Regulating peptides

Marine biologist **Professor Hélène Volkoff**'s research into characterising novel peptides has enabled her to explore the role they play in the lifecycles of fish

Can you share how your career in marine biology has progressed?

I have always been interested in fish. After completing a marine biology BSc degree in Paris, I studied the structure and function of the thyroid gland in elasmobranch fishes (sharks and rays) for my MSc degree in France and then my PhD at Clemson University in the US. I came to Canada as a postdoctoral fellow in Dr Peter's laboratory in Alberta and started research on the endocrine control of feeding in fish, using goldfish as a model. During that time, I acquired expertise in injection and molecular techniques. Now, as a Professor at Memorial University of Newfoundland, I am pursuing research on the endocrine control of feeding and reproduction in freshwater fish and local cold water marine fish.

What are the overall goal and key objectives of your latest work?

My research focuses on the nature of appetite-regulating peptides and their physiological roles in fish. The overall goal is to identify the molecules and physiological mechanisms regulating appetite and food intake in fish, and to examine how they are modulated by feeding status, season and reproductive events. My programme uses a comparative approach to examine endocrine

Controlling appetite

Scientists at the **Memorial University** of Newfoundland are shedding light on what is regulating the desire for food in fish, ultimately informing Canada's aquaculture sector

IN 2010, THE Food and Agriculture Organization of the United Nations (FAO) observed that aquaculture now accounts for 46 per cent of the world's total food fish supply. Correctly feeding farmed fish is critical for achieving appropriate growth and reproduction and consequently maximising the efficiencies in this industry. Work being undertaken by experts in fish endocrinology and physiology at the Memorial University of Newfoundland in Canada is delving into the endocrine mechanisms which regulate fish feeding. Research leader Professor Hélène Volkoff observes that this work may well lead to specific adjustments in fish holding conditions and feeding strategies. It is also important for assisting the aquaculture industry to maximise feeding and minimise waste, which results in lower cost inputs and a reduced accumulation of toxic waste products in the marine environment.

The researchers are specifically interested in understanding more about the function of a variety of hypothalamic and gastrointestinal hormones which are responsible for appetite regulation. This project is known as the 'Characterisation of appetite-regulating peptides in fish' and is exploring peptides that have yet to be understood in fish, such as orexin, cocaine and amphetamine regulated transcript (CART), melanin-concentrating hormone (MCH), ghrelin and amylin. The team is seeking to gain insights into the roles these peptides have in fish feeding and reproduction by examining their actions on both the brain and gastrointestinal tract.

The research team has been very productive over the past 10 years. They have successfully secured funding and had the opportunity to collaborate with prestigious Canadian universities and government institutions such as the Department of Fisheries and Oceans. The researchers have published over 30 papers in peer reviewed journals, including one recently in *Molecular and Cellular Endocrinology* on early investigations into the role of MCH and its receptors in appetite regulation of winter flounder. Their investigations have also been included in some of the latest technical books covering this topic.

DIRECT AND INDIRECT TECHNIQUES

The group is carrying out *in vivo* tests by directly injecting hormones in fish. Brain stereotaxic or peripheral injections are performed on live specimens that have been deeply anesthetised. Once the fish recuperate, the team observes different behaviours and calculates the amount of food the fish is taking in. This method is being used on goldfish and a range of marine fish species, enabling researchers to view the injected fish undertaking a number of activities and to gather some interesting findings: "This technique has resulted in the collection of direct evidence that orexin treatment increases feeding and locomotor behaviour and decreases reproductive behaviour in goldfish," reveals Volkoff. They have also shown, for the first time, that by injecting goldfish with gonadotroping-releasing hormone (GnRH), a reproductive hormone, there is a resultant decrease in food intake.

Another method being employed is a more indirect approach explains Volkoff, using histological and molecular tools to work out how hormones are controlling the feeding of fish that are resource challenged: "We have been examining the gene expression of these factors under different nutritional status or submitted to different environmental conditions". It is important to be cognisant of how environmental factors influence hunger. To accomplish this, the team has examined fish in a number of different environmental conditions, including a range of temperatures, seasons and photoperiods. This has produced some interesting results, including the discovery that winter flounder eat less in winter than summer working in direct correspondence to higher levels of orexin.

regulation. This is important as fish are a diverse phylogenetic group and relatively few fish species have been studied to date, with regards to feeding regulation. The results of these studies might provide new approaches to manage and enhance growth and reproduction in fish. In the long term, based on the strong similarities in the regulatory pathways between fish and higher vertebrates, our results will contribute to the establishment of a fundamental model of the endocrine regulation of feeding in vertebrates.

Have you made any novel discoveries?

My laboratory has significantly advanced our knowledge on the structure of appetite regulating factors by identifying their structure for new species, such as cod and skate and submitted over 50 sequences to gene databases in the past few years. Also, by using our established *in vivo* injection technique in goldfish, we have shown the role of new molecules in the regulation of feeding in fish. For example, we showed for the first time in fish that amylin decreases feeding, whereas apelin-13 and thyrotropin releasing hormone (TRH) increase feeding and that the reproductive gonadotropin-releasing hormone (GnRH) induces a decrease in food intake, which is, in part, due to down-regulation of brain orexin expression.

How do you intend to expand on this knowledge you have gained?

There are still several gaps in our knowledge of the hormonal regulation of feeding in fish. Feeding is a complex behaviour, which is affected by external factors (such as environment, season, time of day and stress) and internal factors (eg. circulating levels of nutrients, such as glucose, or hormones such as insulin and leptin). In addition, interactions between hormones are common, which makes it more difficult to characterise the actions of individual hormones. This is where I am keen to further our research.

Cloning work enables the team to determine cDNAs that encode for appetite regulators and thus infer the protein structures of the peptides. This, in turn, allows the development of molecular tools for further gene expression and enables, by comparing different species, the identification of possible evolutionary changes that are controlling food intake.

have lower metabolic rates and feeding requirements than warm-blooded animals. Whilst the fact that similarities in appetite regulators amongst vertebrates are attractive to researchers, some of the latest evidence indicates significant interspecific differences, and that any cross-transfer of factors needs to avoid 'universal generalisations'.

This technique has resulted in the collection of direct evidence that orexin treatment increases feeding and locomotor behaviour and decreases reproductive behaviour in goldfish

WIDER APPLICATIONS

Fish can provide a useful model for human diseases because they are sometimes easier to use as experimental animals than mammals. Thus, these studies have every potential to be utilised to ascertain possible pharmacological agents. Given that fish have similar mechanisms regulating feeding as other vertebrates, this project will still deliver valuable insights for other species in the near future, and help understand deregulations of energy homeostasis in mammals, such as obesity. However, as Volkoff observes, there are limitations with this kind of transfer of ideas: "When fish are used as models it must be kept in mind that fish and mammals are distinct groups with different physiologies". Fish, as cold-blooded animals, usually

This work might offer the aquaculture sector important alternatives to boost quality and yield and help towards sustainable marine resources. Although direct hormone injections, which tend to be expensive, time-consuming and stressful manipulations of fish might not be a viable option, it would be possible to offer fish feed which include appetite-regulating factors. Volkoff also believes that there is every possibility this research will identify genetic markers which will be valuable to fish farming: "Establishing the nature and functions of the major appetite-related hormones might also help select and develop strains for specific genetic markers". This innovative research offers the hope of producing fast growing transgenic fish that over-express appetite-stimulating genes in the near future, which could have significant economic implications.

INTELLIGENCE

CHARACTERISATION OF APPETITE-REGULATING PEPTIDES IN FISH

OBJECTIVES

To identify the molecules and mechanisms regulating feeding in fish, to assess their interactions and to examine how they are modulated by environmental factors and reproductive events.

COLLABORATORS

Dr Dounia Hamoutene,
DFO Science, Canada

Dr Valerie Booth,
Memorial University, Canada

Dr Vance Trudeau,
University of Ottawa, Canada

Dr Nicholas Bernier,
University of Guelph, Canada

FUNDING

Natural Sciences and Engineering Research Council of Canada

CONTACT

Hélène Volkoff
Assistant Professor

Memorial University of Newfoundland
Departments of Biology and Biochemistry
Rooms SN 3009/3010
Science Building
St John's, NL
A1B 3X9
Canada

T +1 709 737 2140
E hvolkoff@mun.ca

HÉLÈNE VOLKOFF was born of French parents in Benin and grew up in Brazil. After obtaining her BSc and MSc in France she moved to the US to complete her PhD. After pursuing postdoctoral research in Alberta, Canada, she became a Professor at Memorial University in Newfoundland, Canada in 2002.

Comparative Fish Endocrinology Group