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1 **Stronger together: a workflow to design new fish polycultures**

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11 **Running title:** Polyculture design workflow

12 **Abstract**

13 Polyculture is a relevant practice for improving the sustainability of aquaculture, which raises interest
14 in implementing it in a variety of production systems. However, polyculture is a complex approach that
15 can result not only in complementarity among species but also competition among them and animal
16 welfare issues. Potential polyculture benefits can be expected provided that compatibility and
17 complementarity occur among the combined species. This places a premium on identifying the best
18 species combinations for a given aquaculture system. Here, we developed a conceptual integrative
19 workflow to standardise and plan the development of new fish polycultures. This workflow is designed
20 to screen all possible combinations in a set of species based on three successive steps of assessment.
21 Overall, these steps consider the compatibility and complementarity of co-farmed species as well as
22 stakeholder demands, sustainability and fish welfare. Step 1 consists of selecting the most promising
23 compatible species combinations (i.e. “prospective combinations”) as a function of stakeholder
24 opinion and expectations using databases and surveys. Step 2 validates the effectiveness of
25 prospective combinations based on bioassays by considering species complementarity and animal
26 welfare. Step 3 implements the best species combination(s) in aquaculture production, during which
27 prototyping allows the sustainability of the resulting commercial production system to be studied. In
28 conclusion, the workflow aims at being a valuable tool to innovate in aquaculture by exploiting the
29 opportunities and the strengths of polyculture.

30 **Keywords:** applied functional ecology, integrated approach, stakeholders, species compatibility,
31 species complementarity

32

33 **Introduction**

34 Polyculture is a production practice used in agriculture whose definition varies among agricultural
35 sectors and authors ¹, due to differences in the temporal (e.g. successive vs simultaneous production)
36 or physical boundaries (e.g. production in the same system vs on the same farm) of the production
37 systems combined to create the polyculture (e.g. see alternative definitions among the chapters of ¹).
38 Here, we consider polyculture to be any farming system in which at least two species are farmed at
39 the same time, with the objective of producing several products with economic value (adapted from
40 ²). Thus, polycultures based on a combination of species in which one is a beneficial organism without
41 direct economic value (e.g. salmon farms associated with cleaner wrasses ³) do not fall within the scope
42 of this study.

43 In aquaculture, polyculture is an ancient and still widespread practice in which fish, molluscs,
44 crustaceans, echinoderms, annelids, and/or algae are produced together ^{2,4}. In recent decades, it has
45 been somewhat ignored in aquaculture development in certain regions (e.g. in Europe) and for certain
46 species (e.g. Atlantic salmon) in favour of intensive monoculture ⁵. Nevertheless, recent discussion
47 about traditional production systems and new concepts/practices (e.g. integrated multi-trophic
48 aquaculture [IMTA], aquaponics, integrated agriculture-aquaculture [IAA]) have revived interest in
49 polyculture ^{4,6,7}. A search of the Scopus database (31 Aug 2022) of articles and reviews published from
50 1975-2021 in the Life Science Area (i.e. agricultural and biological sciences; environmental science;
51 multidisciplinary) whose title, abstract, or keywords contains the keywords “polyculture and
52 aquaculture”, or “IMTA”, or “IAA” identified 1141 publications and highlighted the increasing scientific
53 interest in the past decade (Fig. 1).

54 Polyculture is increasingly recommended as a way to improve aquaculture by promoting synergies
55 among species and/or compartments of the system ^{2,4,8}. Polyculture can increase farming efficiency by
56 improving use of the resources naturally present or supplied to the agricultural environment ⁹ and/or
57 by recycling co-products into the farmed biomass ^{6,10}. These processes can decrease a farming system’s
58 environmental impacts ¹¹ and increase its resilience ¹². From a socio-economic viewpoint, it can provide
59 benefits by creating new income sources, having lower operational costs and financial risks than
60 monoculture ¹³, and making economically profitable the production of species that would not be
61 profitable in monoculture ². Furthermore, polyculture can improve the welfare of farmed fish ¹⁴, either
62 directly due to the species combined (e.g. ¹⁵) or indirectly due to reduced maintenance operations,
63 performed in part by other farmed species (e.g. ¹⁶). However, polyculture can also result in (i) pathogen
64 spill-overs ¹⁷, (ii) high mortality or decreased growth rate ^{18,19}, or (iii) stress and physical injuries caused
65 by competition for resources ²⁰ or predation, negatively impacting animal welfare ^{4,21}. Actually,

66 potential polyculture benefits can be expected provided that compatibility or, even better,
67 complementarity occur among the combined species⁴. Compatibility is the ability of species to live in
68 the same production system without negative interactions (i.e. amensalism, predation, parasitism) or
69 competition for resources (e.g. trophic, spatial). Complementarity is the ability of species to use
70 different portions of the available resources (including by-products or waste of other co-farmed
71 species) or develop commensal or mutualistic interactions. This places a premium on designing
72 relevant species combinations to maximise the benefits of polyculture.

73 ***Current fish polycultures***

74 Traditional fish polycultures started in China during the Tang dynasty (A.D. 618-907)²² and are still
75 widely used mainly in household-managed ponds and small- to medium-sized enterprises²³. These
76 polycultures combined species that use different natural resources²⁴ such as the four Chinese major
77 carps (*Ctenopharyngodon idella*, *Mylopharyngodon piceus*, *Hypophthalmichthys molitrix*, and
78 *Aritichthys nobilis*) that have different feeding habits (macrophytes, benthos, phytoplankton,
79 zooplankton) and ecological niches (surface, bottom, mid-water)^{2,4}.

80 In Asia, the leading aquaculture zone (i.e. contributing 88% of the world's production of aquatic
81 animals by 2020, of which 64% in China²⁵), polyculture systems still dominate (e.g. in China²⁶). The
82 most common fish polycultures combine the four Chinese major carps (e.g. in China²⁶) or the three
83 Indian major carps (*Catla catla*, *Cirrhinus mrigala*, and *Labeo rohita*) sometimes with Chinese major
84 carps (e.g. in India)^{26,27}. In Europe, fish polyculture is continuously practiced since the Middle Ages
85 (Aubin et al., 2017) and combines predator species (e.g. *Esox lucius*, *Perca fluviatilis*, and *Sander*
86 *lucioperca*) with species from lower trophic levels (e.g. *Cyprinus carpio*, *Rutilus rutilus*, *Scardinius*
87 *erythrophthalmus*, and *Tinca tinca*)^{27,28}.

88 ***The needs for new fish polyculture***

89 To date, the most common species combinations have been shaped by the trial-and-error and
90 cumulative know-how of fish farmers over decades or centuries⁴. However, new species combinations
91 will be needed in the near future to face new challenges to and developments in aquaculture.

92 First, most fish production in the world relies on a few species (i.e. in 2020, 76% relied on 3 marine
93 species and 17 freshwater species;²⁵), which jeopardises (i) human food security, because the heavy
94 dependence on a few species puts aquaculture production at risk, and (ii) economic prospects, because
95 less diversified production limits aquaculture's potential to adapt to changes in the environment or
96 consumer demand (review in²⁹). Thus, a growing number of researchers, national agencies, and
97 international organisations recommend diversifying species while strengthening the well-established

98 species^{30–32}. This strategy can improve the sustainability and resilience of the aquaculture sector,
99 particularly by producing new species that occupy other market segments, such as niche markets
100^{30,31,33}. Meeting this desire for diversification requires developing new species production systems^{25,30}
101 for which polyculture has never been considered but could be advantageous. It could also be an
102 opportunity to improve traditional polycultures by including new species.

103 Second, ongoing global changes are impacting aquaculture^{34,35} and fish communities^{36,37}, which could
104 make certain traditional polycultures unfit in areas where they currently exist if some of the species in
105 them can no longer live in the new environmental conditions³⁵. This challenge requires adapting
106 existing polycultures, for instance by replacing some of their species or designing new polycultures.

107 Third, the past few decades have seen the emergence of new production systems, such as recirculating
108 aquaculture systems (RAS) and aquaponics, which have rarely included fish polyculture⁴. Since fish
109 biology and species interactions depend upon environmental characteristics^{37–39}, traditional fish
110 combinations (e.g. in extensive pond aquaculture) may not be relevant for these new production
111 systems due to their characteristics (e.g. the low diversity of environmental components and *ad libitum*
112 feeding in RAS change species' resource use). Overall, these current and future challenges and
113 developments require selecting existing species communities or designing new fish combinations.

114 Attempting to choose random fish combinations or only traditional combinations for a given
115 production system is not an effective way to develop new fish polycultures because these strategies
116 could fail to identify species combination(s) that are valuable for aquaculture. In comparison,
117 considering all possible species combinations for a given system maximises the probability of providing
118 the best species combination(s) to fish farmers, but this strategy requires considering many species
119 combinations, and not all of them can be assessed empirically due to practical, ethical, temporal, and
120 financial concerns⁴. To address this issue, we developed a conceptual integrative workflow to
121 standardise and plan the development of new combinations of fish species.

122 ***A workflow to develop new fish polycultures***

123 Efficient and sustainable fish polycultures need to (i) maximise resource use in the farming system; (ii)
124 meet regulatory, economic, and environmental expectations; and (iii) ensure fish welfare^{40,41}. The
125 workflow was designed as an operational approach for developing such fish polycultures. The design
126 was based on two paradigms.

127 First, to minimise empirical testing (i.e. replacement, reduction, and refinement of animals used in
128 research, 3 Rs rule;⁴²) and thus ensure the feasibility of the workflow, we first assess fish combinations
129 using data available in the literature and databases.

130 Second, relevant fish combinations during the workflow are developed with all stakeholders (i.e. fish
131 farmers, consumers, researchers, policy makers, non-governmental organisations) to consider their
132 expectations, interest, and expertise. This co-construction approach addresses societal demands and
133 ensures stakeholder support ⁴³⁻⁴⁵. In line with the concept of open innovation ⁴⁶, a variety of
134 stakeholders must be involved to facilitate innovation and increase the chances of success and
135 dissemination of an innovative product ⁴⁷. Along with farmers, researchers, and policy makers,
136 development of new polycultures needs to involve a broader audience, particularly consumers, to
137 better understand their demands and thus design new products that meet them ^{48,49}. More specifically,
138 consumers can become involved by focusing on "lead users" (i.e. those whose demands are further
139 ahead of market trends than those of most users; ⁵⁰) using approaches such as face-to-face surveys ⁵¹.
140 To avoid focusing on a carefully selected, well-informed, and elite group of customers, a wider range
141 of customers can be reached at relatively low-cost using technology (e.g. networked tools, social
142 media).

143 The workflow successively screens all possible combinations of a set of fish species in three steps that
144 have increasingly restrictive filters. Step 1 selects the most promising compatible species combinations
145 (i.e. "prospective combinations") with all stakeholders using databases and surveys, and considering
146 characteristics of the target farming system. Step 2 develops proofs of concept for prospective
147 combinations to verify species compatibility and assess species complementarity and fish welfare. Step
148 3 prototypes and scales-up new polycultures while considering the sustainability of the production.
149 Figure 2 provides a detailed description of the workflow. Figure 3 provides a concise description of the
150 workflow and some practical examples of (i) species combinations that have the characteristics
151 required to develop a polyculture or (ii) studies that show how to carry out the different actions for
152 each step (e.g. feedback from polycultures or experiments).

153 **Step 1 – Highlighting prospective fish species combinations**

154 The objective of Step 1 is to perform a large-scale screening of all combinations of two or more species
155 within a given organism set (potentially all fish species) to highlight the prospective combinations (Fig.
156 2). Because this screening is fed by information from the literature, databases, and stakeholder
157 expectations, it requires no preliminary experiments.

158 Step 1 consists of three tasks. The first two tasks, performed in parallel, discard the fish combinations
159 that have no-compatible species (Task 1a) and that do not meet stakeholder expectations (Task 1b)
160 (Fig. 2). During the last task (Task 2), stakeholders discuss, rank, and prioritise the remaining
161 combinations to select the prospective combinations (Fig. 2).

162 To foster innovation, Tasks 1a and 1b need to be performed simultaneously and independently.
163 Indeed, considering only the combinations that interest stakeholders before estimating whether the
164 species that make them up are compatible (or *vice versa*) limits the discussion of options that can
165 highlight prospective combinations. Conversely, comparing the list of compatible combinations,
166 ranked according to their compatibility degree, to the list of those that interest stakeholders can cause
167 stakeholders to question their preconceptions and lead to development of new options (Fig. 2). For
168 instance, a combination with lower compatibility may interest stakeholders so much that it is
169 appropriate to see whether certain practices could compensate for this lower compatibility. Similarly,
170 a highly compatible combination might be more efficient at the scale of the production system, despite
171 being initially less interesting to stakeholders.

172 To further promote innovation, we recommend not adjusting compatibility estimates for a given type
173 of production system at the beginning of Step 1 (e.g. highlighting combinations only for RAS), because
174 doing so may narrow the range of options that can be discussed with stakeholders (Task 2). For
175 instance, a potentially highly compatible combination may interest fish farmers, who will then want to
176 identify, in collaboration with researchers, the most suitable production system for it. Therefore, the
177 workflow assesses the mean potential species compatibility among all possible fish combinations in
178 any farming environment in Task 1a before refining the combination assessment using stakeholder
179 opinion in Task 2.

180 ***Task 1a - Estimating species compatibility using meta-analyses***

181 The objective of Task 1a is to estimate species compatibility by considering the species' (i) abiotic
182 requirements, (ii) predation risk, and (iii) use of trophic and spatio-temporal resources (Fig. 2). Overall,
183 sought species combinations are those in which species have (i) similarity in abiotic requirements, (ii)
184 no risk of predation, and (iii) dissimilarity in the use of trophic and spatio-temporal resources.

185 *Species compatibility based on similarity in abiotic niche*

186 The ability of multiple species to live in the same farming environment can be assessed by comparing
187 their ecological niches (*sensu* ⁵²). In theory, if their niches overlap at least partially, there is a set of
188 environmental conditions suitable for all of them ⁵³, and combining them in a polyculture can thus be
189 considered.

190 For niche comparison, we recommend focusing on abiotic parameters that are relevant for most
191 farming environments (e.g. ponds, cages, indoor RAS, raceways) and for which high similarity is
192 required to ensure species compatibility (see the review by ⁵³) such as light (i.e. daylight intensity and
193 duration) and the main physicochemical water parameters (i.e. conductivity, current/flow, dissolved

194 oxygen, pH, salinity, temperature, total hardness, total nitrogen, total phosphorus, and turbidity).
195 Species' requirements for these parameters can be obtained from the literature on fish aquaculture
196 (e.g. for Percids⁵⁴) or ecology (e.g.⁵⁵). Alternatively or additionally, data for analysing niches can be
197 done by comparing the occurrences of fish species (e.g. from the Global Biodiversity Information
198 Facility, <http://www.gbif.org>) to locally observed abiotic parameters (species-distribution modelling
199 follows a similar approach; e.g.⁵⁶). These data are available in several databases, such as EarthEnv⁵⁷,
200 WorldClim⁵⁸, and FLO1K⁵⁹ (Fig. 2).

201 Several methods are available to model and compare species niches⁶⁰. For aquaculture, the recently
202 developed tool AquaDesign assesses the degree of overlap among n-dimensional niche hypervolumes
203 (for details, see⁶¹) based on species-distribution and abiotic-parameter datasets from public databases
204⁵³. Once all possible combinations of a set of species are analysed, the tool classifies them by their
205 degree of niche overlap among the species. More overlap means that species can be co-farmed under
206 a larger set of abiotic conditions, which makes their polyculture feasible in a wider set of farming
207 environments and still possible despite abiotic variability (e.g. extreme weather events in ponds).
208 However, species combinations with little niche overlap do not need to be excluded from
209 consideration, as, once the niches of two fish species overlap, the species can be maintained under
210 rearing conditions that are suitable for both, especially in highly controlled systems, such as indoor
211 RAS. Moreover, information about species physicochemical requirements or occurrences may be
212 scarce for certain species or reflect only a realised niche when they are based on occurrence datasets
213 (e.g.⁶²), which can cause the abiotic compatibility of species to be greatly underestimated. Thus, we
214 recommend not using abiotic niche information alone to exclude fish combinations based on statistical
215 thresholds (see examples of statistical estimates of niche differentiation in^{63,64}), except when the
216 niches of at least one pair of species in the combination do not overlap at all (Fig. 2).

217 *Species compatibilities based on fish functional traits*

218 Functional traits are phenotypic characteristics (i.e. behavioural, morphological, phenological, and
219 physiological) of an organism that directly or indirectly impact its fitness and environment^{65,66}. As for
220 other organism groups (e.g.⁶⁷⁻⁶⁹), these traits can be used to predict the potential risk of predation or
221 competition between fish species (e.g.^{70,71}).

222 Most studies of fish functional ecology have focused on species interactions in the wild and/or been
223 based on traits specific to certain taxa or poorly documented in other species groups (e.g.⁷²⁻⁷⁴). As the
224 workflow aims at assessing potential compatibility among all possible combinations of fish species in
225 any farming environment, we recommend considering functional traits that are (i) commonly available
226 in the literature or inferred from proxies that are (i.e. to ensure data availability), (ii) common to most

227 fish taxa (i.e. to ensure potential relevance for any species), and (iii) relevant for all farming systems.
228 Researchers have produced several datasets of fish functional traits, based on several decades of
229 research, that are available in databases (e.g. ⁷⁵⁻⁷⁷), which makes meta-analyses, such as the one
230 needed for this workflow, possible (Fig. 2).

231 Here, we provide an initial list of functional traits relevant for assessing species compatibility that are
232 available in the TOFF database ⁷⁶. The traits and their expressions cited hereafter are those in the TOFF
233 thesaurus, but this list should be adapted or supplemented with additional key traits depending on the
234 species community considered and the data available. As no comprehensive fish functional trait
235 database currently exists ⁷⁶, we recommend compiling information from several databases and
236 inferring unavailable data about key functional traits from relevant proxies (e.g. ⁷⁸).

237 *Predation risk based on fish functional traits*

238 Among harmful interactions, we considered only predation because, to our knowledge, no amensalism
239 or parasitism has been observed among farmed fish species. Considering the (mis-)match between the
240 functional traits of a predator and its potential prey can provide insight into the risk of predation
241 between species (e.g. ⁷⁹). Although predation has been observed between non-piscivorous fish (e.g. in
242 the wild, ⁸⁰), predation is more likely when one or more piscivorous species are co-farmed with other
243 fish species. Therefore, predation risk should be assessed only when the combination contains one or
244 more piscivorous species.

245 Predation involves detecting, approaching, capturing, and handling a prey. However, several (anti-)
246 predatory strategies (e.g. mimicry, flight, freezing) cannot be expressed in all farming systems. For
247 example, some systems have less diversity in their abiotic and biotic components and a smaller volume
248 (e.g. RAS tank) than those of wild environments. Thus, we suggest that predation risk should be based
249 mainly on a species' ability to capture and handle co-farmed taxa, which can be estimated by
250 comparing the range of actual ratios of prey size to predator size (i.e. for which predation is known to
251 occur) to the range of calculated ratios of sizes of the co-farmed species based on literature data (e.g.
252 ⁸¹⁻⁸³). If the range of the calculated ratio overlaps that of the actual ratio for any of the species in the
253 combination, the combination must be discarded. This assessment likely overestimates the predation
254 risk, since certain anti-predatory strategies might be effective in certain farming environments (e.g.
255 mimicry in ponds). However, we recommend conservative estimates to minimise problems with fish
256 survival and welfare. Although predation may be deliberately sought in certain polycultures (e.g. both
257 piscivorous and forage fish in a pond ⁸⁴), on ethical grounds, we argue that species combinations in
258 which one species is likely to prey upon another should be excluded (Fig. 2).

259 *Spatio-temporal and trophic compatibilities based on fish functional traits*

260 Spatio-temporal and trophic compatibilities can be estimated by comparing information about (i)
261 depth preference or feeding location (e.g. benthic or pelagic, or a morphological proxy; e.g.^{85,86}) and
262 activity period (i.e. diurnal or nocturnal) and (ii) diet (e.g. insectivore, planktivore, periphytivore,
263 detritivore, piscivore) and food size, respectively, or their morphological proxies (e.g.⁷⁰). Lower
264 similarity in these traits can increase resource partitioning among co-farmed species (e.g.⁸⁷⁻⁸⁹), thus
265 increasing their compatibility. The risk of spatio-temporal and/or trophic competition can be
266 quantified using models (e.g. a food-fish model^{70,90}) or by estimating functional dissimilarity (e.g.⁹¹),
267 for instance using Rao's quadratic entropy (e.g.⁶⁸) or distance metrics (e.g. Gower distance) between
268 species after standardising the functional traits (i.e. range: 0-1). This risk should be estimated (i) for
269 trophic and spatio-temporal competition independently, to determine whether one of them is larger
270 than the other, and (ii) by pairs of species, to maximise the compatibility of all species combined. Thus,
271 for combinations of more than two species, the highest estimated risk (i.e. the lowest dissimilarity
272 index) among the pairs of species should be assigned to the entire combination.

273 We do not recommend defining a threshold for competition risk above which species combinations
274 would be discarded automatically (Fig. 2), as polyculture can be feasible even for species with similar
275 functional traits if farming practices are adapted (e.g. *ad libitum* feeding could greatly decrease trophic
276 competition). Thus, although combinations with low competition risks are *a priori* better candidates
277 for polyculture, assessments of all possible combinations should be presented to the stakeholders in
278 Task 2 (Fig. 2).

279 *Addressing intraspecific variability in functional trait expressions*

280 Functional trait expressions vary within species due to polymorphism or phenotypic plasticity (e.g.
281^{92,93}). Although this variability can modify species compatibility depending on the population or farming
282 environment considered, it is difficult to predict competition or other negative interactions between a
283 pair of species from particular populations in a given environment, because doing so requires knowing
284 the expressions of functional traits of both species in this environment. This information is rarely
285 available in the literature for most species, especially those that are not sympatric or have not been
286 co-farmed. Thus, we recommend (i) considering intraspecific variability in functional trait expressions
287 by considering their ranges reported in the literature for different populations and environments (i.e.
288 such information are available in databases informing about the measure environments of traits, e.g.
289 TOFF⁷⁶) and (ii) assigning the highest risk in each range to the entire combination.

290 *Compatible combinations and compatibility indicator*

291 Estimating compatibilities allows species combinations with no abiotic niche overlap or with a
292 predation risk between at least one pair of species to be discarded (Fig. 2). All remaining combinations
293 are considered “compatible combinations” (Fig. 2) and are assigned quantitative estimates of their
294 abiotic ($C_{abiotic}$), spatio-temporal ($C_{spatio-temporal}$), and trophic ($C_{trophic}$) compatibilities, which equal the
295 degree of niche overlap and the spatio-temporal and trophic dissimilarity indices, respectively. Each is
296 standardised independently from 0-1 to summarise the information for Task 2 (Fig. 2).

297 These standardised estimates are used to shape a three-dimensional space in which each axis
298 corresponds to an estimate (Fig. 4). Thus, the theoretical optimal fish combination in the species set
299 under consideration has the coordinates (1, 1, 1) (i.e. $C_{abiotic}$, $C_{spatio-temporal}$, and $C_{trophic}$ each equal 1) (Fig.
300 4). This graphical overview of species compatibility is supplemented by a compatibility index (CI), which
301 estimates the overall compatibility among species in a combination as the distance from the origin (i.e.
302 coordinates 0,0,0; corresponding to the worst theoretical combination in the set species considered)
303 and to the combination's position in the same space (Equation 1). Thus, CI can range from 0 to $\sqrt{3}$ (i.e.
304 ca. 1.73). The graphical overview and CI quantify the three types of compatibility of each combination
305 relative to those of other combinations, which allows combinations to be ranked by according to their
306 overall compatibility.

307 (Equation 1)
$$CI = \sqrt{(C_{abiotic})^2 + (C_{spatio-temporal})^2 + (C_{trophic})^2}$$

308 Where:

309 CI is the compatibility indicator of a given combination (fish species community).

310 $C_{abiotic}$ is the position of the combination on the $C_{abiotic}$ axis, corresponding to its standardised niche
311 overlap extent between all species of the combination.

312 $C_{spatio-temporal}$ is the position of the combination on the $C_{spatio-temporal}$ axis, corresponding to its
313 standardised lowest value of interspecific spatio-temporal dissimilarity index observed among all
314 species pairs of the combination (e.g. among the six pairs for a combination of four species).

315 $C_{trophic}$ is the position of the combination on the $C_{trophic}$ axis, corresponding to its standardised lowest
316 value of interspecific trophic dissimilarity index observed among all species pairs of the combination.

317 Since much time may be needed to calculate $C_{abiotic}$, $C_{spatio-temporal}$, and $C_{trophic}$ (especially $C_{abiotic}$; see ⁵³)
318 when analysing many combinations, or combinations with many species, the information obtained can
319 be published in a database to use the results in later applications of the workflow.

320 **Task 1b. Defining combinations of interest to stakeholders**

321 The objective of Task 1b is to define the expectations of all of the stakeholders. We argue that
322 stakeholders are generally looking for species combinations that are socio-economically interest and
323 follow the regulations of their country or region. These expectations depend strongly on which fish
324 species are combined, and not all species are suitable for aquaculture. Some cannot be produced in

325 farming systems (e.g. for technical reasons or because they were never domesticated) or have no
326 socio-economic interest ²⁹. Thus, the workflow discards compatible combinations that contain (i) at
327 least one species that cannot be farmed (i.e. pragmatically, that has never been produced in
328 aquaculture; see cultured species in ²⁵) or (ii) only species with no socio-economic interest (e.g.
329 according to the score of interest determined by stakeholders; see below) (Fig. 2). International,
330 national, and local regulations may also cause some combinations to be discarded (e.g. they contain
331 species that are illegal to rear in the targeted region) (Fig. 2). Additionally, when species combinations
332 are designed mainly to improve the production of a target species (e.g. pikeperch in RAS ⁹⁴), compatible
333 combinations that do not contain the target species can be discarded.

334 For the remaining combinations, considering their species also provides additional key information
335 about their socio-economic interest, which can be estimated using a socio-economic indicator (*SEI*)
336 that should aggregate at least the economic value of the species and their interest to stakeholders.
337 The economic value can be assessed, for instance, as the price per kg of each species averaged for all
338 species combined (\bar{P}) (available in the FAO database GLOBEFISH, [https://www.fao.org/in-](https://www.fao.org/in-action/globefish/prices/en/)
339 [action/globefish/prices/en/](https://www.fao.org/in-action/globefish/prices/en/) or using FAO's FishStatJ software,
340 <https://www.fao.org/fishery/en/topic/166235>). Although the mean profitability of species would be
341 more meaningful, it is not available in public databases for most fish species, to our knowledge. The
342 interest to stakeholders can be assessed using a survey in which each stakeholder rates his/her interest
343 (e.g. range: 0-10) (regardless of the price per kg, already considered in \bar{P}) in rearing or consuming each
344 species in the remaining combinations. The scores for each species given by the stakeholders are then
345 averaged (\bar{I}). \bar{P} and \bar{I} are then independently standardised (range: 0-1) for all combinations to
346 summarise the information for Task 2.

347 These standardised estimates are used to shape a two-dimensional space in which each axis
348 corresponds to an estimate (Fig. 4). Thus, the theoretical optimal fish combination in the species set
349 under consideration has the coordinates (1, 1; \bar{P} and \bar{I} each equal 1; Fig. 4). The *SEI* provides an overall
350 estimate of the socio-economic interest of a species combination, calculated as the distance from the
351 origin (i.e. coordinates 0,0; corresponding to the theoretical worst combination in the set of species
352 considered) to the combination's position in the same space (Equation 2). Thus, *SEI* can range from 0
353 to $\sqrt{2}$ (i.e. ca. 1.41). The graphical overview and *SEI* quantify the socio-economic interest of each
354 combination relative to those of other combinations. To refine the *SEI*, other parameters could be
355 considered, such as the market size (e.g. mean volume of each species consumed at the international,
356 national, or regional level) or the relevance of developing local production to meet national demand
357 (e.g. total volume imported) (relevant datasets are available in GLOBEFISH and through FishStatJ
358 software).

359

(Equation 2)

$$SEI = \sqrt{(\bar{P})^2 + (\bar{I})^2}$$

360 Where:

361 *SEI* is the socio-economic indicator of the combination.

362 \bar{P} is the position of the combination on the \bar{P} axis, corresponding to its standardised mean of the price
363 per kg of the species in the combination.

364 \bar{I} is the position of the combination on the \bar{I} axis, corresponding to its standardised mean of the score
365 of interest of the species in the combination.

366 **Task 2 – Definition of prospective combinations**

367 The objective of Task 2 is to identify prospective combinations through discussion by stakeholders (i.e.
368 co-construction approach) of the information obtained in Tasks 1a and 1b. This discussion aims at (i)
369 refining the *CI* of species combinations by considering the specific characteristics of the targeted
370 farming systems and practices, (ii) potentially questioning preconceptions that have led to *CI* and *SEI*
371 in order to reach a consensus, and (iii) identifying the prospective combinations (Fig 2). We discuss this
372 task in four phases to encourage possible innovation in the development of polyculture.

373 *First phase: preliminary discussion*

374 The graphical overviews and indices of Tasks 1a and 1b are compared, which allows the combinations
375 that are both compatible and of interest to the stakeholders to be kept. However, combinations that
376 contain species with no socio-economic interest should not be discarded during Task 1b if they can
377 increase species complementarity and thus farming system sustainability ⁴. Similarly, very socio-
378 economically interesting combinations discarded during Task 1a deserve to be discussed because a
379 rearing practice could solve a compatibility problem (e.g. minimising the risk of predation by adjusting
380 the size of individuals combined and/or the duration of the polyculture).

381 *Second phase: consider the specific context in which the fish combination(s) will be applied*

382 Considering the farming systems targeted can help to discard some of the combinations. For instance,
383 when a specific environment is targeted (e.g. ponds in a given area), the abiotic conditions required by
384 combinations can be compared to those observed in that environment to exclude combinations that
385 cannot be farmed there. This exclusion can be based either on expert opinion or on assessing whether
386 the abiotic conditions are included in the abiotic niche overlap of all species combined (e.g. using
387 AquaDesign ⁵³).

388 To refine assessment of the remaining fish combinations, it is necessary to consider how the target
389 farming system and its practices could influence species compatibility. This can be estimated at least
390 partly by weighting the relative importance of each type of compatibility in the *CI* and its three-
391 dimensional space using characteristics specific to systems or practices. For instance, if feeding will be

392 *ad libitum*, trophic compatibility may have less influence on the overall compatibility of combinations.
 393 Similarly, it is easier to maintain abiotic parameters in a range suitable for all species combined in a
 394 highly controlled indoor RAS^{95,96}, which allows even species with little abiotic niche overlap to be co-
 395 farmed. Thus, a combination's compatibilities are weighted by the targeted system and its practices to
 396 create an adjusted *CI* (*ACI*) (Fig. 4, Equation 3). Stakeholders, particularly fish farmers and researchers,
 397 can provide insights useful for setting weighting coefficients. Like for \bar{I} , the weighting coefficient can
 398 be defined using a survey in which each stakeholder rates the importance (i.e. from 0-1) of each
 399 estimate. The scores of each estimate given by the stakeholders are then averaged.

400 (Equation 3)
$$ACI = \sqrt{(wc_1 \times C_{abiotic})^2 + (wc_2 \times C_{spatio-temporal})^2 + (wc_3 \times C_{trophic})^2}$$

401 Where:

402 *ACI* is the adjusted compatibility indicator of the combination for a particular farming system and
 403 rearing practices.

404 $C_{abiotic}$ is the position of the combination on the $C_{abiotic}$ axis, corresponding to its standardised niche
 405 overlap extent between all species of the combination.

406 $C_{spatio-temporal}$ is the position of the combination on the $C_{spatio-temporal}$ axis, corresponding to its
 407 standardised lowest value of interspecific spatio-temporal dissimilarity index observed among all
 408 species pairs of the combination (e.g. among the six pairs for a combination of four species).

409 $C_{trophic}$ is the position of the combination on the $C_{trophic}$ axis, corresponding to its standardised lowest
 410 value of interspecific trophic dissimilarity index observed among all species pairs of the combination.

411 wc_1 , wc_2 , and wc_3 are the weighting coefficients (range: 0-1) applied to $C_{abiotic}$, $C_{spatio-temporal}$, and $C_{trophic}$,
 412 respectively.

413 Similarly, the assessment of combinations can be refined by adjusting the *SEI* and its two-dimensional
 414 space into an adjusted *SEI* (*ASEI*) using weighing coefficients reflecting the relative importance that
 415 stakeholders give to \bar{P} and \bar{I} (Fig. 4, Equation 4). These weighting coefficients (i.e. from 0-1) can be
 416 defined in the same way as those for *ACI*.

417 (Equation 4)
$$ASEI = \sqrt{(wc_P \times \bar{P})^2 + (wc_I \times \bar{I})^2}$$

418 Where:

419 *ASEI* is the adjusted socio-economic indicator of the combination according to stakeholders' demands.
 420 \bar{P} is the position of the combination on the \bar{P} axis, corresponding to its standardised average of the
 421 price per kg of the species in the combination.

422 \bar{I} is the position of the combination on the \bar{I} axis, corresponding to its standardised average of the
 423 score of interest of the species in the combination.

424 wc_P and wc_I , are the weighting coefficients (range: 0-1) applied to \bar{P} and \bar{I} , respectively.

425 *Third phase: addressing differences in expectations among stakeholders*

426 Stakeholders may perceive the importance of abiotic, spatio-temporal, and trophic compatibilities
 427 differently, and these compatibilities cannot have the same importance when designing polycultures.

428 Similarly, stakeholders' expectations may differ depending on their business model, geographic area

429 of activity, farming priorities, and consumption habits^{29,43,97}. These differences can be managed by
430 developing consensual design of prospective polyculture or personalising workflow outcomes to
431 certain stakeholder groups. The former can be achieved by surveying stakeholders to identify their
432 expectations and perceptions of polyculture as well as their fish-farming experience before reaching a
433 consensus using, for instance, Quaker-based or spokes council models^{98,99} or weighting of the
434 compatibilities based on survey results¹⁰⁰. The latter implies developing a workflow with customisable
435 options to fit as closely as possible either one stakeholder or a group of stakeholders with similar
436 expectations.

437 *Fourth phase: determining the prospective combinations*

438 Once the compatible combinations of interest are known and adjusted to a targeted production
439 context, the final decisions for Step 1 can be made. Summarising the assessments makes it easier for
440 stakeholders to identify prospective combinations. We recommend performing this summary as
441 follows. First, *ACI* and *ASEI* are standardised independently (range: 0-1) to give them the same weight,
442 which may raise questions from workflow users, depending on their objectives. Second, coordinates
443 of the *ACI* and *ASEI* are used to form a two-dimensional space in which each axis corresponds to an
444 index (Fig. 4). Thus, the theoretical optimal fish combination in the species set under consideration has
445 the coordinates (1, 1). Third, a “polyculture potential score” (*PPS*) is defined, like indexes developed to
446 identify suitable combinations of candidate species for aquaculture (e.g.^{101–103}) and calculated as the
447 distance from the origin to the position of the combination in the same space (Equation 5). Thus, *PPS*
448 can range from 0 to $\sqrt{2}$ (i.e. ca. 1.41).

449
$$(Equation\ 5)\quad PPS = \sqrt{ACI^2 + ASEI^2}$$

450 Where:

451 *PPS* is the polyculture potential score of the combination.

452 *ACI* is the position of the combination on the *ACI* axis, corresponding to its the standardised adjusted
453 compatibility indicator of the combination for a particular type of farming system.

454 *ASEI* is the position of the combination on the *ASEI* axis, corresponding to its the standardised the
455 socio-economic indicator of the combination.

456 Overall, Step 1 creates a short list of fish combinations that is considered later in the workflow (Fig. 2).
457 Although the number of combinations decreases during Step 1, several combinations usually remain
458 at its end. As Step 2 includes time- and money-consuming bioassays, we highlight the importance of
459 ranking the remaining combinations and prioritising the most relevant ones using the *PPS*.

460 Although the *PPS* can be used to select the best combinations, we do not recommend using it as it
461 stands. In fact, a combination with a high *PPS* (e.g. 0.8) could have a high *ACI* but low *ASEI* (e.g. 0.8 and

462 0.2, respectively), while one with a lower *PPS* (e.g. 0.7) could have more equal indices (e.g. 0.5 each).
463 The most promising combinations seem to be those that have relatively similar *ACI* and *ASEI* and the
464 highest possible *PPS*. Thus, we recommend prioritising combinations for Step 2 by discarding less
465 relevant combinations and then ranking those that remain. First, we recommend considering only
466 combinations that lie above minimum thresholds of *ACI* and *ASEI*, which can be determined arbitrarily
467 (e.g. 0.5, Fig. 4) or by considering polycultures already used in the targeted farming system (i.e.
468 reference polycultures, e.g. a combination of Indian major carps for a pond in India). Combinations
469 that have *ACI* or *ASEI* lower than those of the reference polycultures would be discarded, but this
470 approach is difficult to envision if the desired polyculture will be placed into a system in which
471 polyculture has never been practised or will include a species that has never been farmed in
472 polyculture. Second, the remaining combinations are then ranked by their *PPS* (Fig. 4), and those with
473 the highest *PPS* are prioritised and considered as the “prospective combinations” that will be assessed
474 in Step 2. The number of prospective combinations can be decreased based on the *PPS* ranking to
475 decrease the number of combinations to a reasonably testable number in Step 2.

476 **Step 2 – Developing proofs of concept for prospective combinations to identify the best** 477 **combination(s)**

478 The objective of Step 2 is to verify the validity and technical feasibility of each prospective combination
479 and to demonstrate its relevance for real-world production before considering implementing it in
480 aquaculture, as developing new fish production systems requires large amounts of time, money, and
481 fish-farmer training ⁴. After Step 1 of the workflow, production systems based on prospective
482 combinations could still fail due to the step’s limitations. Thus, implementing new fish polycultures still
483 carries high risk for fish farmers. The cost of potential failure can be limited by performing bioassays
484 in smaller, less expensive versions of real-world production systems. Acting as proofs of concept, these
485 bioassays (i) assess the actual value of prospective combinations (i.e. more accurate assessment), (ii)
486 verify that the targeted farming system is appropriate for the prospective combinations, (iii) and
487 highlight limitations of prospective combinations. Even when only one prospective combination is
488 considered, bioassays should be performed because they can identify flawed prospective
489 combinations for a targeted farming system relatively early in the process to avoid further spending
490 (i.e. “fail fast, fail cheap”).

491 ***Why are bioassays still needed?***

492 Minimising animal testing is universally recognised as an ethical and pragmatic cornerstone of
493 biological research. Nevertheless, alternative approaches such as simulation models or virtual

494 laboratories for aquaculture research cannot yet replace bioassays completely (but see ongoing
495 development at <https://ae2020virtuallab.sintef.no>).

496 To develop polyculture, meta-analyses cannot infer all biological interactions (e.g. synergies, trophic
497 interactions, mutualism) or farming system functioning (similarly in biological conservation, see ¹⁰⁴).
498 This is complicated by (i) the context-dependence of species-interaction outcomes ^{105,106}, (ii) non-
499 additive effects (i.e. antagonism and synergy) of multiple environmental stressors on communities ¹⁰⁷,
500 and (iii) interspecific interactions that can modify fish behaviour ¹⁰⁸ or alter species-specific effects on
501 ecosystem functioning ¹⁰⁹. Changes in species compatibility and complementarity due to biological and
502 ecological processes (e.g. differences in growth rates among species; changes in the numbers and
503 proportions of species due to reproduction; changes in turbidity) during the farming period also
504 remains challenging to predict ⁴. Thus, more accurate modelling of the functioning of farming
505 environments should consider planned and associated biodiversity (including micro-organisms, non-
506 fish animals, and plants), taxon density, the species ratio, abiotic components of the environment, and
507 dynamics of farming system components (e.g. ^{110,111}) as well as characteristics of the specific
508 populations of fish species combined ²⁹. This modelling should also represent responses to
509 environmental conditions of each species in the polyculture ^{112,113}, but the necessary datasets and
510 relevant models remain lacking for many environments and species groups. Although these limitations
511 could be addressed in the near future (e.g. developing a trophic-interaction database ¹¹⁴), modelling
512 remains a useful tool for developing polyculture, but is still not sufficient on its own.

513 ***How should bioassays be performed?***

514 *Designing the experimental system*

515 Since species compatibility and complementarity may differ in different environments, the proofs of
516 concept should be based on bioassays under conditions that mimic those of the targeted production
517 system. For the same reasons, management of the fish community during bioassays must be similar to
518 the farming practices that will be applied in the targeted system (e.g. feeding, density, species ratio,
519 maintenance operations). The design of the proof of concept should also consider the species'
520 requirements and, when known, optimal rearing conditions, which may involve making trade-offs to
521 ensure that the abiotic conditions and possibly the diet used during bioassays lie within the range of
522 conditions suitable for all of the species combined.

523 The experimental system chosen for bioassays must be defined with stakeholders to represent as
524 closely as possible the targeted production system, while being feasible (i.e. in time and cost) for
525 several prospective combinations simultaneously (Fig. 2). For instance, if the ultimate objective is to

526 develop a polyculture in a RAS to grow fish out over several months in tanks several cubic meters in
527 size, the bioassays could be performed for a few months in a RAS a few hundred litres in size with the
528 same fish densities, ratio, and rearing practices as those of the targeted system (e.g. a strategy in ⁹⁴).

529 *Assessing the prospective combinations*

530 The bioassays are intended to validate the compatibility estimated in Step 1 and assess species
531 complementarity and fish welfare. Thus, we recommend developing an integrative assessment
532 framework that considers individual, population, community, and system scales ⁴. Here, we provide
533 guidelines and methods to consider in order to assess the proofs of concept.

534 Species compatibility can be investigated further by defining trophic guilds (i.e. groups of species with
535 similar diets based on analysing, for instance, gut contents or stable isotopes; ^{115,116}) or the spatial
536 structure of fish (e.g. ¹¹⁷) in the experimental system. Synergies based on trophic interactions (i.e. in
537 which one species can feed on the waste of another, thus increasing system efficiency ⁴) can be
538 assessed by analysing stable isotopes of prospective combinations (e.g. ^{118,119}). Analysing the behaviour
539 of species interactions (e.g. ¹²⁰) can provide insight into potential complementarity based on
540 commensalism or mutualism ⁴ within prospective combinations. Compatibility and complementarities
541 can also be detected indirectly by comparing fish production in polyculture to that in monoculture ¹²¹
542 for all species combined ¹²². This assessment scores and ranks alternative polycultures ¹²¹ based on
543 traits that are integrative, simple to measure, and inexpensive to analyse (e.g. morphometric
544 measurements, commonly measured physiological parameters, and behavioural traits through direct
545 observations).

546 Ensuring animal welfare ¹²³ in farming systems has become a major requirement for developing food
547 production (e.g. ^{124,125}). Thus, we strongly recommend assessing the welfare of all fish species during
548 bioassays. Although fish welfare in polyculture is related to species compatibility (i.e. low compatibility
549 is likely to negatively impact fish welfare; ⁴), many other parameters of the farming system, regardless
550 of the species co-farmed, can influence it (e.g. enrichment, water quality; ^{126,127}). Additional
551 information should thus be collected to assess fish welfare during bioassays, such as behavioural,
552 health, and physiological analyses of several welfare markers ^{126,128-130}. This information can be
553 aggregated into a welfare index (e.g. ¹³¹) for each species combined to quantify this important concern.

554 ***Decision-making***

555 Step 2 assesses the degree of compatibility, complementarity, and fish welfare observed in each proof
556 of concept. Although difficult and subjective to define, minimum thresholds should be set for each of
557 these categories to make a go/no-go decision for each prospective combination before beginning Step

558 3 (Fig. 2). These thresholds must be defined on a case-by-case basis by and with the stakeholders based
559 on the context in which the future polyculture will be implemented. Nevertheless, we recommend
560 using benchmarks to define them to identify whether the compatibility, complementarity, and animal
561 welfare of the polyculture implemented can be considered as negative, positive, or neutral. Relevant
562 benchmarks for fish compatibility and welfare can be defined by performing, along with polyculture
563 bioassays, monocultures of each species combined^{16,121,122}, which describe the state of each species
564 when it does not interact with others. If one of the species has lower parameters of fish production
565 (e.g. survival rate, growth) or a lower welfare indicator score in polyculture, the prospective
566 combination should be rejected. Moreover, when prospective combinations aim at improving or
567 replacing an existing monoculture or polyculture, the current production system should be assessed in
568 bioassays to provide a benchmark for decision-making: prospective combinations should be better
569 than the current system to be considered in Step 3. Compatibility and welfare can be compared only
570 to a baseline that includes the same species (e.g. welfare indicators cannot be compared among
571 species).

572 After this final discard process, all of the remaining combinations can be considered as of interest for
573 polyculture development, although only one or a few will likely be implemented in real-world
574 production. Using an effective decision-making method that supports multi-criteria decision-making is
575 necessary to determine the most suitable final combination based on the production context and
576 stakeholder expectations, but it is unlikely that one single prospective combination will be the best for
577 all criteria. It is more likely that a combination will be the best for one dimension (e.g. high trophic
578 compatibility, low economic cost-benefit ratio) but the worst for another (e.g. low spatio-temporal
579 compatibility, interspecific aggressiveness rate). We recommend that stakeholders, particularly future
580 fish farmers, make the final decision about trade-offs. Indeed, the prototyping and scaling-up of Step
581 3 will have to be carried out, or at least strongly co-realised, by these producers. Thus, they must make
582 the final decision about the “best combination(s)”.

583 **Step 3 – Implementing the best combination(s) in aquaculture production**

584 Step 3 aims at developing aquaculture production based on the best combination(s) identified in the
585 previous step via (i) prototyping, (ii) developing a pilot, and (iii) adopting and disseminating the pilot.

586 ***From an initial prototype to successful production***

587 The prototyping stage is envisioned as the development of several versions of a polyculture system
588 (i.e. working prototypes), that are progressively optimised (i.e. fine-tuned) based on the best
589 combination under real-world production conditions. Its goal is threefold.

590 First, the prototyping stage up-scales the best combination based on bioassays of the farming system
591 and ensures that it does not have lower compatibility, complementarity, or welfare than the proof of
592 concept (see a similar strategy and concerns about aquaculture development by ¹³²). Doing so requires
593 (i) developing a polyculture at a real-world production scale and (ii) applying some or all of the
594 assessment framework developed in Step 2 to each prototype (e.g. in the SEPURE project:
595 <https://sepure.hub.inrae.fr>).

596 Second, the prototyping stage provides the first opportunity to study the sustainability of the
597 production system using the best combination(s), as it is difficult to assess sustainability using small-
598 scale bioassays. Assessing the sustainability of aquaculture production requires analysing production
599 systems holistically ⁴¹. Sets of indicators and approaches to assess the sustainability of aquaculture
600 production have been developed ^{41,133,134} that consider economic, environmental, and social
601 dimensions of aquaculture and must include stakeholders to be applied effectively. In the context of
602 this workflow, they can be applied to each prototype to identify its strengths and weaknesses. If several
603 prototypes are built based on different best combinations, the polyculture(s) that likely have the
604 highest sustainability based on prototypes can be identified. Several scoring and ranking procedures
605 based on the sustainability of production are available for aquaculture and terrestrial agriculture ^{134,135}.
606 Nevertheless, their results must be considered with caution because the actual sustainability of the
607 production system that could result from each prototype will be strongly influenced by the system's
608 context, which requires complex analyses and large data sets to be accurately assessed (e.g. ¹³⁴).
609 Assessing sustainability and verifying compatibility, complementarity, and welfare are crucial because
610 they help determine whether development of the prototype should continue (e.g. attempt to improve
611 it by developing new versions) or the up-scaling revealed insurmountable problems (i.e. it should be
612 abandoned).

613 Third, the prototyping stage fine-tunes the polyculture under development by exploring alternative
614 fish combinations, practices, and system parameters (e.g. densities, species ratio, the amount and
615 frequency of inputs). For example, species can be co-farmed in different ratios (e.g. ^{16,136}) and using
616 different management practices ^{137,138}, which can change the combination's feasibility or potential
617 benefits of polyculture. This places a premium on optimising system parameters, which could be
618 attempted empirically, but a model-assisted approach is preferable (for a similar strategy for crops,
619 see ^{139,140}, and for aquaculture, see ^{141,142}). The prototyping stage thus first develops an initial prototype
620 of the polyculture that has the same characteristics as those of the bioassays (Step 2), before modelling
621 the functioning of the initial prototype to identify which parameters to optimise in the next version.
622 With each new version of the prototype, the data obtained when assessing the system can be reused
623 to refine the models and increase their relevance.

624 Stakeholders' resistance to change (e.g. consumers' reluctance to consume new fish species, farmers'
625 reluctance to reconsider their practices) is a major obstacle to developing new agricultural practices
626 and production systems ¹⁴³. Although including stakeholders in Steps 1 and 2 minimises this issue,
627 developing a pilot (i.e. a production system available to a subset of the entire audience) with willing
628 fish farmers can demonstrate the relevance of the polyculture developed and convince other farmers
629 to adopt this new production system. This pilot can be the most optimised prototype, used for
630 teaching, demonstration, additional assessment (e.g. economic), and promotion.

631 Adoption and dissemination of an innovation beyond the group that helped design and test (i.e.
632 upscale) it ¹⁴⁴ is complex because it (i) involves competition among supporters of different innovations
633 or technological solutions, (ii) depends on simultaneous upscaling of other complementary practices
634 or downscaling of existing practices, and (iii) is impacted by implications of the innovation for other
635 domains (see the review of ¹⁴⁵). Identification of the best upscaling strategies for aquaculture
636 development, including those for new polycultures, should be entrusted to the research and
637 development organisations that are mandated to do so, which lies beyond the scope of this study.
638 Nevertheless, other aquaculture developments and challenges need to be considered to define the
639 readiness for innovation (see ¹⁴⁵) and suitability of a new polyculture before upscaling it. For instance,
640 creating labels related to animal welfare, environmental impacts, and production of native species ¹⁴⁶
641 based on public perceptions ¹⁴⁷ can increase the socio-economic interest of production based on new
642 fish combinations. Moreover, including the latest advances in aquaculture, such as precision fish
643 farming ¹⁴⁸, may improve the management of production based on new fish combinations, thus
644 facilitating its adoption by fish farmers ⁴.

645 ***Beyond production and towards a community of practice***

646 Implementing a proof of concept in aquaculture production implies transferring knowledge and
647 technology to fish farmers, but many obstacles can hinder these transfers because they imply using
648 physical structure, knowledge, skills, organisation, values, and funding. Many models of technology
649 transfer are available to facilitate development of new production systems (e.g. ¹⁴⁹), but we emphasise
650 the importance of disseminating information and mobilising stakeholders to develop implementation
651 of new fish combinations.

652 Effective sharing of experience, knowledge, and skills among researchers, technological development
653 organisations, and fish farmers can accelerate development of new production systems ¹⁵⁰. This can
654 be done through a community of practice, which is a group of people who share a concern for
655 something they do and learn how to do it better as they interact regularly ¹⁵¹, like those that exist
656 already for IMTA ¹⁵². Thus, we recommend continuing to share information, begun during the initial

657 steps of the workflow, by creating and managing national and international networks of stakeholders
658 involved in fish polyculture.

659 **Workflow limitations and future prospects**

660 The workflow's reliance on third-party databases in Step 1 exposes it to two major obstacles: the
661 current lack of information and the low quality of available datasets. Several abiotic, biogeographic,
662 and fish functional trait databases currently include only fragmentary and incomplete information for
663 certain geographic areas (e.g. EarthEnv stops at 60°N ⁵⁷) or fish groups (e.g. TOFF currently includes
664 few marine species ⁷⁶). Moreover, problems with database quality such as false data and coarse
665 information (e.g. broad classes of fish depth preferences) can (i) bias the assessment in Step 1 if
666 doubtful datasets are not excluded or (ii) worsen the dearth of information if large datasets must be
667 excluded to avoid bias. Nevertheless, quality control of data of databases (e.g. technical validation of
668 TOFF ⁷⁶) already limit problems with false data, while the lack of information is expected to decrease
669 over the medium term due to on-going research on fish. In the meantime, the latter problem can be
670 mitigated by procedures to fill in missing information in databases (e.g. ¹⁵³).

671 Special attention should be paid to the fish stocks used for the bioassays in Step 2, as they will come
672 from a specific location whose environmental conditions may have shaped particular functional trait
673 expressions due to phenotypic plasticity and epigenetic effects (e.g. ¹⁵⁴). These specific characteristics
674 cannot be observed in other fish stocks or over several generations in farming systems, which may
675 make bioassays based on only one fish stock misleading for aquaculture purposes. One solution to
676 minimise this issue consists of performing bioassays of several different fish stocks, but doing so could
677 be difficult because it would increase the workload, cost, and duration of the workflow.

678 Besides potential pragmatic concerns, workflow users should be aware of five methodological
679 limitations that must be addressed in future developments.

680 First, the assessment of abiotic compatibility in Step 1 identifies species that can be farmed in the same
681 environment, but it does not ensure that this abiotic niche overlap matches the optimal rearing
682 conditions of each species. Although this limitation could be avoided by comparing abiotic niches only
683 to species' optimal farming environments, it is not currently feasible because the latter (e.g. ¹⁵⁵) have
684 been determined only for a few fish species.

685 Second, favouring ecological redundancy in farming systems ensures the resilience and adaptability of
686 production ^{156–158}, but the compatibility-based workflow tends to minimise redundancy among the fish
687 combinations selected. Future studies should investigate how to reach a trade-off between (i)
688 ecological redundancy, which increases farming resilience, and (ii) fish compatibility, which decreases

689 competition and thus fish welfare problems. Alternatively, fish polyculture could become resilient at
690 the territorial scale by diversifying fish production with several fish combinations farmed in the same
691 region to increase the ability of local aquaculture to address global changes.

692 Third, the risk of pathogen spill-over between species is an important issue in polyculture. Although
693 veterinary examination before combining several fish species can decrease this risk, even
694 asymptomatic fish (e.g. ¹⁵⁹) can be contagious and host pathogens harmful to co-farmed species, which
695 decreases species compatibility. Thus, we recommend considering potential pathogen spill-overs
696 during Steps 2 and 3 and, if a spill-over is observed, determining whether it is a one-time contamination
697 or a pathogen common in one of the species combined. Unfortunately, there is currently no large-scale
698 approach to predict the risk of pathogen spill-over to fish, but assuming that such an approach will be
699 developed (see an example of ranking the risk of animal-to-human spill-over by ¹⁶⁰), the workflow will
700 need to be improved by considering the risk of pathogen spill-over in Step 1.

701 Fourth, the workflow considers intraspecific variability in fish species by considering the range of
702 functional trait expressions reported in the literature for different populations and in different
703 environments, but cannot assess whether certain populations of a species are more compatible with
704 other species than other conspecific populations. However, fish intraspecific differentiation can shape
705 genetically-based variability in functional trait expression ²⁹, which can influence compatibility with
706 other species ⁴. This intraspecific differentiation can occur among allopatric populations, domestic
707 strains, or populations with different degrees of domestication ^{29,161}, which places a premium on
708 integrating intraspecific classification (e.g. based on strain characterisation or phylogeographic
709 assessment, see review by ²⁹) in fish databases to consider how intraspecific differentiation influences
710 identification of the best fish combinations.

711 Fifth, changes in stakeholder expectations over time may complicate the co-construction approach
712 used in the workflow; thus, the fish combinations considered best may change over time. To adapt fish
713 polycultures more easily to changes in stakeholder expectations, we recommend continuously
714 monitoring the socio-economic and environmental contexts of the stakeholder panel and the
715 community of practices initiated during the workflow. By developing a fish-compatibility database
716 based on Step 1, this monitoring could help adapt the list of prospective combinations over time. Major
717 changes in this list would indicate that a new combination should be sought to meet new expectations.
718 The monitoring would also identify new challenges that could trigger development or adaptation of
719 the workflow.

720 **Practical example of a potential workflow application: development of new fish polycultures in**
721 **ponds in north-eastern France**

722 We here provide an example of a potential workflow application to show how it can be applied in a
723 practical way. Please note that this example is for illustrative purposes only. It has not been carried out
724 in a real case. Only the CI and \bar{P} are based on real data, those present in public databases at the time
725 the example was produced (06 Jun. 2023). \bar{I} , the weighting coefficients of ACI and $ASEI$, and the
726 $ACI/ASEI$ thresholds for PPS , normally defined by all stakeholders, have only been set by the authors
727 of this article. Therefore, the results presented should not be used to develop fish polycultures even
728 in the geographic area mentioned here.

729 In this example, fish farmers in the Grand Est region of France want to develop new polycultures of
730 juvenile fish in ponds by considering species traditionally produced in this region and species that have
731 recently begun to be produced in ponds in France. Prior starting the workflow, a consortium of
732 stakeholders, a list of fish species to be considered, and main stakeholders' expectations are defined.

733 The consortium includes (i) local fish farmers wanting to change their practices, (ii) fish farmer
734 associations (e.g. the *Filière aquacole du Grand Est*; a non-profit association aiming at promoting
735 research, enhancement, and development of continental aquaculture in the Grand Est region), (iii)
736 scientists working in aquaculture research and in social sciences and humanities, (iv) applied research
737 organisations that can ensure the transfer of innovation to the aquaculture sector (e.g. the Technical
738 Institute for Poultry, Rabbit, and Fish Sectors in France), and (v) representatives of policy makers (e.g.
739 the Ministry of Agriculture). A panel of local consumers is indirectly included in the consortium through
740 a survey carried out by the scientists on social networks to define their expectations (see below).

741 The list of fish species to be considered is based on information provided by the consortium's
742 producers, as well as on production statistics for the country (i.e. FAO's FishStatJ software). In this
743 example, the resulting list includes 10 species: six that are traditionally produced in ponds in this region
744 (i.e. *C. carpio*, *E. lucius*, *R. rutilus*, *S. lucioperca*, *S. erythrophthalmus*, and *T. tinca*) and four that are not
745 (i.e. *C. idella*, *Leuciscus idus*, *Micropterus salmoides*, and *Silurus glanis*).

746 Local fish farmers seek combinations of three species for pragmatic reasons (e.g. difficulty in sourcing
747 many different species). As fish farming in this region traditionally combines piscivorous species with
748 other species, stakeholders do not seek to avoid the risk of predation.

749 One hundred and twenty combinations of three species are theoretically possible among the 10 listed.
750 In Step 1, the compatibility of all combinations and the socio-economic interest of species are
751 estimated simultaneously and independently.

752 Consortium scientists estimate abiotic, spatio-temporal and trophic compatibility. The results are
753 available in DATA S1. Abiotic compatibility is estimated using AquaDesign⁵³. This suggests that species

754 can live in the same farming environment in all but two combinations. These two combinations are
755 thus excluded from the rest of the analysis. For the remaining combinations, AquaDesign calculates
756 the degree of overlap of the abiotic niches to obtain the $C_{abiotic}$ for each combination. Spatio-temporal
757 compatibility is estimated on the basis of four traits corresponding to the species use of spatio-
758 temporal resources (i.e. benthic, pelagic, diurnal, nocturnal). Trophic compatibility is estimated on the
759 basis of 13 functional traits corresponding to species diet (i.e. algivore, carnivore, detritivore,
760 insectivore, invertivore, necrophagous, omnivore, oophagous, periphytivor, herbivorous,
761 phytoplanktivore, piscivore, and zooplanktivore). Information about these traits is extracted from TOFF
762 ⁷⁶ and FishBase ⁷⁷. The data is binary coded and used to calculate a distance (i.e. Minkowski distance)
763 between pairs of species. For each species combination, the smallest distance value is assigned to the
764 combination as $C_{spatio-temporal}$ and $C_{trophic}$. Abiotic, trophic and spatio-temporal compatibility data are
765 used to calculate the CI .

766 The socio-economic interest of each species is estimated by consortium scientists based on (i)
767 economic information from FAO and (ii) an interest score obtained from a survey of consortium
768 members and French consumers (see SEI results in DATA S1). In this example, one species is considered
769 to be of insufficient socio-economic interest (i.e. *S. glanis*; a species not consumed locally and
770 controversial for its presumed impact on wildlife ¹⁶²) and therefore all combinations containing it are
771 excluded.

772 For the remaining 82 combinations, the CI and SEI are adjusted to the local context to obtain the ACI
773 and $ASEI$ (DATA S1). In this example, greater importance is given to (i) $C_{abiotic}$ in view of the
774 consequences of climate change (i.e. greater niche overlap between species enables them to be
775 farmed under more variable environmental conditions), (ii) $C_{trophic}$ to maximise the use of available
776 trophic resources in ponds, and (iii) \bar{I} in response to the political will to improve the social acceptability
777 of aquaculture in France ¹⁶³. ACI and $ASEI$ are used to calculate PPS for each of the 82 remaining
778 combinations. In order to prioritise best combinations for Step 2, arbitrary minimum thresholds (0.75)
779 of ACI and $ASEI$ are applied to discard combinations. This reduces the list of initial combinations to five.
780 These five combinations are regarded as prospective combinations to be evaluated in Step 2.

781 In Step 2, the selected prospective combinations are tested and evaluated during bioassays in
782 experimental ponds managed by research institutes and in a few ponds made available by consortium
783 fish farmers. The evaluation carried out by the consortium's scientists focuses on validating
784 compatibility under real-life conditions and detecting any complementarities between species. This
785 evaluation is based on a comparison of each species production in polyculture compared to that in
786 monoculture (i.e. using approach of ¹²¹). The results of the evaluation are analysed and discussed by

787 all consortium members to integrate all the expectations (i.e. economic, social, environmental, and
788 pragmatic concerns). For the purposes of this example, we consider that one of the combinations (i.e.
789 *C. idella*, *C. carpio*, and *M. salmoides*) presents interesting results that satisfy farmers in terms of
790 zootechnical performance. The next step is initiated for this combination.

791 In Step 3, prototyping and piloting are conducted at fish farms by consortium producers, accompanied
792 by applied research organisations and ideally supported by funding from political decision-makers
793 involved in the project to encourage the emergence of new production. The economic, environmental,
794 and social dimensions of this production are assessed by scientists from applied research organisations
795 to evaluate the sustainability of the polyculture. As soon as the results confirm the suitability of the
796 polyculture based on the chosen combination, and the modalities of application begin to be defined
797 (e.g. species ratio, feeding practices; defined during prototyping), communication actions via fish
798 farmers' associations or the communication departments of research and development organisations
799 are launched to disseminate this new polyculture to other producers not involved in the initial
800 consortium.

801 **Conclusion**

802 The workflow is designed to develop fish polycultures that meet the conditions of compatibility, socio-
803 economic interest, animal welfare, and sustainability. It screens all possible combinations for a set of
804 species based on three successive assessment steps, which progressively decrease the number of
805 combinations by gradually (i) increasing the number of criteria considered and (ii) refining the
806 combinations to the context of a specific production system in a given area. Stakeholders engage in
807 dialogue and co-construction throughout the workflow, which aims at guiding the development of new
808 polycultures by assessing the feasibility of alternative scenarios, from proof of concept and prototyping
809 to implementation in aquaculture production.

810 Applied here to the development of fish polyculture, the workflow could include other taxa (e.g.
811 molluscs, echinoderms, crustaceans, algae, plants) to create a workflow to develop polycultures that
812 combine fish and other species. It could also help develop new IMTAs, which are considered a viable
813 strategy to replace traditional monoculture¹⁶⁴ and face compatibility problems similar to those of fish
814 polyculture. Moreover, the many factors (e.g. biological requirements, species behaviour, pathogens)
815 that need to be considered when designing an IMTA increase the number of alternative interaction
816 networks that need to be considered. As with fish polyculture, an analytical approach can assess the
817 potential species combinations needed to plan IMTA development.

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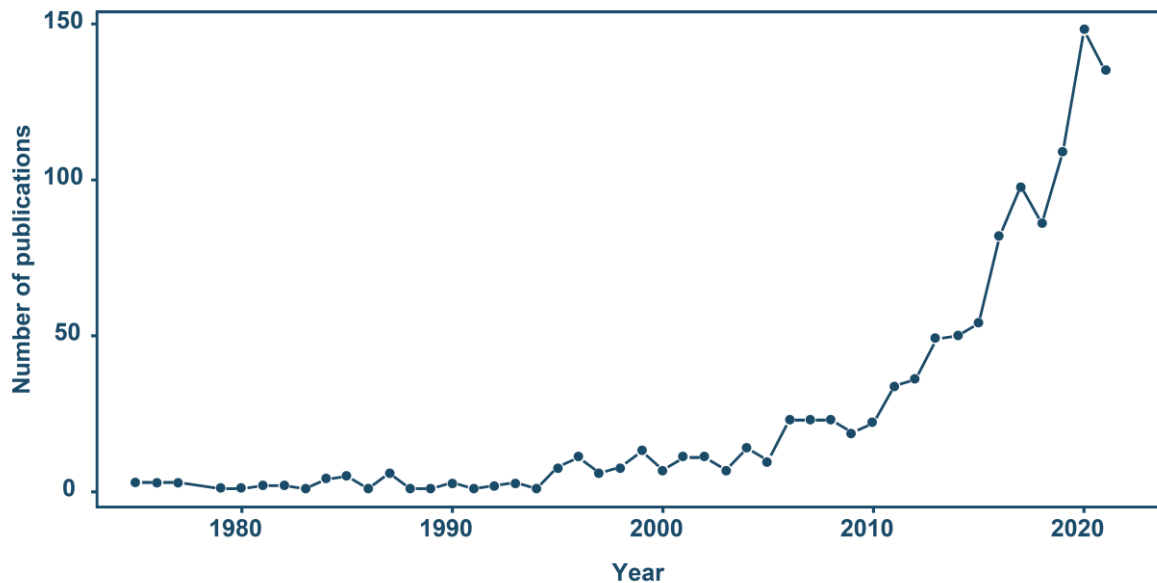
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1302 **Figure**

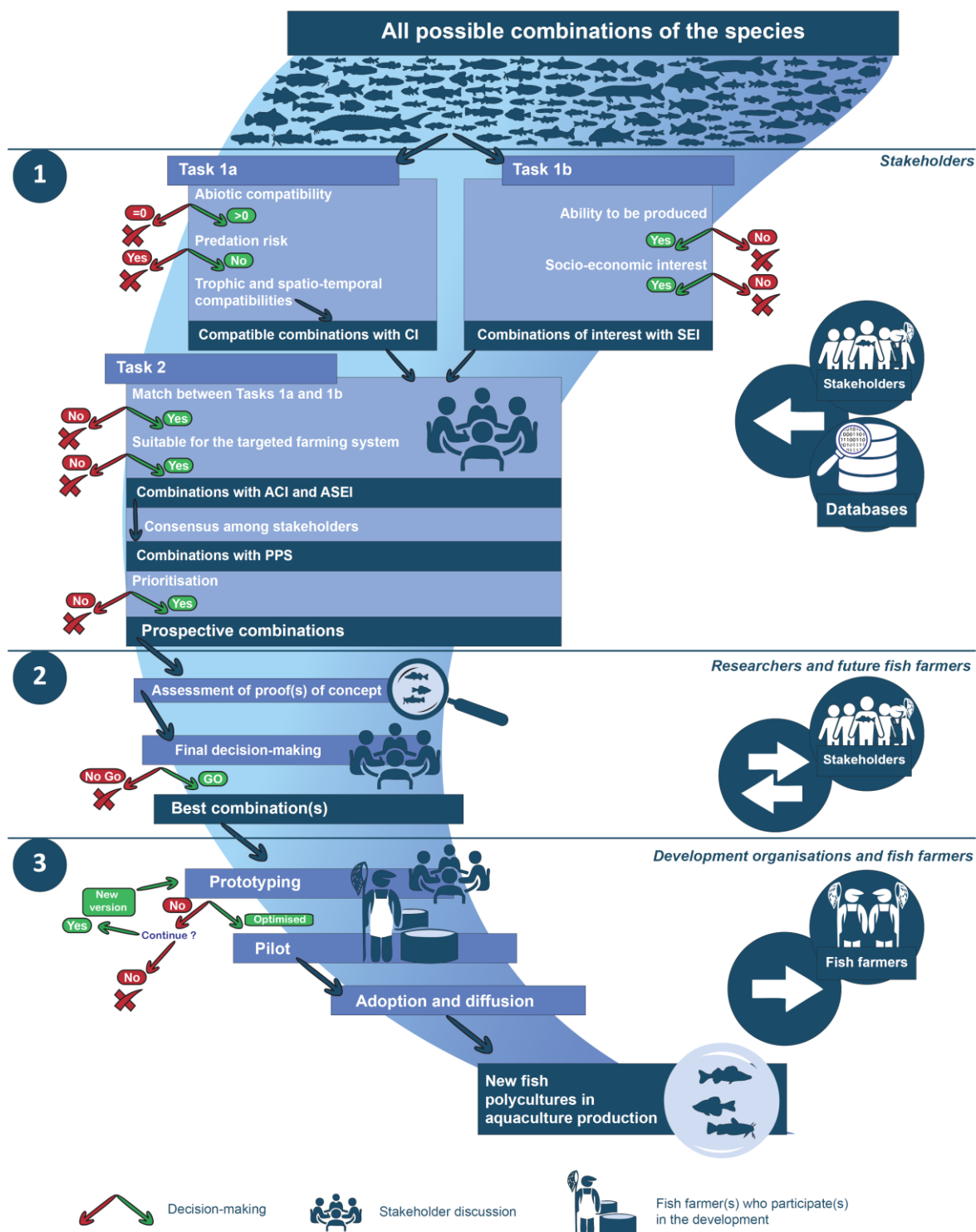
1303 **Figure 1.** Number of publications (reviews and articles) published per year from 1975-2021 whose title,
1304 abstract, or keywords contain the keywords “polyculture and “aquaculture”, or “integrated multi-
1305 trophic aquaculture”, or “integrated agriculture-aquaculture” in the Life Science Area (i.e. agricultural
1306 and biological sciences; environmental science; multidisciplinary) of the Scopus database (accessed on
1307 31 Aug 2022; n = 1141).



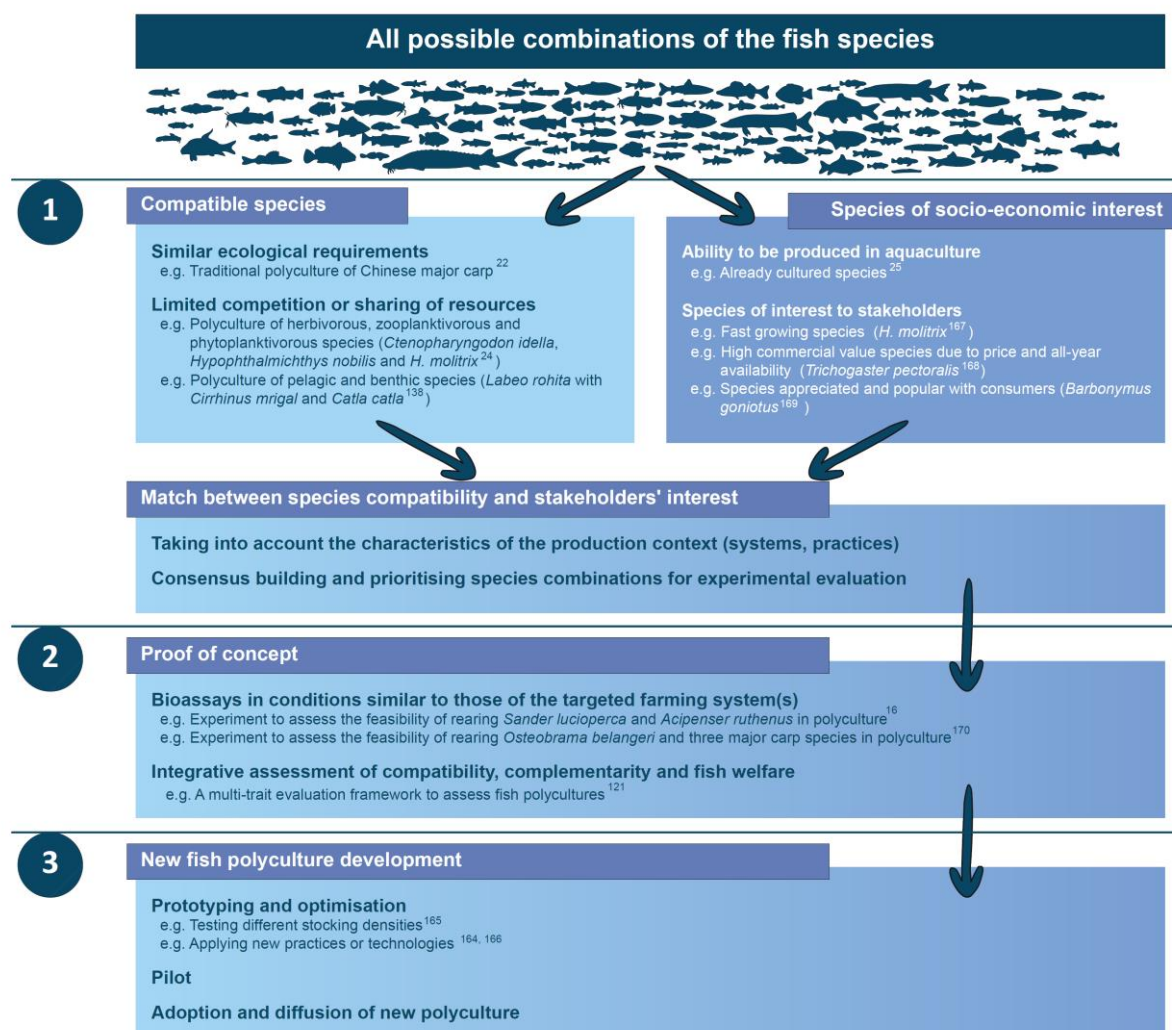
1308

1309 **Figure 2.** A workflow to develop new fish polycultures. The workflow screens all possible species
1310 combinations among a set of fish taxa and then assesses them through increasingly restrictive filters,
1311 decreasing the number of prospective combinations throughout the three steps (numbered on the
1312 left). 1. Determining prospective species combinations based on three tasks. First, led by researchers,
1313 compatible combinations and their compatibility index (*CI*) are determined by assessing abiotic, spatio-
1314 temporal, and trophic compatibilities as well as predation risk between the combined species based
1315 on meta-analyses using datasets in the literature or databases. Second, combinations of interest to
1316 stakeholders (i.e. fish farmers, scientists, political regulators, environmental managers, and consumer
1317 panels) and their socio-economic indicator (*SEI*) are determined based on stakeholder expectations.
1318 Third, prospective combinations are determined by a stakeholder based on a prioritisation approach
1319 that considers the overall polyculture potential score (*PPS*) of each combination (after calculating an
1320 adjusted compatibility indicator (*ACI*)). 2. Developing proofs of concept (bioassays) based on
1321 prospective combinations from which species complementarity and animal welfare are assessed.
1322 Based on this assessment, stakeholders choose the best combination(s) to start developing the new
1323 production system. The assessment is led by researchers in collaboration with other stakeholders. The

1324 best combinations are chosen mainly by future potential fish farmers willing to start a new production
 1325 system. 3. Implementing the best combination(s) in aquaculture production by prototyping and scaling
 1326 up new polycultures. During the prototyping stage, the sustainability of commercial production is
 1327 assessed. This last step is led by development organisations and fish farmers. Pictures with arrows on
 1328 the right side show the flow of information (input and output) of the workflow.



1330 **Figure 3:** A concise description of the three steps to illustrate the overall workflow strategy with
 1331 practical examples of (i) species combinations used in Asian and European polycultures that present
 1332 the sought characteristics and (ii) studies already published showing how to carry out the different
 1333 actions for each step. ^{16,22,24,25,121,138,165–170} refer to cited references.



1334

1335 **Figure 4.** Graphical overviews of the four indices and the polyculture potential score (*PPS*) calculated
 1336 in Tasks 1a, 1b, and 2 of Step 1 (Fig. 2). The compatibility indicator (*CI*) is based on the standardised
 1337 estimates of abiotic ($C_{abiotic}$), spatio-temporal ($C_{spatio-temporal}$), and trophic ($C_{trophic}$) compatibility. The
 1338 socio-economic indicator (*SEI*) is based on the standardised mean price per kg of the species in the
 1339 combination (\bar{P}) and the standardised mean score of the interest in the species in the combination (\bar{I}).
 1340 *ACI* is the adjusted *CI* of the combination for a given farming system and rearing practices (i.e. with
 1341 $C_{abiotic}$, $C_{spatio-temporal}$, and $C_{trophic}$ weighted by stakeholder opinion). *ASEI* is the adjusted *SEI* of the
 1342 combination (i.e. with \bar{P} and \bar{I} weighted by stakeholders). Here, the weighting coefficients of *ACI* and

1343 *ASEI* are arbitrarily set for illustration. The *PPS* is based on standardised *ACI* and *ASEI*. The *PPS* plot
 1344 shows a theoretical example of the prioritisation of candidate combinations. First, minimum values of
 1345 *ACI* and *ASEI* for candidate combinations are set (i.e. "Threshold") to define a set of balanced
 1346 combinations (i.e. green quadrant). Here, the thresholds are arbitrarily set at 0.5 for illustration. The
 1347 *PPS* of these balanced combinations is then calculated as the distance from the origin, and the highest
 1348 *PPS* is the best. All of these combinations can be considered as prospective combinations for Step 2.

