider the
- 150 practicality and sustainability of such methods, including economic, environmental, ethical
- 151 and social considerations.
- 152 As part of the process of drafting an application in response to the call, the project team
- 153 developed a systematic mapping methodology to: address the broad topic and questions set
- 154 by the funder; and to inform the feasibility analysis.

155 Stakeholder engagement

156 The reviewers will engage with 3 stakeholder groups:

- The HSA: initial revising of the scope, questions and search strategy of the map, was
 undertaken through a meeting with the HSA, the research team, and review advisory
 group, to ensure relevancy of the map outcomes with the HSA requirements.
- Review advisory group: comprising academics from disciplines including fish stunning technology, fish biology, health and welfare, and commercial fisheries. This advisory group helped to refine the systematic map protocol, including identifying a list of articles for testing the comprehensiveness of the searches, inclusion criteria and search strategy. The advisory group will not carry out the mapping processes but will provide topic advice, help identify relevant literature through their networks and help interpret findings or set in context findings from the map.
- External stakeholder group: the systematic map forms part of a large project that
 includes a feasibility analysis for the development and use of humane stunning for
 wild-capture fisheries. An external stakeholder group comprising representatives
 from industry, research and non-governmental organisations will be established and
 consulted as part of this project. The stakeholder group will help to identify relevant
 literature, using their networks and interpret or set in context, the findings from the
 project including the systematic map.

174 Demonstrating procedural objectivity

The transparent, objective, and verifiable methods used to create the map will remainrobust to any potential stakeholder bias or undue stakeholder influence [21].

Some of the review and advisory team have authored or worked on research within this
field prior to starting this project. Reviewers who have authored papers which are found
during the searching process will not review these publications to avoid biases towards
these publications. These papers will be screened at both abstract and title screening and
full text screening by an impartial reviewer. Reviewers and stakeholders who have authored
papers will be prevented from providing advice or comments relating specifically to research
papers to which they may have contributed that may bias the outcomes of the map.

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185

186 **Objectives**

- 187 The objective of this systematic map is to catalogue and describe published and grey
- 188 literature relevant to humane stunning in the slaughter of wild-caught fish for food. The
- 189 systematic map will provide a better understanding of the evidence base and will be used to
- identify knowledge gaps that would benefit from primary research, and sub-sets of evidence
- 191 that may be suitable for further secondary synthesis.
- 192 Primary question
- 193 *"What is the evidence for humane stunning in the slaughter of wild-caught fish for food?"*
- 194 The primary question will be framed using the Population, Intervention, Comparator and
- 195 Outcome (PICO) key elements.
- 196 Table 1. PICO key elements for the primary question

Key Element	Key Element Descriptor
Population	Wild-caught fish for food
Intervention	Humane stunning methods and devices
Comparator	No stunning; different stunning methods/devices; no comparator
Outcomes	Fish welfare; Post-stunning flesh quality; Economic, environmental,
	ethical, social, socio-economic or practical considerations

197

198 Secondary questions

199 The following secondary questions will be address using the literature gathered for the

200 primary question:

a. Is there any evidence of the commercial use of or testing of stunning

- 202 devices/methods on-board wild-capture fishing vessels? What types of stunning
- 203 devices/methods have been used or tested on-board for different species of wild-
- 204 caught fish? Where have these devices/methods been used or tested205 geographically?

207	b.	What types of stunning methods/devices have been tested for different fish species
208		under laboratory or farmed conditions that are of relevance to wild-capture
209		fisheries? Where have these studies been carried out geographically?
210	c.	What fish welfare outcomes are reported?
211	d.	What evidence is there about the impact of stunning on flesh quality?
212	e.	Is there any evidence about the feasibility or economic viability of the use of
213		stunning for wild-caught fish?
214	f.	Are there any studies or methods that investigate the process between capture and
215		application of stunning method to minimise suffering prior to stunning?
216	g.	Are there any studies about the cost implications of the use of stunning in wild-
217		capture fisheries on product price?
218	h.	Are there any studies on the likelihood of uptake?
219	i.	What other environmental, social, socio-economic, economic or practical
220		implications are reported in studies about stunning in wild-capture fishing?

221 Methods

222 The systematic map will follow the Collaboration for Environmental Evidence Guidelines and

223 Standards for Evidence Synthesis in Environmental Management [22] and it conforms to

ROSES reporting standards for systematic map protocols [23] (see Additional file 1). The

protocol also conforms to sections 1-11 (Additional file 2) which are relevant to systematic

226 maps of the PRISMA P checklist [24].

227 A request for public comment on the draft protocol (draft version available on request) was

made between 26.08.20 and 09.10.2020 using the following platforms: Systematic

229 Reviews for Animals and Food (SYREAF), ResearchGate and the Centre for Evidence-Based

Agriculture Harper Adams University webpage. Edits to the protocol were made in response

to comments received. This is the final version of the protocol that will be used to guide the

232 systematic mapping process.

233

234 Searching for articles

- 235 A comprehensive search to capture an un-biased sample of published and grey literature
- will be undertaken using multiple information sources. We will search bibliographic
- 237 databases using a tested and iteratively modified search string (Additional file 3). The search
- string was tested for sensitivity by comparing a benchmark list of 10 articles known to be
- relevant to the review team and topic experts (see Additional file 3). This search string will
- 240 be adapted according to each database's input syntax.
- 241 Searches will be carried out in English and Spanish language using subscriptions from Harper
- Adams University. The following search string will be used, where possible, to search all
- 243 bibliographic databases:
- 244 (*Fish*) AND (stun* OR slaughter* OR welfare OR electronarcosis OR euthan* OR "electric
- shock") NOT (stunt* OR pig* OR swine OR pork OR cattle OR cow* OR beef OR chicken* OR

poultry OR turkey* OR lamb* OR sheep OR calf OR calves OR bull* OR jellyfish* OR crab* OR

- 247 trematode*)
- 248 Bibliographic database searches
- 249 1. Scopus
- 250 2. Food Science Source
- 251 3. CAB abstracts
- 252 4. Web of Science Core Collections
- 253 5. Electronic Theses Online Service (EThOS),
- 254 6. Digital Access to Research Theses (DART-Europe E thesis)
- 255

256 Web-based search engines

- 257 Attempts to identify grey literature will include searches of Google Scholar which has been
- demonstrated to be effective in identifying traditional academic and grey literature [25].
- 259 Results will be sorted by relevance, and the first 500 exported into Endnote.

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261 Organisational websites

- Additionally, the websites of 19 key organisations will be searched for relevant studies by
- using built-in search facilities and by searching the sites 'by hand' (i.e. focusing on any
- 264 'Publications' pages and examining site maps where available). These websites will include:
- 265 Fish Count [http://fishcount.org.uk/]
- 266 Centre for Environment Fisheries and Aquaculture Science [https://www.cefas.co.uk/]
- 267 Defra online databases [https://www.gov.uk/government/organisations/department-for-
- 268 environment-food-rural-affairs]
- 269 Food and Agriculture Organization [http://www.fao.org/]
- 270 UFAW [https://www.ufaw.org.uk/]
- 271 SINTEF Fisheries and Aquaculture [https://www.sintef.no/]
- 272 WWF [https://www.wwf.org.uk/]
- 273 International Marine Ingredient Association [https://www.iffo.net/]
- 274 Sea Fish [https://www.seafish.org/]
- 275 WorldFish [https://www.worldfishcenter.org/]
- 276 Marine Stewardship Council [https://www.msc.org/home]
- 277 Compassion in World Farming [https://www.ciwf.org.uk/]
- 278 EFSA [http://www.efsa.europa.eu/]
- 279 Wageningen University [https://www.wur.nl/en.htm]
- 280 Nofima [https://nofima.no/en/]
- 281 Ace Aquactec [https://aceaquatec.com/]
- 282 Fair Fish International FishEthobase [http://fishethobase.net/]
- 283 Humane Slaughter Association [https://www.hsa.org.uk/]
- 284 European Commission http://publications.europa.eu/
- 285 Royal Society for the Protection of Animals [https://www.rspca.org.uk/]

286 Public call for evidence

Finally, a public call for relevant studies and sources of studies that may not be readily identified will be made via the expert advisors' and stakeholders' networks and social media.

A record of each search will be made including: date the search was conducted; database name; search term used; how the database was searched (e.g. by title, abstract and keyword); number of hits; and notes. This information will be provided as additional information to be published alongside the systematic map.

294 After the search results have been collated in reference management software EndNote,

295 duplicates will be removed using a combination of EndNote, and systematic review

296 management software EPPI-Reviewer 4 [26]. The review will be managed within EPPI-

297 Reviewer 4.

298

299 Article screening and study eligibility criteria

300 Screening process

The final set of deduplicated search results will be screened for relevance against inclusion 301 302 criteria in a 2-stage process: (i) title and abstract (screened concurrently for efficiency), (ii) 303 full text. We will attempt to retrieve full texts of relevant abstracts using Harper Adams University library subscriptions and inter-library loan requests. Where articles cannot be 304 sourced this way authors will be contacted directly with requests for full text either via 305 306 email or through social networking sites for scientists and researchers. Articles that cannot be located or accessed at full text will be recorded. The number of articles included and 307 excluded at each stage will be recorded and reasons for exclusion of articles at full text 308 309 recorded. This information will be provided as additional information to be published 310 alongside the systematic map.

Prior to commencing screening, a random sub-set of 10% of articles will be screened at title
and abstract by all reviewers and the level of agreement (consistency) calculated using
Cohen's Kappa analysis [27]. The same process will be carried out at the full text screening

stage. All disagreements will be discussed in detail and inclusion criteria definitions 314 improved where necessary. Where there is uncertainty about inclusion of an article, all 315 reviewers will examine the text and a consensus agreement will be made. A Kappa statistic 316 317 of 0.6 or higher will be considered acceptable [27]. Where the level of agreement is low 318 (below 0.6 agreement), further consistency checking will be performed on an additional set 319 (10%) of articles. Reviewers that have authored papers which are found during the 320 searching process will not review these publications to avoid biases towards these publications. These papers will be screened at both abstract and title screening and full text 321 322 screening by an impartial reviewer.

323 Eligibility criteria

The following inclusion criteria will be used to assess relevance of studies identified throughsearching.

Eligible population(s): wild finfish, caught on commercial scale, from inland and marine waters, that are intended for consumption by humans and/or animals. Any species or group of mixed species will be considered. Studies from fish farms and laboratory-based studies deemed relevant to wild-caught fish will be included (i.e. the species is caught commercially in the wild). Studies about fish caught for recreational purposes will not be included.

331 Eligible intervention(s): any stunning or stun/kill methods used in the slaughter of fish defined as humane by the OIE [3]. This includes: percussive stunning (mechanical or 332 333 manual), spiking or coring, free bullet, and electrical stunning (dry, semi-dry, wet). These methods may be applied alone or in combination. The following methods: chilling with ice in 334 335 holding water, carbon dioxide (CO₂) in holding water; chilling with ice and CO₂ in holding water; salt or ammonia baths; asphyxiation by removal from water; exsanguination without 336 stunning, have been shown to result in poor fish welfare [3]. Studies solely investigating 337 these less humane methods will not be included. Studies that compare humane and less 338 339 humane methods will be included, and detail about both interventions recorded. Any novel 340 or modified stunning methods that are potentially humane but not yet recognised by the 341 OIE will be categorised separately.

342 Eligible comparator(s): no stunning; different stunning methods/devices; no comparator

Eligible outcome(s): outcomes will include the impact of the intervention on fish welfare
(e.g. time between capture and death, or loss of consciousness; time to reach
unconsciousness post stunning; duration of unconsciousness); post-stunning flesh quality
(e.g. haematoma, spinal damage) and any practical, economic, social, socio-economic,
ethical or environmental implications resulting from stunning (e.g. ease of implementation,
cost of equipment, labour requirements etc). Outcomes will be captured iteratively as they
are identified in the relevant literature.

Eligible data type: we will include both quantitative and qualitative research, including both primary empirical research and secondary research (reviews will be catalogued in a separate database).

Eligible study design: we will include all types of primary empirical and secondary research including: randomised controlled trials; quasi-experimental designs; observational studies; systematic reviews; traditional reviews; and meta-analyses. Reviews will be screened for relevant studies to ensure they have been collated in the searches. Commentaries will not be included.

358 Geographical limitations: none

359 Date restrictions: none

Language: English language will be used to search for literature from bibliographic and grey
 literature sources (key papers identified in other languages may be translated if resources
 allow).

363

364 Study validity assessment

This systematic map will not assess study validity, which follows the guidance for systematic maps by CEE [22]. Some of the extracted meta-data and coding will however relate to internal validity, to aid any subsequent secondary synthesis conducted on the map's outputs.

369

371 Data coding strategy

- 372 We will extract and code a range of variables, (see Additional File 4 for full data coding
- 373 strategy). All meta-data and coding will be included in a detailed systematic map database,
- 374 with each line representing one study-location (i.e. each independent study conducted in
- ars each independent location). The format of the database will be a Microsoft Access
- 376 database.
- 377 Meta-data extraction and coding will be performed by multiple reviewers following
- 378 consistency checking on an initial coding of subset of between 10% full texts, discussing all
- disagreements. The remaining full texts will then be coded. If resources allow we may
- 380 contact authors by email with requests for missing information.

381 Study mapping and presentation

We will display the results of the systematic mapping process using a ROSES flow diagram [23]. We will narratively synthesise the relevant evidence base in our systematic map using descriptive plots and tables showing the number of studies identified across the variables described above. For more complex data, we will use heat maps to display the volume of evidence across multiple variables (see "Knowledge gap and cluster identification strategy", below).

388 We will also display the contents of our systematic map database in an Evidence Atlas; an 389 interactive, web-based geographical information system showing all meta-data and coding 390 on a cartographic map.

The systematic map database will be accompanied by a narrative synthesis of key findings describing the volume and nature of the evidence base for both the primary and secondary questions. The narrative synthesis will highlight evidence gaps and sub-sets of evidence that may be suitable for further secondary synthesis, as well as implications for policy and practice, and research.

396 Knowledge gap and cluster identification strategy

We will use interactive heat maps (cross tabulations of key descriptors, e.g. interventionsand outcomes, interventions and populations/settings) to display the volume of evidence

- 399 across multiple dimensions of meta-data in order to identify knowledge gaps (sub-topics un-
- 400 or under-represented by evidence) and knowledge clusters (sub-topics with sufficient
- 401 evidence to allow full synthesis). This will be performed by a methodology expert of the
- 402 review team (i.e. not a subject expert to avoid preconception bias).
- 403

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485 Funding

486 This work is funded by the Humane Slaughter Association HSA WCF-01/20.

487 Authors' contributions

- 488 KLJ produced a first draft of this manuscript, with input from NJ for the coding strategy and
- 489 background. All authors edited, and commented on the draft. All authors read and approved
- 490 the final manuscript.

491 **Registration of protocol**

- 492 This protocol is available on the <u>Centre for Evidence-Based Agriculture</u>, Harper Adams
- 493 University, project website pages and has been published on the platform <u>Systematic</u>
- 494 <u>Reviews for Animals and Food (SYREAF).</u>

495 Acknowledgements

- 496 Not applicable.
- 497 Ethics declarations
- 498 Ethics approval and consent to participate
- 499 Not applicable.
- 500 **Consent for publication**
- 501 Not applicable.
- 502 Availability of data and materials
- 503 Not Applicable
- 504 Competing interests
- 505 The authors declare that they have no competing interests.

506 Supplementary information

- 507 Additional File 1. ROSES for Systematic Map Protocols checklist
- 508 Additional File 2. PRISMA P checklist
- 509 Additional File 3. Scoping searches and benchmark articles to test comprehensiveness of
- 510 search strategy.
- 511 Additional File 4. Coding strategy