Stronger together: A workflow to design new fish polycultures

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Running title: Polyculture design workflow

Abstract

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

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

Introduction

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

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

Polyculture is increasingly recommended as a way to improve aquaculture by promoting synergies among species and/or compartments of the system [2,4,8]. Polyculture can increase farming efficiency by improving use of the resources naturally present or supplied to the agricultural environment [9] and/or by recycling co-products into the farmed biomass [6,10]. These processes can decrease a farming system’s environmental impacts [11] and increase its resilience [12]. From a socio-economic viewpoint, it can provide benefits by creating new income sources, having lower operational costs and financial risks than monoculture [13], and making economically profitable the production of species that would not be profitable in monoculture [2]. Furthermore, polyculture can improve the welfare of farmed fish [14], either directly due to the species combined (e.g. [15]) or indirectly due to reduced maintenance operations, performed in part by other farmed species (e.g. [16]). However, polyculture can also result in (i) pathogen spill-overs [17], (ii) high mortality or decreased growth rate [18,19], or (iii) stress and physical injuries caused by competition for resources [20] or predation, negatively impacting animal welfare [4,21]. Actually, potential polyculture benefits can be expected provided that compatibility or, even better, complementarity occur among the combined species [4]. Compatibility is the ability of species to live in the same production system without negative interactions (i.e. amensalism, predation, parasitism) or competition for resources (e.g. trophic, spatial). Complementarity is the ability of species to use different portions of the available resources (including by-products or waste of other co-farmed species) or develop commensal or mutualistic interactions. This places a premium on designing relevant species combinations to maximise the benefits of polyculture.

Current fish polycultures

Traditional fish polycultures started in China during the Tang dynasty (A.D. 618-907) [22] and are still widely used mainly in household-managed ponds and small- to medium-sized enterprises [23]. These polycultures combined species that use different natural resources [24] such as the four Chinese major carps (*Ctenopharyngodon idella*, *Mylopharyngodon piceus*, *Hypophthalmichthys molitrix*, and *Aritichthys nobilis*) that have different feeding habits (macrophytes, benthos, phytoplankton, zooplankton) and ecological niches (surface, bottom, mid-water) [2,4].

In Asia, the leading aquaculture zone (i.e. contributing 88% of the world's production of aquatic animals by 2020, of which 64% in China [25]), polyculture systems still dominate (e.g. in China [26]). The most common fish polycultures combine the four Chinese major carps (e.g. in China [26]) or the three Indian major carps (*Catla catla*, *Cirrhinus mrigala*, and *Labeo rohita*) sometimes with Chinese major carps (e.g. in India) [26,27]. In Europe, fish polyculture is continuously practiced since the Middle Ages (Aubin et al., 2017) and combines predator species (e.g. *Esox lucius*, *Perca fluviatilis*, and *Sander lucioperca*) with species from lower trophic levels (e.g. *Cyprinus carpio*, *Rutilus rutilus*, *Scardinius erythrophthalmus*, and *Tinca tinca*) [27,28].

The needs for new fish polyculture

To date, the most common species combinations have been shaped by the trial-and-error and cumulative know-how of fish farmers over decades or centuries [4]. However, new species combinations will be needed in the near future to face new challenges to and developments in aquaculture.

First, most fish production in the world relies on a few species (i.e. in 2020, 76% relied on 3 marine species and 17 freshwater species; [25]), which jeopardises (i) human food security, because the heavy dependence on a few species puts aquaculture production at risk, and (ii) economic prospects, because less diversified production limits aquaculture's potential to adapt to changes in the environment or consumer demand (review in [29]). Thus, a growing number of researchers, national agencies, and international organisations recommend diversifying species while strengthening the well-established species [30–32]. This strategy can improve the sustainability and resilience of the aquaculture sector, particularly by producing new species that occupy other market segments, such as niche markets [30,31,33]. Meeting this desire for diversification requires developing new species production systems [25,30] for which polyculture has never been considered but could be advantageous. It could also be an opportunity to improve traditional polycultures by including new species.

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

Third, the past few decades have seen the emergence of new production systems, such as recirculating aquaculture systems (RAS) and aquaponics, which have rarely included fish polyculture [4]. Since fish biology and species interactions depend upon environmental characteristics [37–39], traditional fish combinations (e.g. in extensive pond aquaculture) may not be relevant for these new production systems due to their characteristics (e.g. the low diversity of environmental components and *ad libitum* feeding in RAS change species' resource use). Overall, these current and future challenges and developments require selecting existing species communities or designing new fish combinations.

Attempting to choose random fish combinations or only traditional combinations for a given production system is not an effective way to develop new fish polycultures because these strategies could fail to identify species combination(s) that are valuable for aquaculture. In comparison, considering all possible species combinations for a given system maximises the probability of providing the best species combination(s) to fish farmers, but this strategy requires considering many species combinations, and not all of them can be assessed empirically due to practical, ethical, temporal, and financial concerns [4]. To address this issue, we developed a conceptual integrative workflow to standardise and plan the development of new combinations of fish species.

A workflow to develop new fish polycultures

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

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

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

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

Step 1 – Highlighting prospective fish species combinations

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

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

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

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

Task 1a - Estimating species compatibility using meta-analyses

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

Species compatibility based on similarity in abiotic niche

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

For niche comparison, we recommend focusing on abiotic parameters that are relevant for most farming environments (e.g. ponds, cages, indoor RAS, raceways) and for which high similarity is required to ensure species compatibility (see the review by [53]) such as light (i.e. daylight intensity and duration) and the main physicochemical water parameters (i.e. conductivity, current/flow, dissolved oxygen, pH, salinity, temperature, total hardness, total nitrogen, total phosphorus, and turbidity). Species' requirements for these parameters can be obtained from the literature on fish aquaculture (e.g. for Percids [54]) or ecology (e.g. [55]). Alternatively or additionally, data for analysing niches can be done by comparing the occurrences of fish species (e.g. from the Global Biodiversity Information Facility, http://www.gbif.org) to locally observed abiotic parameters (species-distribution modelling follows a similar approach; e.g. [56]). These data are available in several databases, such as EarthEnv [57], WorldClim [58], and FLO1K [59] (Fig. 2).

Several methods are available to model and compare species niches [60]. For aquaculture, the recently developed tool AquaDesign assesses the degree of overlap among n-dimensional niche hypervolumes (for details, see [61]) based on species-distribution and abiotic-parameter datasets from public databases [53]. Once all possible combinations of a set of species are analysed, the tool classifies them by their degree of niche overlap among the species. More overlap means that species can be co-farmed under a larger set of abiotic conditions, which makes their polyculture feasible in a wider set of farming environments and still possible despite abiotic variability (e.g. extreme weather events in ponds). However, species combinations with little niche overlap do not need to be excluded from consideration, as, once the niches of two fish species overlap, the species can be maintained under rearing conditions that are suitable for both, especially in highly controlled systems, such as indoor RAS. Moreover, information about species physicochemical requirements or occurrences may be scarce for certain species or reflect only a realised niche when they are based on occurrence datasets (e.g. [62]), which can cause the abiotic compatibility of species to be greatly underestimated. Thus, we recommend not using abiotic niche information alone to exclude fish combinations based on statistical thresholds (see examples of statistical estimates of niche differentiation in [63,64]), except when the niches of at least one pair of species in the combination do not overlap at all (Fig. 2).

Species compatibilities based on fish functional traits

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

Most studies of fish functional ecology have focused on species interactions in the wild and/or been based on traits specific to certain taxa or poorly documented in other species groups (e.g. [72–74]). As the workflow aims at assessing potential compatibility among all possible combinations of fish species in any farming environment, we recommend considering functional traits that are (i) commonly available in the literature or inferred from proxies that are (i.e. to ensure data availability), (ii) common to most fish taxa (i.e. to ensure potential relevance for any species), and (iii) relevant for all farming systems. Researchers have produced several datasets of fish functional traits, based on several decades of research, that are available in databases (e.g. [75–77]), which makes meta-analyses, such as the one needed for this workflow, possible (Fig. 2).

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

Predation risk based on fish functional traits

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

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

Spatio-temporal and trophic compatibilities based on fish functional traits

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

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

Addressing intraspecific variability in functional trait expressions

Functional trait expressions vary within species due to polymorphism or phenotypic plasticity (e.g. [92,93]). Although this variability can modify species compatibility depending on the population or farming environment considered, it is difficult to predict competition or other negative interactions between a pair of species from particular populations in a given environment, because doing so requires knowing the expressions of functional traits of both species in this environment. This information is rarely available in the literature for most species, especially those that are not sympatric or have not been co-farmed. Thus, we recommend (i) considering intraspecific variability in functional trait expressions by considering their ranges reported in the literature for different populations and environments (i.e. such information are available in databases informing about the measure environments of traits, e.g. TOFF [76]) and (ii) assigning the highest risk in each range to the entire combination.

Compatible combinations and compatibility indicator

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

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

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

Where:
CI is the compatibility indicator of a given combination (fish species community).
$C_{abiotic}$ is the position of the combination on the $C_{abiotic}$ axis, corresponding to its standardised niche overlap extent between all species of the combination.
$C_{spatio-temporal}$ is the position of the combination on the $C_{spatio-temporal}$ axis, corresponding to its standardised lowest value of interspecific spatio-temporal dissimilarity index observed among all species pairs of the combination (e.g. among the six pairs for a combination of four species).
$C_{trophic}$ is the position of the combination on the $C_{trophic}$ axis, corresponding to its standardised lowest value of interspecific trophic dissimilarity index observed among all species pairs of the combination.

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

Task 1b. Defining combinations of interest to stakeholders

The objective of Task 1b is to define the expectations of all of the stakeholders. We argue that stakeholders are generally looking for species combinations that are socio-economically interest and follow the regulations of their country or region. These expectations depend strongly on which fish species are combined, and not all species are suitable for aquaculture. Some cannot be produced in farming systems (e.g. for technical reasons or because they were never domesticated) or have no socio-economic interest [29]. Thus, the workflow discards compatible combinations that contain (i) at least one species that cannot be farmed (i.e. pragmatically, that has never been produced in aquaculture; see cultured species in [25]) or (ii) only species with no socio-economic interest (e.g. according to the score of interest determined by stakeholders; see below) (Fig. 2). International, national, and local regulations may also cause some combinations to be discarded (e.g. they contain species that are illegal to rear in the targeted region) (Fig. 2). Additionally, when species combinations are designed mainly to improve the production of a target species (e.g. pikeperch in RAS [94]), compatible combinations that do not contain the target species can be discarded.

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

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

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

Where:
SEI is the socio-economic indicator of the combination.
$\bar{P}$ is the position of the combination on the $\bar{P}$ axis, corresponding to its standardised mean of the price per kg of the species in the combination.
$\bar{I}$ is the position of the combination on the $\bar{I}$ axis, corresponding to its standardised mean of the score of interest of the species in the combination.

Task 2 – Definition of prospective combinations

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

First phase: preliminary discussion

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

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

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

To refine assessment of the remaining fish combinations, it is necessary to consider how the target farming system and its practices could influence species compatibility. This can be estimated at least partly by weighting the relative importance of each type of compatibility in the *CI* and its three-dimensional space using characteristics specific to systems or practices. For instance, if feeding will be *ad libitum*, trophic compatibility may have less influence on the overall compatibility of combinations. Similarly, it is easier to maintain abiotic parameters in a range suitable for all species combined in a highly controlled indoor RAS [95,96], which allows even species with little abiotic niche overlap to be co-farmed. Thus, a combination's compatibilities are weighted by the targeted system and its practices to create an adjusted *CI* (ACI) (Fig. 4, Equation 3). Stakeholders, particularly fish farmers and researchers, can provide insights useful for setting weighting coefficients. Like for $\bar{I}$, the weighting coefficient can be defined using a survey in which each stakeholder rates the importance (i.e. from 0-1) of each estimate. The scores of each estimate given by the stakeholders are then averaged.

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

Where:
ACI is the adjusted compatibility indicator of the combination for a particular farming system and rearing practices.
$C_{abiotic}$ is the position of the combination on the $C_{abiotic}$ axis, corresponding to its standardised niche overlap extent between all species of the combination.
$C_{spatio-temporal}$ is the position of the combination on the $C_{spatio-temporal}$ axis, corresponding to its standardised lowest value of interspecific spatio-temporal dissimilarity index observed among all species pairs of the combination (e.g. among the six pairs for a combination of four species).
$C_{trophic}$ is the position of the combination on the $C_{trophic}$ axis, corresponding to its standardised lowest value of interspecific trophic dissimilarity index observed among all species pairs of the combination.
wc_1, wc_2, and wc_3 are the weighting coefficients (range: 0-1) applied to $C_{abiotic}$, $C_{spatio-temporal}$, and $C_{trophic}$, respectively.

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

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

Where:
ASEI is the adjusted socio-economic indicator of the combination according to stakeholders' demands.
$\bar{P}$ is the position of the combination on the $\bar{P}$ axis, corresponding to its standardised average of the price per kg of the species in the combination.
$\bar{I}$ is the position of the combination on the $\bar{I}$ axis, corresponding to its standardised average of the score of interest of the species in the combination.
wc_P and wc_L, are the weighting coefficients (range: 0-1) applied to $\bar{P}$ and $\bar{I}$, respectively.

Third phase: addressing differences in expectations among stakeholders

Stakeholders may perceive the importance of abiotic, spatio-temporal, and trophic compatibilities differently, and these compatibilities cannot have the same importance when designing polycultures. Similarly, stakeholders' expectations may differ depending on their business model, geographic area of activity, farming priorities, and consumption habits [29,43,97]. These differences can be managed by developing consensual design of prospective polyculture or personalising workflow outcomes to certain stakeholder groups. The former can be achieved by surveying stakeholders to identify their expectations and perceptions of polyculture as well as their fish-farming experience before reaching a consensus using, for instance, Quaker-based or spokes council models [98,99] or weighting of the compatibilities based on survey results [100]. The latter implies developing a workflow with customisable options to fit as closely as possible either one stakeholder or a group of stakeholders with similar expectations.

Fourth phase: determining the prospective combinations

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

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

Where:
PPS is the polyculture potential score of the combination.
ACI is the position of the combination on the ACI axis, corresponding to its the standardised adjusted compatibility indicator of the combination for a particular type of farming system.
ASEI is the position of the combination on the ASEI axis, corresponding to its the standardised the socio-economic indicator of the combination.

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

Although the *PPS* can be used to select the best combinations, we do not recommend using it as it 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

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

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

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

Why are bioassays still needed?

Minimising animal testing is universally recognised as an ethical and pragmatic cornerstone of biological research. Nevertheless, alternative approaches such as simulation models or virtual laboratories for aquaculture research cannot yet replace bioassays completely (but see ongoing development at https://ae2020virtuallab.sintef.no).

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

How should bioassays be performed?

Designing the experimental system

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

The experimental system chosen for bioassays must be defined with stakeholders to represent as closely as possible the targeted production system, while being feasible (i.e. in time and cost) for several prospective combinations simultaneously (Fig. 2). For instance, if the ultimate objective is to develop a polyculture in a RAS to grow fish out over several months in tanks several cubic meters in size, the bioassays could be performed for a few months in a RAS a few hundred litres in size with the same fish densities, ratio, and rearing practices as those of the targeted system (e.g. a strategy in [94]).

Assessing the prospective combinations

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

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

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

Decision-making

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

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

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

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

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

From an initial prototype to successful production

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

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

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

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

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

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

Beyond production and towards a community of practice

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

Effective sharing of experience, knowledge, and skills among researchers, technological development organisations, and fish farmers can accelerate development of new production systems [150]. This can be done through a community of practice, which is a group of people who share a concern for something they do and learn how to do it better as they interact regularly [151], like those that exist already for IMTA [152]. Thus, we recommend continuing to share information, begun during the initial steps of the workflow, by creating and managing national and international networks of stakeholders involved in fish polyculture.

Workflow limitations and future prospects

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

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

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

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

Second, favouring ecological redundancy in farming systems ensures the resilience and adaptability of production [156–158], but the compatibility-based workflow tends to minimise redundancy among the fish combinations selected. Future studies should investigate how to reach a trade-off between (i) ecological redundancy, which increases farming resilience, and (ii) fish compatibility, which decreases competition and thus fish welfare problems. Alternatively, fish polyculture could become resilient at the territorial scale by diversifying fish production with several fish combinations farmed in the same region to increase the ability of local aquaculture to address global changes.

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

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

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

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

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

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

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

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

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

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

Consortium scientists estimate abiotic, spatio-temporal and trophic compatibility. The results are available in DATA S1. Abiotic compatibility is estimated using AquaDesign [53]. This suggests that species can live in the same farming environment in all but two combinations. These two combinations are thus excluded from the rest of the analysis. For the remaining combinations, AquaDesign calculates the degree of overlap of the abiotic niches to obtain the $C_{abiotic}$ for each combination. Spatio-temporal compatibility is estimated on the basis of four traits corresponding to the species use of spatio-temporal resources (i.e. benthic, pelagic, diurnal, nocturnal). Trophic compatibility is estimated on the basis of 13 functional traits corresponding to species diet (i.e. algivore, carnivore, detritivore, insectivore, invertivore, necrophagous, omnivore, oophagous, periphytivorous, herbivorous, phytoplanktivore, piscivore, and zooplanctivore). Information about these traits is extracted from TOFF [76] and FishBase [77]. The data is binary coded and used to calculate a distance (i.e. Minkowski distance) between pairs of species. For each species combination, the smallest distance value is assigned to the combination as $C_{spatio\text{-}temporal}$ and $C_{trophic}$. Abiotic, trophic and spatio-temporal compatibility data are used to calculate the *CI*.

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

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

In Step 2, the selected prospective combinations are tested and evaluated during bioassays in experimental ponds managed by research institutes and in a few ponds made available by consortium fish farmers. The evaluation carried out by the consortium's scientists focuses on validating compatibility under real-life conditions and detecting any complementarities between species. This evaluation is based on a comparison of each species production in polyculture compared to that in monoculture (i.e. using approach of [121]). The results of the evaluation are analysed and discussed by all consortium members to integrate all the expectations (i.e. economic, social, environmental, and pragmatic concerns). For the purposes of this example, we consider that one of the combinations (i.e. *C. idella*, *C. carpio*, and *M. salmoides*) presents interesting results that satisfy farmers in terms of zootechnical performance. The next step is initiated for this combination.

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

Conclusion

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

Applied here to the development of fish polyculture, the workflow could include other taxa (e.g. molluscs, echinoderms, crustaceans, algae, plants) to create a workflow to develop polycultures that combine fish and other species. It could also help develop new IMTAs, which are considered a viable strategy to replace traditional monoculture [164] and face compatibility problems similar to those of fish polyculture. Moreover, the many factors (e.g. biological requirements, species behaviour, pathogens) that need to be considered when designing an IMTA increase the number of alternative interaction networks that need to be considered. As with fish polyculture, an analytical approach can assess the potential species combinations needed to plan IMTA development.

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Author contributions

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Figure

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

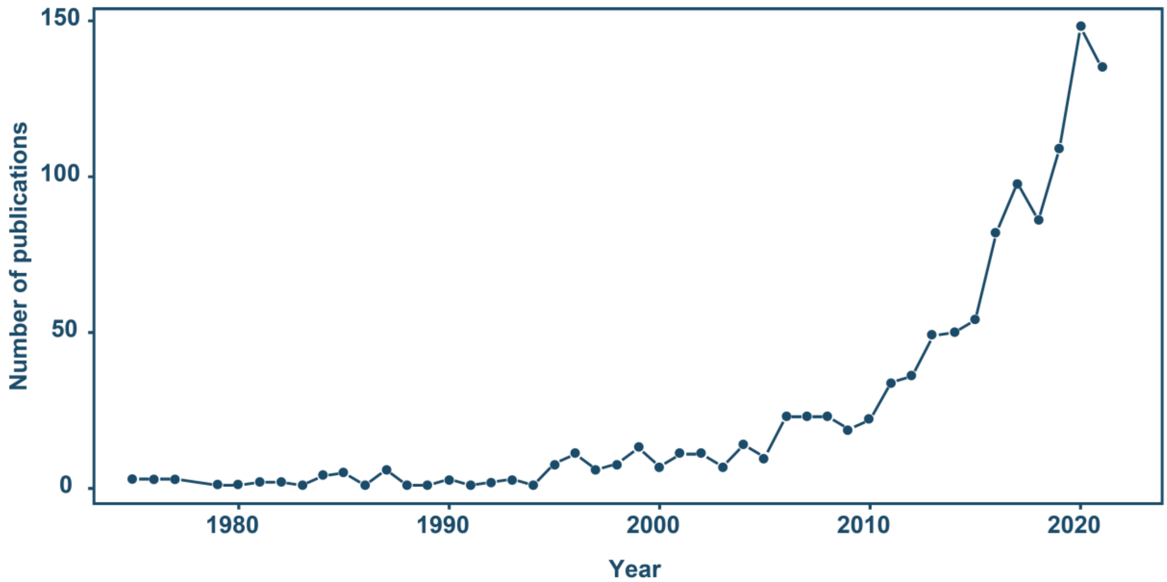

Figure 2. A workflow to develop new fish polycultures. The workflow screens all possible species combinations among a set of fish taxa and then assesses them through increasingly restrictive filters, decreasing the number of prospective combinations throughout the three steps (numbered on the left).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

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

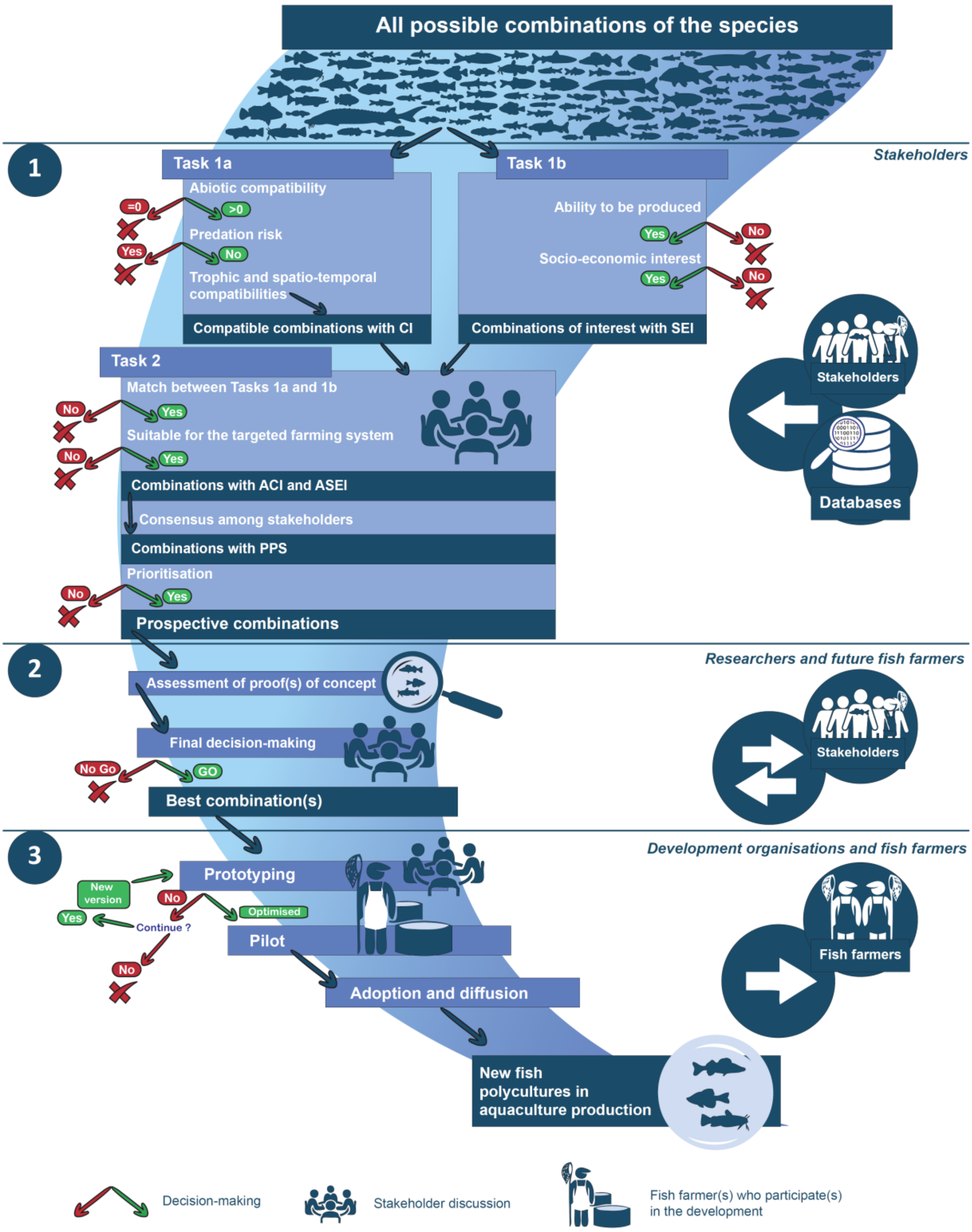

Figure 3: A concise description of the three steps to illustrate the overall workflow strategy with practical examples of (i) species combinations used in Asian and European polycultures that present the sought characteristics and (ii) studies already published showing how to carry out the different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

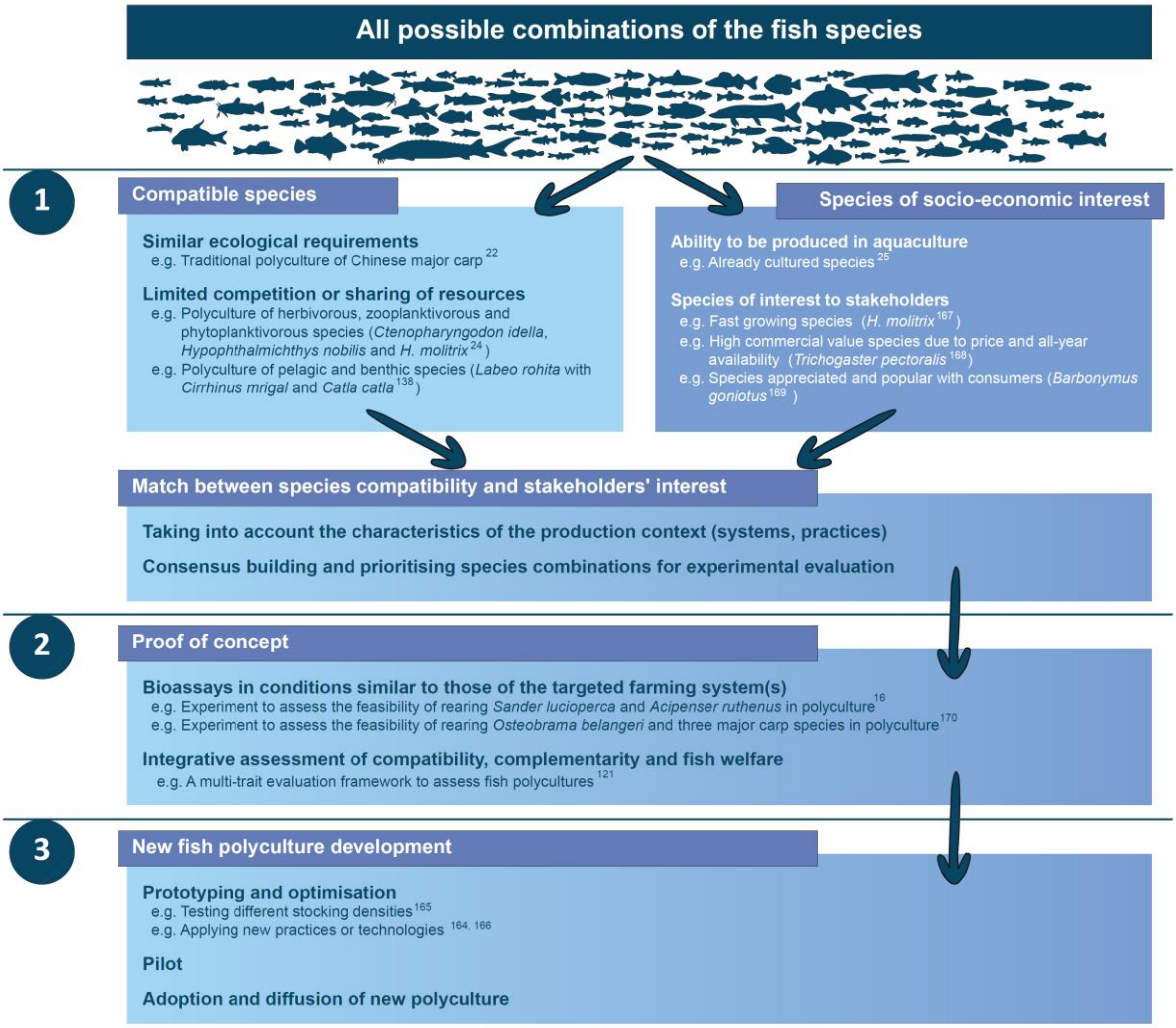

Figure 4. Graphical overviews of the four indices and the polyculture potential score (*PPS*) calculated in Tasks 1a, 1b, and 2 of Step 1 (Fig. 2). The compatibility indicator (*CI*) is based on the standardised estimates of abiotic ($C_{abiotic}$), spatio-temporal ($C_{spatio\text{-}temporal}$), and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

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