New ways to assess stress in fish are urgently needed in aquaculture

With an increasing demand in fish products, aquaculture is fast becoming a main priority towards achieving sustainable fish production. In this context, an accurate and consistent way to evaluate fish stress levels is essential to ensure high standards of welfare. Dr Pedro Miguel Rodrigues and Msc Cláudia Raposo de Magalhães, based at the University of Algarve, CCMAR, Portugal, believe current methods are unreliable and a new approach is urgently needed. For the researchers, the best option is to use cutting-edge new technologies, like metabolomics and proteomics to find markers which can be used to assess the level of stress in fish.

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Humans have explored bountiful oceans for millennia. For generations, fishermen deemed it to be infinitely vast, but it's becoming increasingly apparent that our waters are not as indestructible as we once believed. The oceans cannot continue to cover our growing demand for fish products. Responsible and sustainable fishery is crucial to move forward, and aquaculture may be the best tool to fill this gap.

However, aquaculture can have devastating effects on animal welfare. The industry and the legislators have attempted to implement codes of practice to ensure companies take fish welfare seriously and promote good husbandry conditions. The Federation of European Aquaculture Producers, the World Organisation for Animal Health and even the European Union all have their own guidelines and practical indications on how to manage fish stocks to ensure their welfare.

These guidelines, however, are based on limited knowledge about fish biology. Dr Pedro Miguel Rodrigues and Msc Claudia Raposo de Magalhães, based at the University of Algarve, Portugal, believe that, to develop appropriate welfare protocols, it is essential to understand the physiology of each species.

A wide-ranging protocol doesn't suit the several species of fish used in aquaculture, each one with its own anatomical, physiological and behavioural characteristics. Instead, the researchers defend that the industry needs to develop ways to assess welfare, considering ideal conditions for each species.

CAN FISH FEEL HAPPY OR SAD?

Up until now, the idea of fish welfare has mostly been synonymous with merely keeping the animals alive and in good health. However, recent research is starting to unveil that these animals need more than that. It turns out that, despite lacking self-awareness and cognitive abilities on the same level as mammals, fish can nevertheless experience pain and fear and are even capable of expressing rudimentary emotions.

For Dr Rodrigues and Raposo de Magalhães, this research means that fish can adapt and evolve if the environment around them changes, and a stressful situation arises. In fact, the researchers suspect fish have a "Goldilocks" approach when it comes to stress: too much will distress the animals, and they will struggle to cope, but too little will not stimulate them and they'll get bored. On the other hand, an intermediate level of stress is just right to trigger a sense of reward and teach the animals how to cope with future stressful situations.

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For Dr Rodrigues and Raposo de Magalhães, welfare is not just a matter of looking at the animal's health but also includes what the fish is experiencing. "It is necessary to gain a deeper understanding of the mental processes in fish and explore to what extent fish are able to make conscious choices based on positive or negative expectations", said the researchers.

ASSESSING STRESS IS NOT EASY

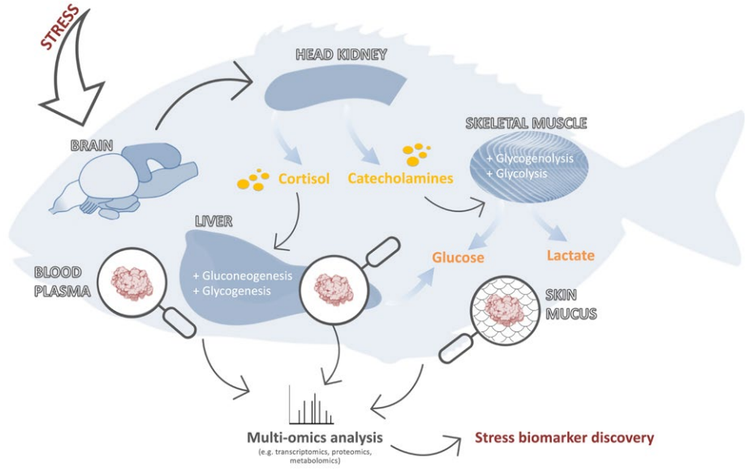
This ability to adapt to the environment should really not be that surprising. In the wild, fish are exposed to a variety of situations which may cause stress. From injuries and parasites to predators and storms, fish need coping mechanisms to deal with such adverse conditions. The animals may be temporarily stressed, but that does not mean they are suffering.

In contrast, what we consider harmless practices in aquaculture – such as repetitive handling or high fish density – can be severe stress factors, sabotaging coping responses and disturbing these animals' welfare.

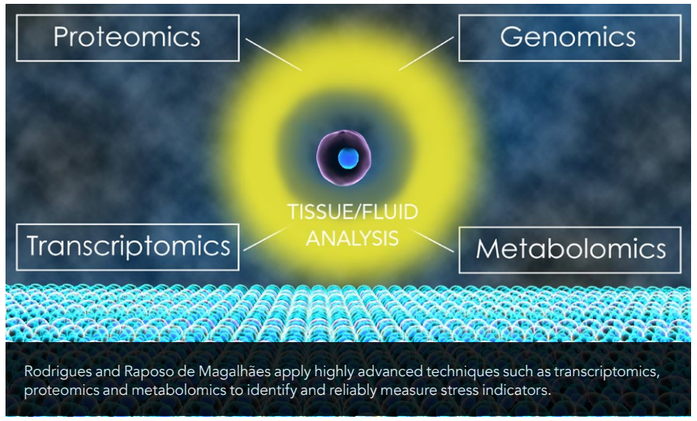
It's impossible to say whether a situation is stressful or not without actually evaluating the stress level in fish. For Dr Rodrigues and Raposo de Magalhães, this is paramount to ensure sustainable production. "Improving the quality of husbandry practices and the measurement of welfare to reduce stress should not be seen only from a productive point of view but also from a more holistic, ecological and ethical perspective", says Dr Rodrigues.

There is a need to establish new methods that will promote productivity and consumer's acceptance of farmed fish.

The problem now is finding an accurate and reliable way to achieve this. High levels of plasma cortisol are currently used as the primary indicator of stress in fish, but this has limited value. Dr Rodrigues and Raposo de Magalhães believe this information is not reliable enough to check fish welfare. This hormone can be influenced by many different factors, including the frequency of exposure and animal genetics. For example, although most fish show a pattern of increased cortisol levels after exposure to a source of stress, followed by a return to normal a few hours later, it's virtually



Integration of different 'omics technologies can provide a holistic overview of potential alterations in the fish biological system under stressful situations, thus allowing the discovery of reliable stress indicators.



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Rodrigues and Raposo de Magalhães apply highly advanced techniques such as transcriptomics, proteomics and metabolomics to identify and reliably measure stress indicators.

impossible to define what 'normal' is for each animal.

The reliability of cortisol levels becomes even worse when fish are subjected to prolonged and chronic stress. For these animals, cortisol may stay elevated for days or even weeks or may return to low levels even though the animal remains stressed. It's impossible to predict how each animal reacts, but most likely the situation will lead to stunted growth, low performance, impaired reproductive function and poor health, disease and ultimately death.

In theory, there are other compounds – such as glucose and lactate – that can reflect conditions of poor welfare. However, the same issues arise, and results must be interpreted with care, considering extrinsic factors such as diet, life stage, time since last feeding and season of the year, to name just a few.

Dr Rodrigues and Raposo de Magalhães defend none of these compounds is a reliable indicator of stress due to their high biological variability. "We believe these methods are unreliable", they explain, "there is a need to establish new methods that will promote productivity and consumer's acceptance of farmed fish".

NEW OPTIONS TO MONITOR STRESS

In an effort to find the Holy Grail of stress indicators, many scientists have started using highly advanced techniques such as transcriptomics, proteomics and metabolomics. In a nutshell, these 'omics technologies cover the study of genes, proteins, and compounds involved in normal metabolism to understand better the structure and function of anything from a single cell to a whole organism.

This approach has already yielded incredible results in farm and wild animals, and Dr Rodrigues and Raposo de Magalhães believe there is no reason why these procedures cannot be applied to aquaculture as well. These methods can be used to study not only nutrition, reproduction and health but also welfare and effects of stress.

Identification of reliable biomarkers of fish welfare would greatly improve our knowledge regarding responses to stress.

Undoubtedly, proteomics has been the most common method used in aquaculture so far. Studies looking at the impact of diseases, water temperature, stocking densities, handling procedures and others have uncovered exciting changes in the type and abundance of specific proteins produced in stressed fish.

One of the main aims of this work is to find a few of these proteins that can reliably work as stress indicators. There are some good news in this front, as some potential markers have been identified. The list includes proteins involved in transporting iron, protecting against oxidative damage or cleaning up blood clots, to name just a few. There's still a long road ahead to validate these proteins, and not all of them will be useful, but it's a good starting point.

For Dr Rodrigues and Raposo de Magalhães, the other 'omics approaches are also likely to come into play in the future. A combined effort using transcriptomics, proteomics and metabolomics "provide a wider vision of the physiological function of interest while pointing out the direction for future research", said the researchers. "In the next years, with the development of more cost-effective technologies and the increase in proteomic databases, the value of omics technologies in fish biology research will be enhanced".

FUTURE DIRECTIONS

Consumers are getting increasingly savvy and aware of animal welfare issues. Keeping up with high welfare standards is probably the most critical factor in how consumers perceive the aquaculture industry.

This is where science comes in. Welfare is no longer a matter of designing adequate husbandry procedures to ensure fish are in good health. It has to go further than that, it has to provide an environment where fish can thrive and grow as if in the wild. Fish can deal with a certain amount of stress in their lives, but how much is too much? Dr Rodrigues and Raposo de Magalhães believe in-depth knowledge of fish biology is the best pathway to finding the most effective welfare assessment tools.

In this context, "identification of reliable biomarkers of fish welfare would greatly improve our knowledge regarding responses to stress", conclude the researchers "and contribute to the development of more sustainable fish rearing protocols with concern to a more holistic, ecological and ethical perspective".

Behind the Research

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Research Objectives

Pedro Rodrigues and Cláudia Raposo de Magalhães investigate reliable and standardised tests for farmed fish welfare assessment.

Detail

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Both Pedro Rodrigues and Cláudia Raposo de Magalhães are members and researchers of the Aquaculture Research group at CCMAR, University of Algarve, Portugal. They both work on fish welfare with several publications in the area.

Funding
This work has been financially supported by project WELFISH (Ref. 16-02-05-FMP-12, "Establishment of Welfare Biomarkers in farmed fish using a proteomics approach" MAR 2020), and the Foundation for Science and Technology (FCT) through project UID/Multi/04326/2020.

Cláudia Raposo de Magalhães acknowledges an FCT PhD scholarship, Ref SFRH/BD/138884/2018.

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References

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Personal Response

In your opinion, how far are we from a reliable and effective marker to measure stress in fish?

"Public perception plays a major role in science. Since consumers are getting increasingly aware of fish sentience, the farming conditions and sustainability, we believe research on farmed fish welfare will significantly increase in the next couple of years and go to towards the use of more cutting-edge high-throughput technologies. Several proteins have already been identified as potential candidates of impaired welfare markers in different fish species. Still, all these need to be further validated, and the link between these proteins and fish performance should also be clarified for an effective welfare assessment."