

WORKSHOP SUMMARY

Promoting stemness and proliferation in fish cell cultures

2023

Hosted by The Good Food Institute



Executive summary

GFI identified slow proliferation rates and unreliable cell culture performance of fish cell lines as a key barrier to early-stage R&D and scale-up efforts for cultivated fish. To address this issue, GFI identified stakeholders with the knowledge and interest to brainstorm the most effective ways to make routine fish cell culture more rapid and reliable and invited them to an online workshop. Attendees were split up into breakout rooms to explore different strategies for improving fish cell culture performance: optimization & selection of starting cell lines, culture media optimization, and transdifferentiation of easy-to-grow cell types. Workshop attendees' key insights on each topic are summarized in this report.

From these insights emerged four key themes:

Theme 1: A lack of validated antibodies, annotated genome sequences, and other fairly basic research tools for fish species is a key barrier for both academics and industry scientists.

Theme 2: Many of the big challenges in fish cell culture are interrelated. For example, cell line development is hampered by the lack of optimized media formulations, and media optimization is hampered by the lack of cell lines.

Theme 3: We do not understand the basic biology of fish cells as well as we should. By better understanding the metabolism, cell types, and key molecular pathways in fish cells, we can speed up cell line development and media optimization and investigate the potential of transdifferentiation-based approaches.

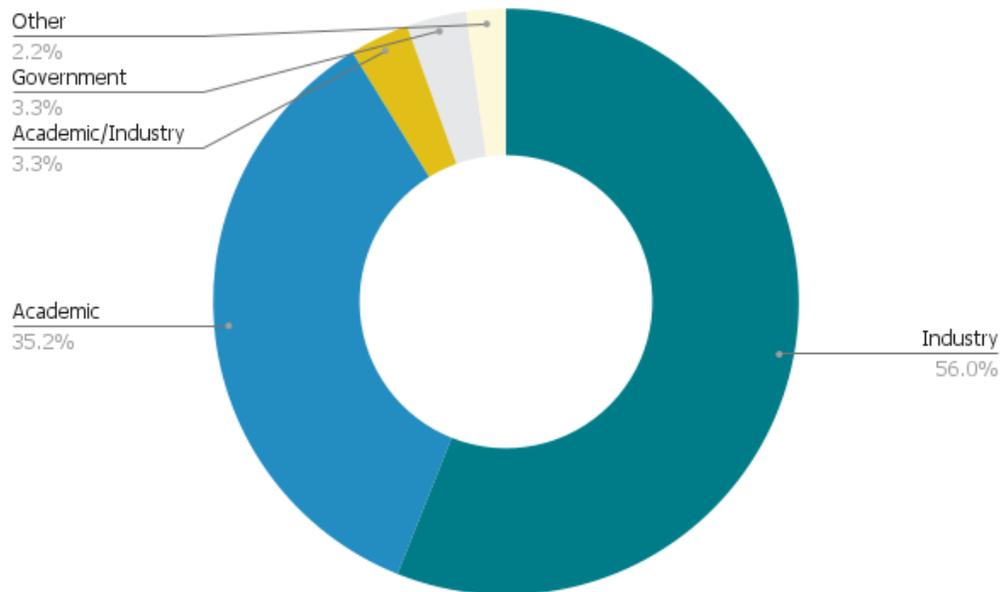
Theme 4: Collaboration within the field of cultivated seafood, including across the boundary between academia and industry, will be crucial to the ultimate success of both groups. Access to funding, especially open-access funding that benefits the whole industry, remains a key barrier.

Table of contents

Promoting stemness and proliferation in fish cell cultures	1
Executive summary	2
Table of contents	3
Workshop participant demographics	4
Workshop background and motivation	4
Workshop insights	4
Broadly-applicable scientific insights	5
Insights on cell lines	6
Insights on culture media & culture conditions	9
Insights on transdifferentiation	10
Insights on systemic barriers & solutions	12
What's next?	15
Appendix	17
Current challenge	17
Proposed solution	17
Anticipated impact	18
Related efforts and resources	19
Contact information	19

Workshop participant demographics

The workshop was held on January 24, 2023. There were 91 attendees (excluding 13 GFI staff in attendance) from 52 organizations (excluding GFI) across 18 countries. The organization types and number of attendees affiliated with each are as follows, where “other” was typically a non-profit organization:



Please note that this summary includes anonymized and collective insight from group discussions and therefore does not represent the opinions or strategic priorities of individuals, companies, or organizations named.

Workshop background and motivation

For more information, please see the [appendix](#) or the following GFI resource:

- [GFI solution: Promoting stemness and proliferation in fish cell cultures](#)

Workshop insights

This workshop convened stakeholders to explore strategies for improving the efficiency and reliability of fish cell cultures across three topics:

1) Optimization & selection of starting cell lines. What strategies can be used to develop cell lines with the desired properties or to improve the properties of existing cell lines?

2) Culture media optimization. What needs to be done to identify better culture media formulations to enable rapid and reliable proliferation of fish cells? This session also discussed strategies for optimizing other aspects of the culture conditions (e.g., temperature, bioprocess design) to achieve these goals.

3) Transdifferentiation of easy-to-grow cell types into myogenic and adipogenic lineages. As an alternative to extensive optimization of myogenic and adipogenic cell lines and their growth conditions, might it be feasible to focus on transdifferentiating cells that are inherently easier to grow, such as fibroblasts?

The key questions and insights from each of these topics are summarized below.

Broadly-applicable scientific insights

Key Takeaways: One of the most frequently mentioned scientific challenges across all three breakout topics was the lack of validated antibodies and other basic research tools for fish cell culture. Participants also discussed the fact that current systems and incentive structures result in negative results often going unshared, leading to duplication of effort by other researchers.

Solutions: The problem of tool availability could be addressed by improving communication between cultivated fish scientists and life science companies, contracting the development of new antibodies, systematic testing of existing antibodies on fish cultures, and the development of non-antibody-based cell type identification methods. GFI recommends the use of existing tools for sharing negative results, and can help by aggregating these reports for the alternative protein community (see “**Next steps**” section).

- A key theme across all three breakout topics was that the lack of validated antibodies, other tools for cell type characterization, annotated genome sequences, PCR primers, CRISPR tools, gene delivery systems, and other fairly basic research tools for fish species is a barrier for both academics and industry scientists.
 - Even for antibodies that appear to work on fish tissue, it's important to perform negative controls to verify that the staining

is specific. However, published papers do not always include the appropriate controls.

- It is not always entirely clear if cell-type specific expression of common cell type markers is shared between fish and mammalian cells.
- Because fish cell culture is more niche, there may be a lack of financial incentives for a company to produce fish-specific antibodies.
 - 💡 Making it easier for scientists to communicate their needs to life science companies could help.
- 💡 It was suggested that GFI or another similar entity could contract out fish-specific antibody development for use by the entire community. This could be especially helpful if focused on conserved domains to improve the chances of cross-species applicability. 💡 Another suggestion was a project to systematically test the efficacy of existing antibodies in various fish species and cell types and publish the results in an open-access database. This could be a relatively non-sensitive area where companies might be willing to collaborate with academics by making their in-house cell lines available for testing.
- Where antibodies are unavailable, other approaches for identifying cell types may be used, e.g., morphology or lectin staining. There are opportunities for cell type identification methods that don't rely on antibodies, we just need to get a bit more creative!
 - 💡 Small grants for student or postdoc projects using or developing histochemical methods are [available from the Histochemical Society](#) and might be applicable for cultivated seafood.
- Participants expressed quite a bit of interest in omics-based methods, including the use of transcriptomics for cell type identification, but also noted that the lack of basic tools such as fully-annotated genomes (see [Lu & Luo 2020](#) and the [AQUA-FAANG](#) project) is a barrier to realizing the potential of these methods.
 - 💡 The functional annotation tool [EggNogMapper](#) was recommended for use with fish species that lack a complete genome annotation.

-
- Several participants mentioned the need for better negative results sharing.
 - Mechanisms already exist for sharing these types of information, including various [journals focused on negative results](#), [Biorxiv](#), and one's own blog.
 - However, informally-published negative results may not be easy to find by other researchers. 💡 There are some small ways GFI can help here (see **“Next steps”** section).

Insights on cell lines

Key Takeaways: There are numerous barriers that contribute to the difficulty in creating and accessing cell lines. Key challenges include species differences and our limited understanding of fish biology.

Solutions: Large, collaborative efforts may be needed to address challenges in fish cell line development. This could include efforts by multiple companies to co-fund cell line development by academic groups, the creation of “core facilities” to support cell line development at multiple institutions, and the development of hands-on or video courses on cell line development.

- Wide fish species diversity makes cell line development more challenging, as insights from one species often don't translate directly to others.
 - Some species seem to be more likely to spontaneously immortalize than others.
 - Fast-growing species might produce fast-growing cell lines.
 - In the experience of one participant, cell lines from marine fish are easier to establish in conventional (i.e., mammalian) media compared to freshwater fish.
 - Warm water species may be preferable, as they tend to grow faster.
 - Temperature needs can become a challenge, e.g., maintaining the correct temperature during imaging for species that require cold temperatures.
 - Common reagents are optimized for mammalian cell culture, meaning that additional steps are necessary to adapt protocols to fish, e.g., adjusting the osmolarity of phosphate-buffered saline (PBS) and culture media to account for the needs of fish cells.

-
- Alternatives to trypsin that don't impact cell viability would be helpful.
 - Cell sourcing remains a challenge. Participants mentioned working with fishermen, buying samples from supermarkets, and acquiring juvenile fish from aquaculture companies as strategies they have used.
 - Isolation from younger fish fairly consistently gives better results.
 - There are challenges related to the isolation process.
 - Controlling contamination can be a challenge for fish cell cultures.
 - Automating cell isolation could be helpful. Third-party companies offer high-throughput services for cell line development, but growth at 37°C is often assumed.
 - Optimized protocols for enzymatic tissue dissociation are lacking.
 - Cell sorting methods optimized for fish, especially those that don't rely on protein expression, could help isolate the cell type of interest from a larger population.
 - We don't understand the biology of fish cells as well as we should, and we don't fully understand the pathways involved in proliferation and differentiation. Many of these pathways *may* be shared with mammals, but this is often unclear, and species differences within fish are not well understood.
 - Predicting differentiation potential is not always straightforward.
 - Transcriptomics and proteomics were mentioned as useful tools for understanding the behavior of a particular cell line or cell isolation. Bioinformatics tools can help us understand the key transcription factors and other regulators of proliferation and differentiation pathways.
 - Based on what is already known about the molecular mechanisms of pluripotency or multipotency, it may be possible to predict what pathways need to be activated or inhibited.
 - Machine learning could enable these sorts of predictions (💡 possible Ph.D. project).
 - Direct comparisons of non-immortalized and immortalized cells from the same species could shed light on mechanisms of immortalization.
 - The properties of cell lines can change over time due to selection or spontaneous mutation, making it necessary to re-do characterization and optimization steps.
 - Cell diversity within a culture can decrease over time due to “survival of the fittest” mechanisms.

-
- Methods for high-throughput screening can enable the development of lines with desirable properties.
 - Adapting fish cells to suspension culture is difficult.
 - Initiating cultures in 2D inherently selects for anchorage-dependent cells. 💡 One suggestion was that it might be possible to initiate cultures under suspension conditions.
 - Pluripotent fish cells have not been extensively studied, and conditions for expanding cell numbers while maintaining pluripotency are not very well established. Mechanisms of pluripotency are poorly understood.
 - Developing non-integrative methods for iPSCs would be helpful.
 - Lack of optimized basal media and defined culture conditions (see next section) is a bottleneck for cell line development.
 - Isolating cell lines directly into serum-free media would be ideal.
 - 💡 It could be helpful to have a joint fund where multiple companies can contribute funding, mentorship, and protocols to enable students to develop non-proprietary cell lines. This should be primarily driven by industry, with non-profits like GFI playing a supporting role to contribute to a “rising tide lifts all boats” effect.
 - Logistical challenges make cell line development and acquisition difficult.
 - Depending on the country of origin, ordering cell lines can be very difficult and time-consuming due to paperwork and shipping.
 - 💡 It may be necessary for cell line owners to deposit in multiple repositories to truly make their line accessible.
 - Cell line development represents a huge amount of overhead for new companies or labs. 💡 A consortium of labs or a “fish cell culture core facility” with the necessary equipment and trained personnel could lower barriers to entry.

Insights on culture media & culture conditions

Key Takeaways: Our lack of a detailed understanding of fish biology is an obstacle to media development. A variety of approaches, from large-scale screening to targeted approaches based on transcriptomics or metabolomics, can address this challenge.

Solutions: A combination of many smaller projects (Ph.D. project or single lab) and larger, systematic collaborations can come together to address this challenge. At the same time, it will be necessary to consider how the developed media formulations will be scaled up and how future supply chains will be built.

-
- Currently-used basal media formulations are simply those that have been shown to work “well enough” in fish, but they are not optimized, and it is unclear whether all components of these formulations are necessary.
 - Metabolic needs of fish differ from those of mammals, e.g., inositol as an essential amino acid.
 - Media osmolarity may need to be adjusted, especially for bony fish versus cartilaginous fish.
 - The need for specific growth factors and other components of serum is poorly understood.
 - We need to understand the composition of fish serum from different species, the responses of fish cells to various growth factors, and the properties of fish growth factors themselves (💡 possible Ph.D. project).
 - Mass spec and NMR can be used to understand serum composition.
 - Fractionation can be used to narrow down where the active components are.
 - Hydrolysates have some efficacy in replacing FBS, but their performance is not identical.
 - It may be necessary to look beyond “traditional” media components to meet the needs of fish cells—e.g., omega-3 fatty acids, cholesterol, and microalgae-derived compounds.
 - We need to better understand the species specificity of fish cells’ needs regarding media and culture conditions.
 - Focusing on one or a few species can be helpful as a way to narrow the problem space.
 - Optimizing oxygen concentration may be helpful, as fish are adapted to lower-oxygen environments, and environments with too-high oxygen concentrations can lead to reduced proliferation, multipotency, and myogenic capacity ([Knežić et al. 2022](#)).
 - A systematic comparison of responses to culture conditions across multiple cell lines from fresh and saltwater fish could be a good fit for a 💡 Ph.D. project (would depend on cell line availability).
 - Fish physiologists and electrophysiologists may have relevant insights into the metabolic needs of different fish species.
 - We need to better understand the basic biology:

-
- Mechanisms of pluripotency, satellite cell maintenance, myogenesis, adipogenesis, etc.
 - Sequencing data from different stages of differentiation.
 - Composition of the natural environment of a given cell type.
 - Spent media analyses (e.g., [O’Neill et al. 2022](#)) and metabolomics could be helpful.
 - Creating genome-scale metabolic models could be used to guide rational media design. 💡 This would be best suited to a large, collaborative project.
 - Real-time measurement of nutrient and metabolite concentrations would be helpful.
 - Tools like DOE, AI, and high-throughput screening methods can aid optimization efforts.
 - For cultivated seafood development, it will be important to consider the impacts of media formulations on end product properties, e.g., omega-3 content.
 - Lack of cell lines to test media formulations is a barrier (see previous section).
 - Working with the incumbent industry may be helpful.
 - 💡 It may be possible to work with the fisheries and aquaculture industry to use byproducts, enabling scale-up. However, batch-to-batch variability may present a challenge.
 - 💡 Aquafeed suppliers could be well-positioned to become suppliers of media components.
 - Food safety of media components, including residues, is essential.
 - There is a need for transparent, publicly-available data about the composition and safety of the inputs to cultivated seafood to ensure that consumers feel comfortable with these new foods.

Insights on transdifferentiation

Key Takeaways: While transdifferentiation may be a promising approach for cultivated fish, the most immediate need is to clearly understand and be able to identify the relevant cell types.

Solutions: Short-term research priorities should include developing antibodies and other cell characterization tools (see “**Broadly-applicable scientific insights**”), mapping out meat-relevant cell types in commonly-consumed fish species using transcriptomics or other methods, and correlating fine-scale cell type differences—including differences in maturation state—to sensory

differences. These experiments may lay the groundwork for successful investigations into fish cell transdifferentiation.

- There is a clear need to better understand cell type identities and basic biological processes in fish.
 - A clear definition of what constitutes a fish fibroblast, myoblast, adipocyte, etc., is somewhat lacking.
 - A finer-grained understanding of cell type may be necessary in many cases, e.g., red vs. white vs. pink muscle (and subtypes thereof) as opposed to the broad category of “muscle cell.”
 - Transcriptomics can help us better understand the muscle and fat differentiation process in fish and the boundaries between cell types (e.g., [Farnsworth et al. 2020](#)).
 - There are no standards for characterizing the differentiation process in the context of cultivated fish. Ultimately what matters is taste, texture, and nutrition, but when working at small scales, it is unclear whether it is sufficient to monitor the process using morphology and lipid accumulation or whether specific biomarkers are necessary.
 - Fat is important for flavor ([Shahidi & Hossain 2022](#)), but we don’t understand the sensory differences between fully-differentiated adipocytes versus cells that have taken up lipids and taken on an “adipocyte-like” phenotype. 💡 Comparing these could be a good Ph.D. project.
 - Funding for academic fish studies comes from aquaculture, so the incentive is to study the whole organism, not the molecular mechanisms of differentiation.
 - A better understanding of the mechanisms behind regeneration in certain fish species could inform efforts at transdifferentiation.
 - Genome duplication makes understanding genetics harder.
 - Antibodies and other tools for cell type characterization (see section on “**Broadly-applicable scientific insights**” above) are another big part of this issue.
- There is no clear answer as to whether GM or non-GM approaches to transdifferentiation are preferable. Food safety, regulation, and public perception must be considered when choosing an approach.
 - For non-GM (i.e., based on small molecules or changes to culture conditions as in [Pasitka et al. 2022](#) and [Tsuruwaka & Shimada 2022](#)) approaches, it is necessary to take into account regulatory

-
- and food safety considerations when choosing transdifferentiation reagents. (The same is true for reagents used to induce differentiation from stem cells).
- Minimizing the number of exogenous substances added to a cell line will mean an easier regulatory process.
 - Negative public perceptions of GMOs and regulatory hurdles limit the approaches that are feasible, especially in certain geographic regions such as Europe and New Zealand. Participants expressed a wish for efforts to change public perceptions of GMOs generally.
 - Material cues (grooves, stiffness, etc.) were suggested as a possible alternative to GM or small molecule-based approaches.
 - Participants brought up the challenge of designing scalable systems that allow for differentiation in 3D tissues.
 - Participants highlighted that many cultivated meat patents focus on fibroblasts and speculated that this is related to the cost of working with iPSCs.
 - There are limitations to using methods like FACS on stem cells due to the fragility of these cells.

Insights on systemic barriers & solutions

Key Takeaways: Funding, especially funding for open-access research, is a major need. Both industry scientists and academics are eager to collaborate, but there remains a need for coordination to ensure these collaborations can happen.

Solutions: There are opportunities to identify shared needs between for-profit entities, which can be helpful for solving some challenges. However, some of the basic research needs within cultivated fish ultimately will be best solved by open-access funding from government agencies. Facilitating collaboration across the industry is one area where GFI can play a key role. Some areas, such as in-person cell line isolation workshops, may be best led by academic or industry groups with the necessary facilities and equipment, with GFI playing a supporting role.

- Funding remains a key bottleneck.
 - There is a lack of funding generally and substantial regional variation.
 - There is a lack of funding for basic research, such as cell line development, in most regions.

-
- However, in some regions with more funding availability, the primary bottlenecks may be more related to the need to get established researchers with the necessary expertise to work on cultivated seafood.
 - Importance and short-term profitability are not always aligned, so government support is crucial.
 - Solving certain challenges can immensely benefit the field, but these are not always profitable problems to solve. A different funding model is needed to tackle these shared challenges across the field.
 - Participants mentioned NIH programs and agricultural subsidies as examples of the U.S. government recognizing a need not best served by profit-driven solutions alone and stepping in to fill the gaps.
 - 💡 Training and funding programs administered by NIH and USDA could accelerate the development of open-access knowledge on cultivated meat and seafood.
 - One barrier to grant funding for basic research is that reviewers may be unfamiliar with the state of cultivated seafood science and have an inaccurate understanding of what further research is needed.
 - This can lead funding bodies to demand overly-ambitious solutions that are at odds with the true needs of the industry.
 - 💡 It was suggested that GFI could help by developing educational materials for grant reviewers to better understand the field.
 - In cases where the needs of the cultivated seafood industry align with the needs of conventional seafood, medicine, or other industries, there may be opportunities to make the development of these solutions profitable, thereby incentivizing profit-driven actors to develop the necessary data and tools.
 - The same could apply to shared needs for research tools with other fields of academia. For example, a similar idea was suggested related to 💡 developing cell lines from endangered fish species that would be useful for studying ecology and fish biology.
 - Participants expressed a strong desire for more collaboration and information sharing within the field of cultivated fish.

-
- Participants pointed out that the success of any one company depends on the success of the industry as a whole. This should provide a strong incentive for collaboration within the field.
 - Participants also expressed concern about the potential for the pressure for short-term results to compromise the quality of research, especially in industry. In the long run, being careful, transparent, and honest is the fastest way to retain talent and investment and find long-term success!
 - Scientific integrity can also be bolstered by the publication of peer-reviewed research by industry players.
 - More collaboration between biologists and engineers can help academia to work more efficiently.
 - Lack of vertical integration in companies can lead to siloing. Companies choosing to specialize in one part of the value chain still need to understand how their product fits with the rest of the industry.
 - GFI can be a matchmaker between companies, investors, and scientists.
 - 💡 Participants would like to see more events that foster discussion and collaboration.
 - 💡 Establishing working groups proactively can help researchers to be prepared when relevant funding opportunities arise.
 - 💡 Hands-on workshops for cell isolation and other key methods would be very helpful. Since GFI does not have wet lab space, workshops could be organized by academic groups or companies.
 - X-ray crystallography was mentioned as an example of a field that benefited a lot from this approach.
 - 💡 Since travel to in-person workshops can be a barrier, video courses could help to democratize the space.
 - 💡 Academic-industry training programs at the graduate and postgraduate levels can help with workforce development.
 - 💡 Because of the scale of the challenge, large multi-institutional projects focused on cultivating fish will be essential.
 - NIH [COBRE](#) programs were mentioned as an example of a model that can be effective in accelerating both research and training.
 - Intellectual property concerns can make information sharing more difficult.

-
- The right incentive structure is essential for building academic/industry collaborations, whether small one-off projects or large collaborative research centers.
 - 💡 Investors who choose to invest in multiple companies have an incentive to encourage information sharing among the companies they are invested in. This is ultimately beneficial for these companies and their investors.
 - Companies would like to collaborate with academics but may be hesitant to share protected information like media formulations. Companies will need to think carefully about what they can offer their academic partners without revealing what they don't want to reveal.
 - In pharma, it is common for academics to test de-identified compounds and publish without revealing the identities of the compounds.
 - If this allows a collaboration that otherwise wouldn't have happened, some public data is better than none.
 - Companies choosing not to patent key innovations with the potential to benefit the whole field can be part of the equation, and there is precedent for this. [Volvo's choice to make its patent on the three-point seat belt available](#) to other companies without license fees was mentioned as an example.
 - The [Horizon consortium](#) was mentioned as a positive example of a model that facilitates collaboration.
 - Because pilot plants are expensive, there can be an incentive for joint investment into shared facilities.

What's next?

Our goals in organizing this workshop were three-fold:

1. Inform GFI's internal strategy for how we will support the industry in addressing the challenge.
2. Provide an opportunity for potential collaborators to meet.
3. Provide a forum for the people who will solve the problem to develop their ideas for how they will do so.

Related to GFI's next steps, we gained valuable insights into how GFI can support the industry. We surveyed participants on what they felt would be the

most impactful next steps for GFI. The highest-rated categories (based on 33 responses) were:

1. Funding research via a dedicated RFP on this topic (81.8%)
2. Advocating for more government funding of this research (75.8%)
3. Hosting networking sessions to bring together potential collaborators (72.7%)
4. Explaining the state of the science & research needs via blog posts, videos, concept notes, technical deep dives, or review papers (45.5%)

We have a lot of thinking to do about how we can best move forward based on these survey results and the specific ideas discussed during the workshop. We'll have more specifics to share in the coming weeks and months. In the meantime, a couple of quick ways we may be able to help are:

- There was a lot of discussion about sharing information about service providers, such as antibody manufacturers, and whether commercially-available antibodies work or don't work in specific species. We already track this information in our [Research Tools Database](#), though we are sure we're missing a lot! We welcome submissions to the database—whether it's letting us know about a paper we missed or sharing your own positive or negative results—to help us make it more comprehensive!
- Another common theme was the need for researchers to share negative results. We encourage researchers to share this information using existing channels such as [bioRxiv](#), and we are happy to serve as an “aggregator” of alternative protein-specific negative results. If you have shared negative results elsewhere, send us a link, and we can include your work in the [Alternative Protein Literature Library](#).

Related to connections between potential collaborators, we hope that the connections made between workshop participants will lead to meaningful collaborations in the future. To support further connections between participants and others interested in this topic, we created a Slack channel where further discussions can take place. GFIdeas members can find the community in the channels list (just search “workshop”). For those who are not already part of GFIdeas and would like to be, you can join for free [here](#).

Related to next steps for participants, many of the ideas discussed during the workshop are beyond the scope of what GFI is poised to accomplish internally. We hope that workshop participants will develop their own set of next steps

based on what was discussed and that others may identify new ways to get involved as a result of this report. One clear theme from the workshop was the need for collaboration, so we encourage anyone inspired by an idea from this report and looking for a collaborator to post in the Slack channel (see above) and see if someone else is interested in the same thing!

Appendix

Current challenge

Reports of continuous myogenic, adipogenic, mesenchymal stem cell (MSC), and embryonic stem cell (ESC)-like lines from fish in academic literature are relatively sparse, and their reported doubling times tend to be long compared to mammalian cell types. Many fish cell lines have doubling times of several days, whereas mammalian cells typically require only approximately one day. For example, the doubling time of the C2C12 mouse myoblast line is [approximately 20 hours](#). Long doubling times pose a major challenge scaling up production of cultivated seafood to commercially-relevant levels. Slow growth also encumbers lab-scale research efforts into cultivated seafood.

In addition to the challenges posed by slow cell growth, media formulations that [avoid using serum](#) and other animal-derived components are necessary for cultivated seafood to become economically viable. Substantial progress has been made in cultivated terrestrial meat with formulations like [Beefy-8](#) and [Beefy-R](#). However, research on animal-free media formulations for fish cell culture still needs to catch up. Serum-free growth of medaka cells was [achieved using IGF2](#). However, the growth rates under those conditions were slower than the serum-containing control, suggesting that IGF2 only partially substituted for serum. Current efforts to produce high-performance, serum-free media for fish cell culture are underway, including at [Virginia Tech](#) and [Defined Bioscience](#), but additional research is needed. Even in the presence of serum, spontaneous differentiation is observed in many pluripotent fish cell lines ([Chen et al. 2003a](#); [Chen et al. 2003b](#); [Parameswaran et al. 2007](#)). Premature differentiation presents an additional challenge to large-scale cell production by depleting the pool of proliferative cells.

Proposed solution

Researchers may employ various strategies to achieve rapid and reliable proliferation of relevant cultivated seafood cell types. These may be broadly categorized based on the production step they most closely align to:

Cell line development and optimization: Optimization of the source cells themselves—either by direct manipulation or by selecting for desirable

phenotypes within a heterogeneous cell population—may help to produce cell lines with the desired characteristics.

Optimization of culture media formulation and culture conditions for proliferation: Likely the most important set of tools available to researchers attempting to improve proliferation rates and other metrics for cultivated seafood will be optimization of culture conditions, especially culture media formulations.

Differentiation: By better understanding the differentiation potential of various fish cell types, additional starting cell types may be added to the menu of possibilities. If easy-to-grow cells such as fibroblasts could be easily transdifferentiated, issues related to cell line development and media optimization may become much more straightforward.

This workshop served as a brainstorming session to further develop a vision of how this proposed solution may be achieved and what kinds of support from GFI are likely to be impactful.

Anticipated impact

In the short term, identifying more reliable conditions for cell proliferation will mean that academic researchers and early-stage companies will be able to spend their time on downstream problems, such as the development of better scaffolds, bioprocess optimization, and end product characterization. In the longer term, more rapidly proliferating and more efficient cells will allow a given amount of cultivated fish to be produced less expensively and with lower environmental impacts. Early techno-economic modeling efforts suggest that the metabolic efficiency of the cell lines used for cultivated meat and seafood is likely to be one of the biggest factors in determining the cost of production ([Humbird 2021](#)). Cell lines and media formulations that maintain high proliferation rates and appropriate differentiation capacity over many passages will allow for the production of large amounts of cultivated meat from a single biopsy. If transdifferentiation is a viable strategy for fish, the issues of both cell line availability and culture conditions could become much less challenging without adding the additional regulatory burdens sometimes associated with genetic manipulation. Unlike myogenic, adipogenic, and pluripotent lines, fibroblast-like lines are available from a reasonably wide variety of fish species (see the Indian [National Repository of Fish Cell Lines](#)). Fibroblasts are also

relatively easy to work with compared to other cell types, meaning that less complex and costly media formulations may be required. Similar to advances in cell line development and media optimization, advances in differentiation techniques that expand the possible menu of starting cell types will make it easier to conduct basic research into various areas related to cultivated fish.

Related efforts and resources

- [GFI solution: Promoting stemness and proliferation in fish cell cultures](#)
- [GFI research grant: Lowering the cost of growth factors](#)
 - [Venkatesan et al. \(2022\)](#) compared the performance of growth factors produced in-house based on gene sequences from various animal species (related to grant mentioned above).
- [Expanding access to cell lines](#)
- [Anticipated growth factor costs and volumes](#)
- [PISCES/ATLAS: Aggregating data for alternative seafood](#)
- [Algae and tobacco plant researchers among winners of €400,000 prize to commercialize cultivated meat.](#)
- [Liu et al. \(2022\)](#) improved proliferation rates of large yellow croaker satellite cells by adding a p53 inhibitor and a Yap activator

Contact information

For comments or questions please feel free to contact:

Claire Bomkamp, Ph.D.

Lead Scientist—Cultivated Meat & Seafood Specialist, The Good Food Institute

✉ claireb@gfi.org

About GFI

The Good Food Institute (GFI) is a 501(c)(3) nonprofit working internationally to make alternative proteins like plant-based and cultivated meat delicious, affordable, and accessible. GFI advances open-access research, mobilizes resources and talent, and empowers partners across the food system to create a sustainable, secure, and just protein supply.