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Perspective

A More Open Approach Is Needed to Develop Cell-Based Fish Technology: It Starts with Zebrafish

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The global demand for fish is rising and projected to increase for years to come. However, there is uncertainty whether this increased demand can be met by the conventional approaches of capture fisheries and fish farming because of wild stock depletion, natural resource requirements, and environmental impact concerns. One proposed complementary solution is to manufacture the same meat directly from fish cells, as cell-based fish. More than 30 ventures are competing to commercialize cell-based meat broadly, but the field lacks a foundation of shared scientific knowledge, which threatens to delay progress. Here, we recommend taking a research-focused, more open and collaborative approach to cell-based fish meat development that targets lean fish and an unlikely but very attractive candidate for accelerating research and development, the zebrafish. Although substantial work lies ahead, cell-based meat technology could prove to be a more efficient, less resource-intensive method of producing lean fish meat.

Introduction

Over the next 30 years, the global population is projected to increase from 7.7 billion to as many as 9.7 billion people, with rapid growth and urbanization in less developed parts of the world.[1] Along with more people needing food, diets in developing countries are expected to consist of more meat, including finfish,[2] which, moving forward, we refer to as "fish." Based on these trends, we likely face an expanding demand for meat for years to come, and meeting this demand with more efficient use of resources presents a key challenge.[2]

Fish and seafood—which includes fish, crustaceans, mollusks, and other aquatic animals, but excludes reptiles, seaweeds, and other aquatic plants—already supports 20% of the global demand for consumed animal protein.[3] This percentage is even higher, on average, in the Global South, ranging from 16.8% to 56.5% in low- or middle-income Asian and African countries.[4] Among several roles that it plays in our food system, including substantial income generation, particularly in the Global South,[5] fish meat provides essential nutrition. As an animal protein, fish is a source of high-quality, easily digested protein,[2] and supplies micronutrients not found as readily in plant-derived foods such as iron, zinc, vitamin A, and vitamin B_{12}.[6]

However, despite the current and growing importance of fish and seafood in food security and nutrition for billions of people, our ability to meet increased demand for fish is being called into question. Pressure on fisheries stocks through capture fisheries has increased over the past 40 years to the point that 33.1% of stocks are overfished beyond biological sustainability and some populations are in decline.[5] Although some projections propose that, with improved management and policies in place, wild fisheries could produce almost 20% more today, and nearly all (98%) depleted stocks could recover globally by 2050,[7] other reports indicate that output from capture fisheries has stagnated and will not increase.[8] Based on this current understanding and information, it remains unclear as to whether conventional capture fishery methods can generate additional output to help meet the growing demand for fish protein.

The other primary source of fish protein is fish farming, as part of aquaculture, which has been expanding in recent decades, partially in response to dwindling wild fish stocks.[5] Aquaculture, now the fastest-growing food production system by total volume,[5] is predicted to double its output by 2050.[3,5,9] Although aquaculture might be able to keep pace with rising demand, there are concerns regarding its resource requirements and resulting pollution and

Box 1. Beyond Plant-Based Meats

One proposed solution to address the increasing demand for meat is plant-based meat, which uses plants and other non-animal ingredients to create products that look, smell, taste, and feel like animal meat. The plant-based meat industry has received media and investment attention for its measurable impact on sustainability[20] and commercial successes, most notably for plant-based hamburgers (ground meat) from food technology companies Impossible Foods and Beyond Meat.[21,22] Because the production of plant-based meats does not involve the conversion of plant proteins into muscle proteins, their protein retention is high, approximately 72%.[23] With a growing set of products, plant-based meats (including plant-based fish products) are providing consumers with more sustainable choices. Unfortunately, the contribution of plant-based meat production to improved planetary and food sustainability, although measurable, might not actually be so pronounced when considering resource requirements for production.[23,24] Also, cultural associations with meat are difficult to change.[23] Moreover, the range of uses for alternative meats is limited, as they are not functionally equivalent to conventional meats.[25] Made of multiple ingredients, plant-based meats cannot act as replacements for the single ingredient of meat in the manufacturing process of existing products. Thus, alternative proteins are generally restricted to new products, giving consumers more choices but not offsetting products that use traditional meat.

habitat loss.[10] As with all farming of meat that requires feed, fish farming is essentially a method of transforming one source of protein (that contained in feed) into another (the fish meat). In doing so, aquaculture requires tracks of arable land to grow soy and corn[11] or the depletion of finite forage fish stocks to produce fish meal, all for feed.[12] This is an inefficient form of protein conversion, as, on a weighted average basis, the protein retention of aquaculture is 19% (ranging from 14% to 28%)[11] (Figure S1), analogous to discarding more than 80% of the feed protein inputs during production. Although more sustainable feed input options are being pursued (e.g., insects, food wastes, and fisheries by-products), there remains a great deal of uncertainty as to whether these can be scaled to meet demand.[12] Given the concerns around output for capture fisheries and sustainability for fish farming, it seems prudent to investigate complementary solutions to help meet the rising demand for fish meat.

One innovative approach that has gathered increased attention and investment of late is cell-based meat,[13] intended as a direct meat replacement, unlike plant-based meat, which is a meat alternative (Box 1). Grounded in a hypothesis articulated by Winston Churchill in 1932 that it will someday be possible to produce meat more efficiently and economically by growing it directly from animal cells,[14] cell-based meat proposes to do precisely this. With current know-how in regenerative medicine, stem cell science, muscle biology, tissue bioengineering, and bioprocessing, some suggest that commercial production of cell-based meat might soon be possible.[15] Proponents contend that cell-based fish and seafood might mitigate some of the sustainability issues associated with capture fisheries and aquaculture, including uncertain supply, land demands, and stock depletion.[16] To support development, cell-based meat has been backed primarily by private investors within a venture capital model, and there are more than 30 funded startups (as of early 2020) pursuing end-to-end food solutions in a system based on competition.[13,17] An amalgam of different factors, including an emphasis on impact, and unique intellectual property has resulted in cell-based fish ventures selecting what are commonly known as fatty species of fish, such as coho salmon and bluefin tuna,[18,19] while overlooking what are likely to be fundamentally simpler approaches. The private system has also resulted in a severe lack of transparency, to the extent that current technological progress is almost entirely unknown.[13] Therefore, a different path to cell-based meat development seems warranted.

In this Perspective we, a multidisciplinary team of authors, propose a simpler approach to cell-based meat development grounded in a philanthropic, more open model. We outline the case for why lean fish, a set of species with low fat content in their muscle,[26] should be a focus for cell-based meat development as a potentially more tenable first manufacturing solution. Here, we summarize findings (see Table S1) from a conceptually based investigation and a literature-based review. We start with a minimalist framework for cell-based lean fish production, cell to fork, that includes core processing requirements. This is followed by what are likely to be the benefits of selecting fish generally and then lean fish specifically, over other consumed species with respect to these production requirements. We then present a first target lean fish species, zebrafish, that is best suited to accelerate research and development, as the most understood and studied fish species in all the life sciences by far.[27–29] We conclude this Perspective with recommendations for enabling the success of the larger, non-profit, participatory effort required to establish the science for later large-scale production of cell-based lean fish.

Finally, because the grand challenge of manufacturing skeletal muscle at scale is so large, multidisciplinary, and complex, this Perspective uses simple terminology and figure imagery intended to maximize accessibility while maintaining scientific integrity. We chose words and illustrations that are accurate and consistent, but differ at times from terminology for a particular field.

A Framework for Cell-Based Lean Fish Production

We are proposing a conceptual framework based on some starting technology elements that exist, but in order to meet the objective of producing full skeletal muscle at a scale necessary for mass consumption, we have to base our work on what is known in the public domain and start with high-level system requirements. Here, we run through an idealized manufacturing solution to meet this need.

In this solution, cell-based lean fish will be produced by creating many identical cells (step 1) that are then directed to grow into skeletal muscle (step 2). These two steps are part of "bioprocessing," the stage during the overall manufacturing process where cells are alive. After bioprocessing, cell functioning is no longer supported.

The cell-to-fork process is segmented as inputs, bioprocessing, and output (Figure 1). The primary inputs are starter

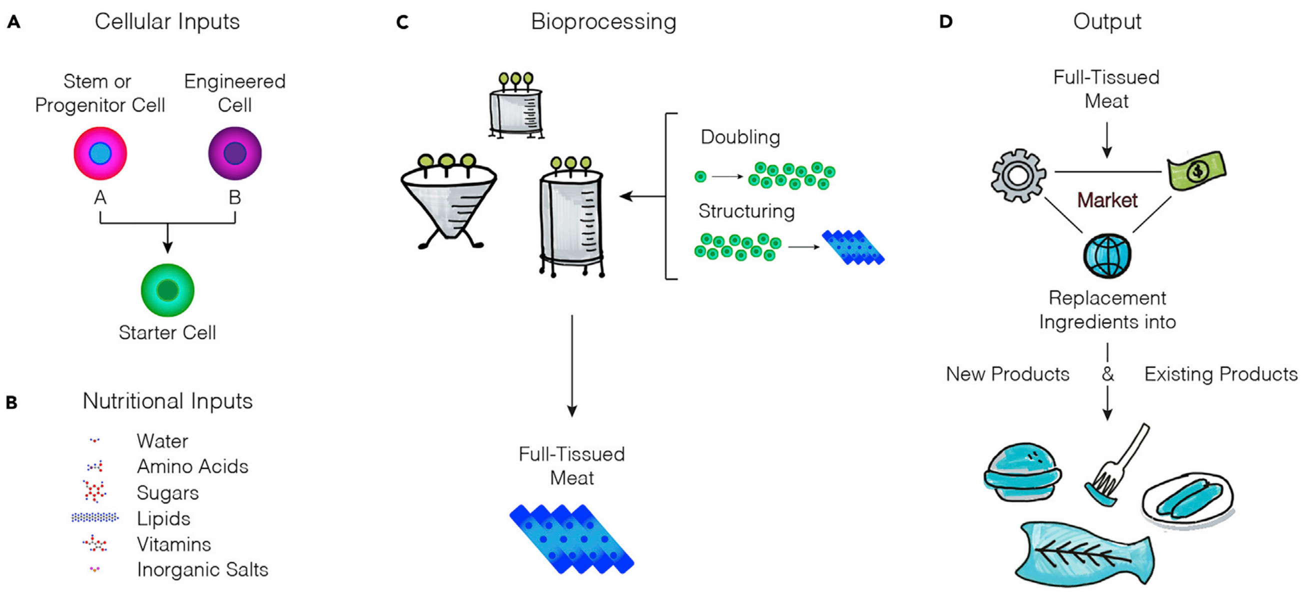

Figure 1. Conceptual Framework for Cell-Based Lean Fish Production, Cell to Fork

As cellular inputs (A), there are one of two types of starter cells, a naturally occurring stem or progenitor cell, or an engineered cell, depending on future research. Nutritional inputs (B) generally support the functioning of the cells, whereas amino acids are primarily assembled into the muscle in full-tissued meat. In bioprocessing (C), starter cells are directed through many rounds of doubling to create identical starter cells. Structuring involves directing starter cells to differentiate down specific pathways into muscles that, with exercise (e.g., using electromechanical stimulation), develop into full-tissued meat. Bioprocessing occurs in industrial cell-based meat bioreactors, liquid-containing vessels that support the large-scale processing and production of live animal cells. For fish cells, bioreactors use three gas inputs, oxygen, air, and nitrogen (indicated as solid green circles). The output (D), full-tissued meat, is minimally refined and then enters the world's markets to become fully functional single-ingredient replacements in new and existing food products globally.

cells sourced from a small tissue sample from an animal (Figure 1A) and nutritional inputs, such as sugars and lipids (Figure 1B), which support cell functioning and contain all the amino acids (the building blocks of proteins) entering the system. In bioprocessing (Figure 1C), the two steps that starter cells undergo are doubling, whereby starter cells are produced on a large scale (in high numbers), and structuring, whereby cells already created are transformed into what we call "full-tissued meat." By "full-tissued," we are implying cell-based meat that retains all the essential qualities, including taste and texture, of the conventional meat it aims to replace as one ingredient. We call it "meat" and not "a fillet," as the size and shape of the skeletal muscle output from the solution has not yet been determined.

For the basic requirements of the first of the two bioprocesses, doubling, starter cells are anticipated to be suspended in a liquid composed primarily of water and other nutritional inputs of non-animal origin (Figure 1B), probably sourced from plant or microbial materials or harvested from other bioprocesses.[30] Signaling molecules (e.g., growth factors and hormones) are also required. Here, starter cells are directed to replicate repeatedly in a bioreactor (Figure 1C). With each doubling, a starter cell becomes two starter cells, each able to perform identically to the original. The doubling process must maintain starter cell health and performance capability both for continued doubling and for subsequent processing steps.

An effective doubling bioprocess is essential to support the very high number of starter cells that cell-based meat manufacturing will require. As a ballpark estimate, assuming a single nucleus wet muscle cell mass of ca. 3.5×10^{-12} kg/cell[31] and disregarding the negligible presence of adipocytes (fat cells) in lean fish muscle, approximately 45.2 billion starter cells must be grown in the doubling bioprocess to produce a standard, daily portion (5.5 oz/155.9 g) of lean fish[32] (see Table S2). Using these numbers, when starting from a single cell, feeding a million people a 5.5-oz (155.9-g) portion of lean fish[32] requires at least 64 doublings to produce close to 17 quintillion starter cells, the number 17 followed by 18 zeros. To produce these numbers, bioreactors will need to be designed at the maximum scale at which an effective doubling bioprocess can be maintained.

In the second bioprocess, structuring, billions of cells from the doubling bioprocess will be seeded on "scaffolding," a set of surfaces meant to mimic the extracellular matrix in the species and skeletal muscle tissue of interest.[33] Among several roles, the scaffolding should offer mechanical strength, oxygen and nutrient input transport, and waste product removal,[34] supporting the overall functioning and development of the cells.[33] In a process that is anticipated to require days rather than the months needed to raise farmed fish, starter cells will be directed to differentiate and fuse (forming multinucleated cells) by exposure to specific chemical cues (growth factors and hormones),[35] physical cues, and nutrient inputs,[36] and will subsequently develop into skeletal muscle tissue.[37]

With respect to the critical metric of high protein retention,[11] bioprocessing is required to convert a large percentage of amino acid mass input into full-tissued lean fish meat. In this pursuit, bioprocessing is anticipated to occur in as closed a system as possible (i.e., one where waste products are minimized and at least partially reutilized to regenerate some of the inputs). With this approach, it might be possible to design doubling and structuring bioprocesses that achieve high efficiencies and outputs,

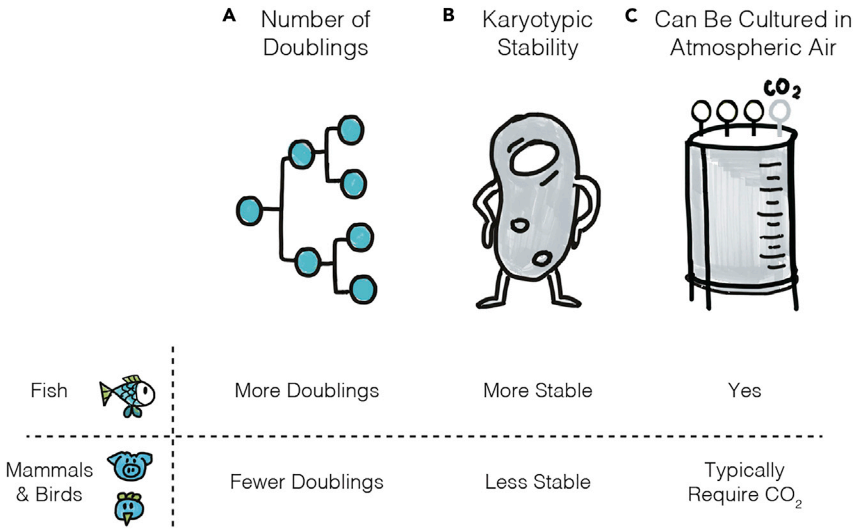

Figure 2. Three Potential Advantages of Fish Cells for Cell-Based Meat Production

Fish cells have shown the ability to undergo more doublings before they senesce (A). Fish cells also tend to maintain their correct number of chromosomes, doubling to doubling, a quality known as karyotypic stability (B), which, in general, improves the reliability of cell functioning. Fish cells can also be cultured in atmospheric air (C), likely simplifying gas-handling requirements. See Table S1 for a summary and references.

and reduce associated resource use and waste production challenges. Thus, cell-based meat bioprocessing has the potential to be less resource intensive than current animal protein production systems. However, this must, ultimately, be determined later, following considerably more research, development, and design work, and after key limitations are overcome (see A Few Hurdles below).

Potential Advantages of Targeting Fish

Beyond the case for developing cell-based fish meat to help meet growing demand, targeting fish offers engineering advantages over other mammalian and avian cell-based meat approaches. For the doubling bioprocess, whereby bioreactors are used to deliver very high numbers of starter cells, the research literature indicates that fish cells are more advantageous in a number of ways.[16] Three potential advantages are summarized in Figure 2, with additional detail on the particular suitability of fish cells (and fish in general) for cell-based production outlined in the summary table (Table S1).

First, fish cells might offer advantages over mammalian and avian cells with regard to the main requirement of cell replication. After repeated divisions, cultured mammalian and avian cells (excluding embryonic stem cells) have been well documented to eventually undergo senescence,[38–41] a phenomenon that causes cells to lose the ability to replicate. Some studies suggest that cultured fish cells exhibit senescence and have proliferation potential similar to mammalian cells.[42–44] However, other studies have found that fish cells are less susceptible to senescence and are able to undergo many more doublings[45–53](Figure 2A). With specific regard to muscle stem cells, adult fish satellite cells isolated from trout have been shown to be proliferative *in vitro*, in contrast to quiescent satellite cells in adult mice.[54,55]

Given that cell-based meat starter cells must undergo many rounds of doubling and endure extended bioprocessing runs without aberration, cell stability is an important consideration. The advantage here also goes to fish cells, which are known to maintain the chromosome number, doubling to doubling,[56–61] better than cells of mammalian and avian species (Figure 2B).[62,63] This feature is known as karyotypic stability.

A third likely advantage of fish cells for the doubling bioprocess relates to gas handling. In cell culture bioreactors, gas inputs from air, oxygen, and nitrogen, along with agitation, are used to manage dissolved oxygen and pressure inside the vessels.[64] With mammalian and avian cell culture systems a fourth gas input, carbon dioxide, in conjunction with bicarbonate, is usually required for pH control.[65,66] Because fish cells (1) can readily grow in atmospheric air[67,68] (Figure 2C) and (2) have high intracellular buffering capacity,[16] doubling these cells will likely involve managing only three gas inputs, oxygen, air, and nitrogen, and their associated impact on process dynamics inside the bioreactor. Managing three gases is less complex than managing four gases and bicarbonate buffering, simplifying process scale-up challenges such as CO_2 stripping[69] (see Note S1).

Lean Fish as a First Fish Meat

Complexity is often an impediment to scaling. A key element of a meat's complexity relates to the presence of fat cells (adipocytes) within its primary muscle tissue matrix. Lean fish (e.g., cod, pollock, haddock, tilapia) usually have 1%–2% fat content in their muscles and an abundance of liver lipids,[26] whereas fatty fish (e.g., salmonids and tunas) typically have 10%–20% muscle fat content with low retention of lipids in the liver.[26] Meanwhile, beef steak could be called lean even if it contains nearly 10% fat,[32] and, even when excess fat is trimmed away, the fat content of chicken (2.6%),[70] beef (2.69%),[71] and pork (2.17%)[72] exceeds the literature-based definition of 1%–2% fat content in lean fish muscle.

Because lean fish get most of their lipid needs met by their livers, with relatively few fat cells in the meat,[26] we think it likely (and make the assumption) that intermuscular fat does not play a key biological role in lean fish muscle growth, and, therefore, that structured cell-based lean fish meat can be manufactured without having to process live fat cells. This could make the meat simpler to produce, as challenges have been reported with co-culturing fat and muscle cells. For one, muscle and fat cells adhere differently to growth substrates,[73] which would make growing fat and muscle together in one scaffolding system more challenging. Another impediment relates to "crosstalk" between muscle and fat cells. Specifically, secretions of fat tissue have been shown to reduce the doubling and differentiation performance of adjacent muscle cells.[74]

As additional evidence to support the hypothesis that lean fish meat will be the simplest to structure into full-tissued meat, consider the visual appearance of lean fish compared to a piece

Figure 3. Meat as Skeletal Muscle
Three different cuts of meat are shown with visible patterning and varying fattiness: a piece of Pacific cod, sashimi from Atlantic salmon species, and a ribeye steak. Images are not to scale.

of fatty fish and a beef steak. For this, we draw your attention to Figure 3, which shows the structured skeletal muscle of these three types of meat that need to be recreated by cell-based meat technology.

The meat of Pacific cod, considered a lean fish or "white fish," is low-fat muscle with 15.27 g of protein and 0.41 g of fat in a 100-g serving.[75] It has consistent, repeated patterning recognizable to the naked eye. Consistent patterning is also readily visible in the sashimi of salmon, a "fatty" fish[26] (Figure 3) with 20.42 g of protein and 13.42 g of fat in a 100-g serving.[76] However, what is not readily visible is that muscle and fat cells are well interspersed throughout the peach-colored sections in the salmon sashimi,[77] unlike the more singular nature of lean fish meat. Therefore, from an engineering perspective, it is easy to appreciate that structuring might be more complex for the fatty meat of salmon than for the lean meat of cod.

Even when trimmed of some fat, a ribeye steak contains visible fat and marbling (16.9 g of fat and 19.55 g of protein in a 100-g serving)[78] in a less regular-looking, interwoven pattern (Figure 3). It is easy to see how recreating a whole steak, with all its complex structuring, including the fat content, might prove a tougher challenge. This is particularly apparent when comparing the steak with the relatively simpler structures of the sashimi of salmon and the meat of cod, the simplest.

Beyond minimizing manufacturing complexity, a selection of a lean fish species for cell-based meat development offers unexpected potential for impact, as illustrated in Figure 4. Lean fish meat represents a very large existing market: the world's most captured fish species by mass is Alaska pollock (Figure 4A),[5] whereas Nile tilapia is one of the top four most farmed fish species in the world.[5] Other highly consumed lean fish include cod, haddock, and hake.[5] Lean fish meat also has similar sensory qualities across species (Figure 4B) and is virtually interchangeable in recipes and foodstuffs across the world (Figure 4C).

The claim that lean fish meat has similar sensory qualities across species (Figure 4B) is supported by evidence that lean fish muscle tissue has less variation in flavor, species to species. In a comparative study of the flavor profiles of 17 species of North Atlantic fish by a trained taste panel, the lean species, having 1%–2% fat content, were generally grouped away from those species with a higher fillet fat content.[79] This attribute suggests that a single or small number of successfully developed cell-based lean fish species could support demand for lean fish broadly.

In addition to lean fish having similar flavor, species to species, it should also be simpler to recreate a sensory experience fully recognized as lean fish meat rather than one recognized as tuna or salmon, which have been shown to have more complex, volatile flavor profiles.[80] Targeting meat with a simpler flavor profile could reduce the development time and cost. Our hypothesis is further supported by noting how the lean fish meats of multiple species are often used interchangeably across a broad range of mass-market fish products (Figure 4C). Examples of such products include fish sticks, fish fillet sandwiches, fish and chips, fish tacos, fish kebabs, fish burgers, fish nuggets, fish rolls, fish squares, fish fingers, fish chowder, and surimi.[81,82]

Of note, lean fish meat is among the purest forms of animal protein from a macronutrient perspective, providing high protein content with virtually no fat (often <1%) and no carbohydrates.[75,83–85] Lean fish consumption has also been shown to confer comparable health benefits to omega-3-rich fatty fish on risk factors for cardiovascular disease and type 2 diabetes.[83,86] Considering that cell-based meat will also contain no environmentally derived toxins or contaminants such as mercury and plastics, cell-based lean fish could be an ideal, single-ingredient health and wellness animal protein.

Overall, cell-based lean fish meat could be a healthy animal protein that is easier to produce as full-tissued meat than other cell-based equivalents. It could result in more impact than meets the eye,[5] potentially providing a less resource-intensive replacement for the meat of several mass-market captured and farmed lean fish species. Because lean fish meat is virtually interchangeable across species, we next consider a nuanced question: "Which species will most accelerate research and development for cell-based lean fish production?" As we return briefly to Figure 4A, the answer becomes obvious: zebrafish.

Meet Zebrafish, an Ideal First Species for Research and Development

A popular aquarium fish native to South Asia and formally known as *Danio rerio*, zebrafish is a small freshwater fish approximately 30–50 mm in length.[87,88] In addition to serving as pets, zebrafish are sold and consumed as food at roadside huts in India (Dr. John Postlethwait, personal communication). These supporting roles aside, zebrafish's largest contribution is in science as the most researched fish in the world by far (Figure 5).

With over 3,250 institutes in over 100 countries studying the species,[89] a dedicated peer-reviewed journal,[90] and data maintained and curated by the Zebrafish Information Network,[91] no other fish species comes close to matching zebrafish's large knowledge base and devoted research community. This is illustrated in Figure 5, comparing the number of results returned when searching the National Institutes of Health PubMed database of publications for frequently studied species of fish, where the 40,322 publications returned for zebrafish is much greater than for any other species (see Table S3).

As 82% of genes associated with human diseases and disorders have a zebrafish counterpart,[92] zebrafish are studied extensively to unlock potential cures and treatments for a broad range

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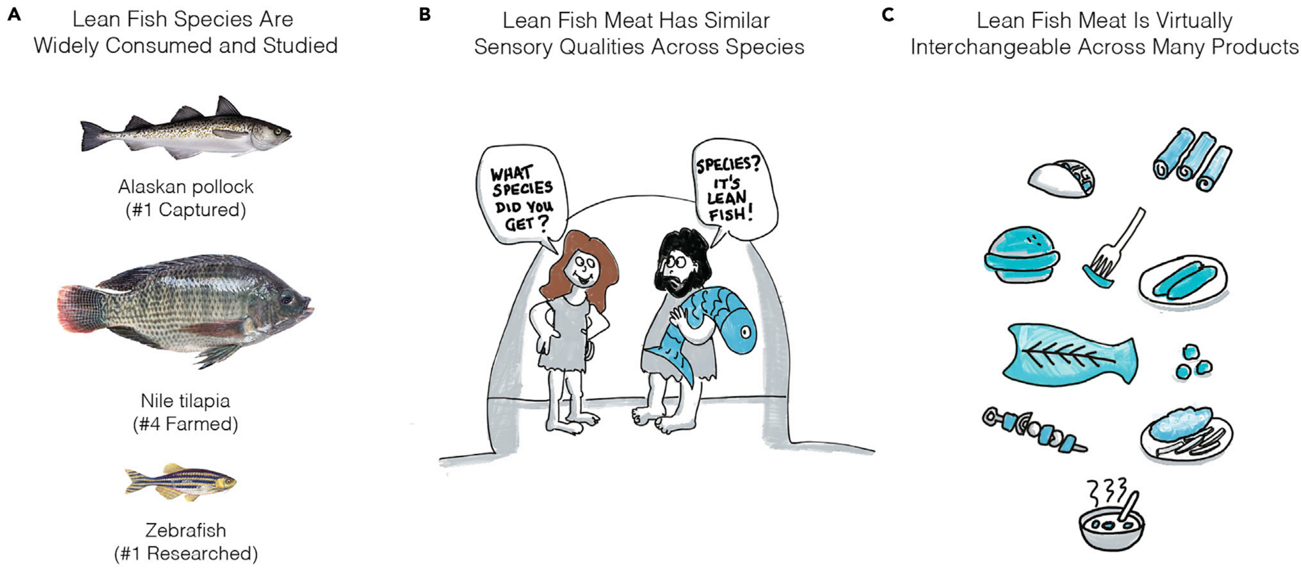

Figure 4. The Impact and Versatility of Cell-Based Lean Fish

Lean fish species (A) include the most captured species (Alaska pollock), the fourth most farmed species of fish (Nile tilapia; top three, in order, are grass carp, silver carp, and common carp), and easily the most understood and studied of all fish species in the life sciences, zebrafish, used extensively for medical and other scientific research. Because lean fish meat has similar sensory qualities across species (B), it is virtually interchangeable as input material for many products (C). Images not to scale. [Zebra Danio]/[Encyclopedia Britannica] via Getty Images, [Nile tilapia] via Shutterstock, [Alaska pollock] via NOAA.

of diseases.[93] Autism, epilepsy, amyotrophic lateral sclerosis, and numerous cancers are all active targets of scientific investigation.[94,95] In particular, research into muscular dystrophy has contributed to a deep understanding of zebrafish muscle development.[96]

In addition to all this knowledge that can be borrowed to help establish cell-based lean fish science and technology, decades of zebrafish investigations have resulted in a powerful research toolkit that is extremely relevant to cell-based meat development. Experimental procedures have been defined for isolating different starter cell types from zebrafish, such as embryonic stem cells[97,98] and muscle stem cells.[99] For muscle stem cell isolation, established genetic markers exist that can help ensure cell population purity during the procedure.[100–103] Protocols also exist to generate muscle cells from zebrafish starter cells on the lab bench.[97,98] Additionally, an extensive genetic engineering toolkit is available to turn genes on and off within the zebrafish genome.[27] This toolkit should allow for zebrafish starter cells to be genetically engineered for enhanced performance for both doubling and structuring bioprocessing. Among the contents of the zebrafish toolkit, there are numerous fluorescence imaging techniques available to provide cell-based meat scientists with real-time optical cues on cell status and viability.[104] These make it easy to distinguish muscle cells from other cells.[105] With all these benefits, the expansive zebrafish toolkit has the potential to simplify and accelerate research and development and to facilitate eventual production of cell-based meat for consumption.

Another set of benefits of choosing zebrafish is especially pronounced in the initial stages of research, where it will be invaluable to have a supply of embryos and fish readily available to source live starter and other cells. Zebrafish meets this need well, as the species is raised in labs around the world,[89] produces many offspring, and has a short generation time.[106] The selection of an aquaculture-raised or wild species for research would require frequent trips to hatcheries or natural waterways, adding considerable cost, complexity, and delays to cell-based research.

Ensuring starter cell health will also be critical for cell-based meat development and production. Fortunately, zebrafish cell sources can be obtained from facilities designated specific-pathogen-free (SPF),[107] a term for laboratory animals that are guaranteed to be free of particular infectious agents. Additionally, the process for establishing a zebrafish SPF facility and cell line is well defined.[107] SPF-sourced cells will eliminate the need for numerous safety and quality tests by the research and development teams, and should reduce the likelihood of incidental research findings caused by contamination events.

Our findings leave little doubt that to accelerate the development of cell-based fish it is best to start simpler, saving more complex variations for later. Furthermore, starting with zebrafish is a clear choice for research and development, and is likely the fastest path possible to producing full-tissued, cell-based fish meat.

A Few Hurdles

Numerous technological challenges exist, and although a full list of the known obstacles is outside the scope of this Perspective, we highlight a few key areas for development. A successful manufacturing solution will require a complete integration of these elements and others.

Starter Cells

With comparatively few fish cell lines available overall,[16] it will be essential in the long term to have starter cells to support the wide variety of fish meats consumed globally. In the short term, a new set of starter cells must be established from zebrafish embryos and live fish. Unfortunately, currently available zebrafish starter cells are not well suited to large-scale cell-based lean fish

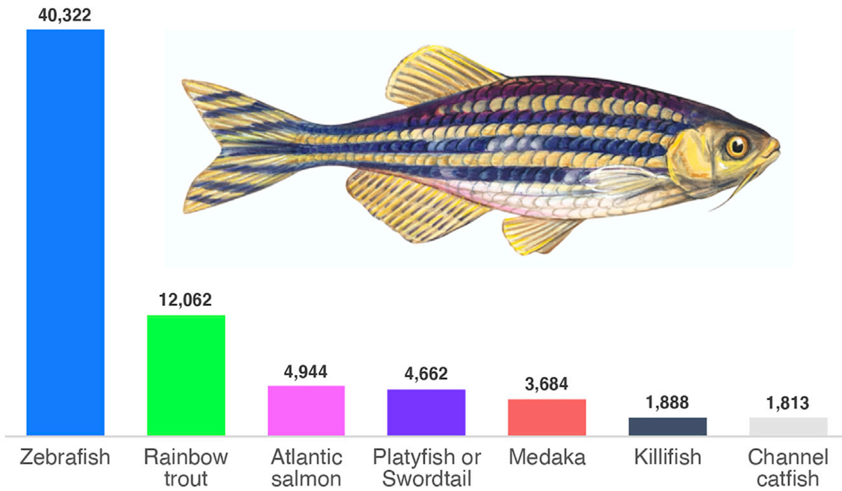

Figure 5. Zebrafish Is the Most Studied Fish Species in the Life Sciences
Search results are shown for fish commonly featured in research within the PubMed database of the US National Library of Medicine National Institutes of Health. The search query included the common OR species name, where the search terms were indicated with quotations for specificity (e.g., "Zebrafish" OR "Danio rerio;" "Rainbow trout" OR "Oncorhynchus mykiss"). Zebrafish (*Danio rerio*) revealed 40,322 publications, and the comparative publication counts for other fish are shown. Scientific illustration is of a zebrafish. [Zebra Danio]/[Encyclopedia Britannica] via Getty Images.

production, as they require animal serum for growth.[59,108] This poses challenges for bioprocessing control and scalability, as the composition of animal serum can vary batch to batch, in addition to ethical concerns.

Signaling Molecules

At present, the cost of signaling molecules (i.e., growth factors and hormones) is prohibitively high, given the amounts needed for mass production.[109] Potential solutions might include (1) redesigning signaling molecules to enhance effectiveness and lower costs,[110] (2) genetic engineering of starter cells so that they generate their own growth factors in large enough quantities,[109] and (3) use of other non-consumed cells (e.g., microbes) in the bioprocess to serve as growth factor production factories.[110] A combination of these solutions might be used within the doubling and structuring steps in cell-based lean fish manufacture.

Bioreactors

With little existing know-how for growth of fish cells in bioreactors[16] and no existing operational full-scale or pilot production plants for cell-based fish (or cell-based meat for that matter), bioreactor design is a major focus of current and future research efforts.[31] Customized designs will be needed for the doubling step and the structuring step, each of which might require a series of bioreactors. Overall, bioreactor design needs to support the efficient conversion of inputs into maximal amounts of full-tissued lean fish meat with minimal waste production.

Scaffolding

Although some small-scale tissue engineering applications exist today,[16] a scaffolding solution set for full-tissued meat has yet to be demonstrated or openly developed. Scaffold designs might need to be customized for specific lean fish species and tissue types.[33] This work, required before a full manufacturing solution for lean fish meat can be developed, will include computer modeling and prototyping to optimize scaffolding mechanical strength, nutrient transport, and waste product removal.[111]

Life-Cycle Assessment

Early-stage predictive life-cycle assessments do exist today but are not the same across the board and compare different animal protein production systems.[30,112–114] Nonetheless, a main conclusion from these predictive pieces, although imperfect, is that the energy burden of cell-based meat is high,[30,113,114] which could be reduced through the use of decarbonized energy sources such as renewables. However, it is only after the commercial-scale cell-based lean fish approach is further developed that accurate assessments can be made, which also consider inputs such as water, and cellulose that humans cannot directly digest.

Unknowns, Limitations, and Assumptions

The selection of lean fish for production and zebrafish for research and development is straightforward, but the path ahead is not. A manufacturing solution for cell-based lean fish meat will integrate many existing, enhanced, and new-to-the-world technologies. Before all this technology is designed and built, extensive knowledge must be developed through experimentation. Although the gaps in knowledge are fewer and smaller for zebrafish than for any other species of fish, the future is full of unknowns. One major unknown is whether the production of full-tissued cell-based meat (with any species) is even feasible for a single manufacturing facility, let alone at the scale required to support global demand.

Other unknowns that cannot be addressed until a solution is much further along include "How much will a serious research effort cost?," "How long before a manufacturing facility is operational?," "Will cell-based lean fish manufacturing costs and prices be low enough for global, mass-market appeal and disruption?," and "How will cell-based lean fish be approved and regulated under different frameworks around the world?" Ultimately, the answers to these questions influence whether cell-based lean fish will realize its potential as a complementary solution to the increasing demand for fish.

As a limitation, the cell-to-fork framework indicates that one set of starter cells is needed to develop structured meat. Lesser amounts of other terminal cell types (fibroblasts to provide connective tissue, for example) might also be required, which would add some bioprocessing complexity. Regardless, lean fish meat should be simpler to structure than meat with a greater presence of adipocytes (fat cells). Another potential input, animal serum (e.g., fetal bovine serum) as a source of hormones, growth factors, and nutrition, is not mentioned in our proposed manufacturing solution as, for one, animal serum composition varies batch to batch, hindering process scale-up.

Also, because bioprocessing needs to achieve efficient conversion of protein inputs into fish meat, our proposed framework focuses on the mass of amino acids that enter and move through

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the manufacturing process to be assembled and structured into skeletal muscle. It excludes from consideration other inputs and outputs, such as energy, water (the largest contributor to meat's mass), and waste streams, all of which will factor into the financial and environmental costs of producing cell-based meat.

A complete discussion on the sustainability of meat consumption is outside the scope of this Perspective, as food production and its impact on the environment involve complex systems with high variability.[24,115,116] Some of this variability is captured across such standardized metrics as greenhouse gas emissions, land use, and water use.[24,115] Nevertheless, as authors, we align with scientists who report that the production of animal protein, overall, is putting the future health of the planet at risk.[8,12,115,117] We see that cell-based lean fish technology has the potential to be a less resource-intensive method of making fish meat than conventional methods.[16] However, whether this potential can be realized is unknown. To make a fair assessment, research and development must be much further along, and standardized approaches for comparing conventional animal protein production must be established, given the influence of animal growth stage, animal size, and feed composition on protein retention.[118,119]

Additionally, we have offered only limited detail regarding the potential nutritive benefits of cell-based lean fish, noting that conventional lean fish can provide health benefits similar to those from omega-3-rich fatty fish.[83,86] A full nutritional and compositional analysis of cell-based lean fish must be undertaken later as soon as sufficient biomass is produced.

With no information available on the sensory attributes of zebrafish meat, we assume that full-tissued cell-based zebrafish meat will receive widespread consumer acceptance, given that lean fish meat has similar sensory attributes, species to species, and because zebrafish is eaten at roadside huts in India (J. Postlethwait, personal communication). If not, we expect this foundational zebrafish research and development to expedite progress on cell-based equivalents to commonly consumed species of lean fish, more than making up for any lost time.

Finally, an important topic not discussed in this Perspective is animal welfare. Although the author team hypothesizes that fish are sentient and can suffer, sentience is not provable.[120] Nevertheless, we assume that a manufacturing solution for cell-based lean fish will make positive contributions to animal welfare because it will not require the mass capture or farming of fish.

Conclusions and Recommendations

With as many as 2 billion more people expected on our planet in the next 30 years,[1] the demand for animal protein, and fish specifically, is likely to rise. There is, however, high uncertainty as to whether conventional capture fisheries can support this increased demand[5,7,8] or whether fish farming can sustainably expand outputs given its input requirements.[9,11,12] A potential complementary solution to the growing demand for fish meat is to investigate cell-based fish, making the same meat directly from fish cells. More than 30 private ventures have been launched in pursuit of cell-based meat broadly, and at least six are specifically devoted to cell-based fish.[17]

Unfortunately, it would seem that for-profit interests got involved too soon, resulting in a host of systemic impediments to successfully and rapidly advancing cell-based meat technology. Among these, cell-based meat ventures are incentivized to generate and carve out know-how in the form of specific intellectual property (IP), despite the strong need for building knowledge and advancing basic research first. Without a shared foundation of know-how, development teams must each "reinvent the wheel" to solve many of the same technical challenges, which is highly inefficient system-wide for such an important and urgent planetary challenge. Also, because they are businesses, ventures are under considerable pressure to focus on future revenue streams and food products instead of advancing the research and development needed beforehand.

Limited access to transdisciplinary expertise is yet another systemic problem with the current approach, as cell-based ventures lack the resources or openness to engage the wide range of world-class scientists, engineers, and technologists they (and Earth) need in tackling this critical work. In addition to species-specific expertise, support is needed right now from thought leaders in regenerative medicine, stem cell science, muscle biology, tissue bioengineering, bioprocessing, and computational modeling. Environmental economics, food science and nutrition, and large-scale process engineering are among other fields that will also certainly play important roles.

Bringing all these pieces together to build out and realize a cell-to-fork framework requires substantial investment. Unfortunately, the current venture model for cell-based meat, which targets minimal spending and maximal returns, has resulted in total funding that is less than 0.03% or 3/10,000 of the annual global market for meat.[121,122] Given what is at stake and the need to co-create all this knowledge, this for-profit model does not seem appropriate in the short term.

As scientists, we conclude that a generosity-based approach should be pursued right now to accelerate cell-based fish research and development. Instead of protecting early know-how as IP, we recommend beginning anew and opening the door to the co-creation of knowledge. For maximum speed and efficiency, we further suggest society pool its resources to advance what is likely a simpler first solution, lean fish.

On this journey, we pledge to share our work more openly, including hypotheses, methods, protocols, formulations, findings, results, failures, and other learnings. We also promise to hold ourselves to the highest academic and research standards and publish our work through vetted, peer-reviewed venues. By sharing our knowledge, information, and ideas freely, we aim to participate in and demonstrate the kind of collaborative knowledge building, insight discovery, and innovative thinking required to accelerate planet-critical solutions.

To further engage the appropriate breadth and level of talent, we strive to partner with leading individual researchers, universities, and research institutes. Because growing lean fish meat and regenerating human and zebrafish muscle are similar pursuits, we look to accelerate lean fish research and development side-by-side with regenerative medicine. Aided by a more open approach, we aspire to freely engage all the expertise needed to advance the fastest path to a manufacturing solution for cell-based lean fish.

Instead of adopting a for-profit model before the science has been established, we suggest society fund and accelerate knowledge development through philanthropy, as a gift to our shared future. With unconditional grants, we aim to place decision-making for planet-critical science and technology in the hands of scientists, outside of special interests.

Ultimately, we see that developing cell-based lean fish technology has the potential to support the global demand for fish more sustainably than current production methods. We also see that a concerted, collaborative effort, based on simpler solutions built from the ground up, is best suited for this endeavor. With this, we come together to conserve planetary health and contribute to a world with enough food for all. Who will join us?

SUPPLEMENTAL INFORMATION

Supplemental Information can be found online at https://doi.org/10.1016/j.oneear.2020.06.005.

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Conceptualization, A.R., G.P., A.S.T.S., N.T.K.V., J.M., L.M., D.L.M., W.W., and A.B.; Methodology, A.R. and G.P.; Formal Analysis, A.R. and G.P.; Investigation, G.P., A.R., A.S.T.S., J.M., and D.L.M.; Resources, D.L.M. and J.M.; Data Curation, A.R. and G.P.; Writing – Original Draft, G.P. and A.R.; Writing – Review & Editing, A.R., G.P., A.S.T.S., N.T.K.V., J.M., L.M., D.L.M., W.W., and A.B.; Visualization, J.M., A.R., and G.P.; Supervision, A.R.; Project Administration, A.R. and G.P.; Funding Acquisition, A.R.

DECLARATION OF INTERESTS

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