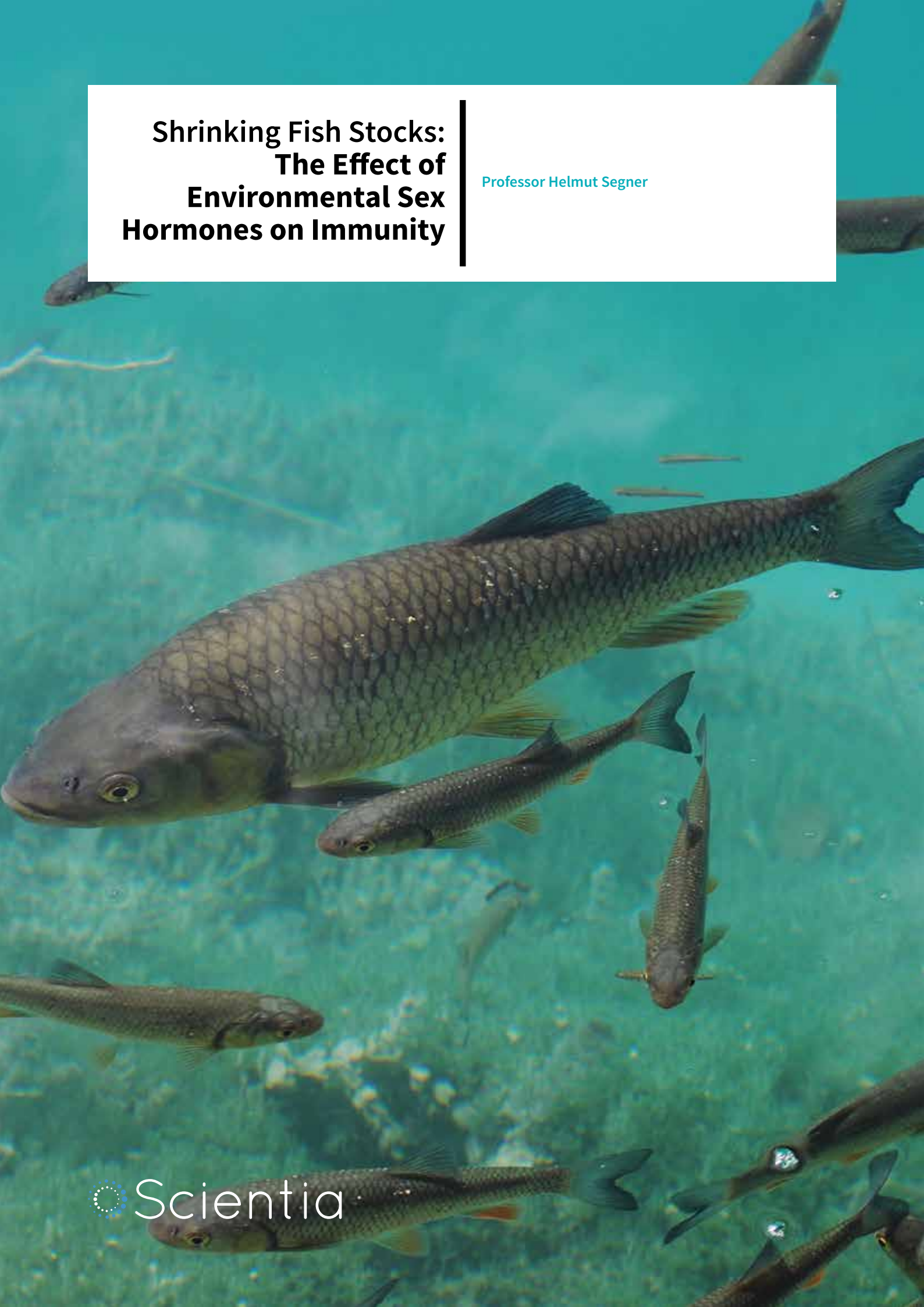
Shrinking Fish Stocks: **The Effect of Environmental Sex Hormones on Immunity**

Professor Helmut Segner



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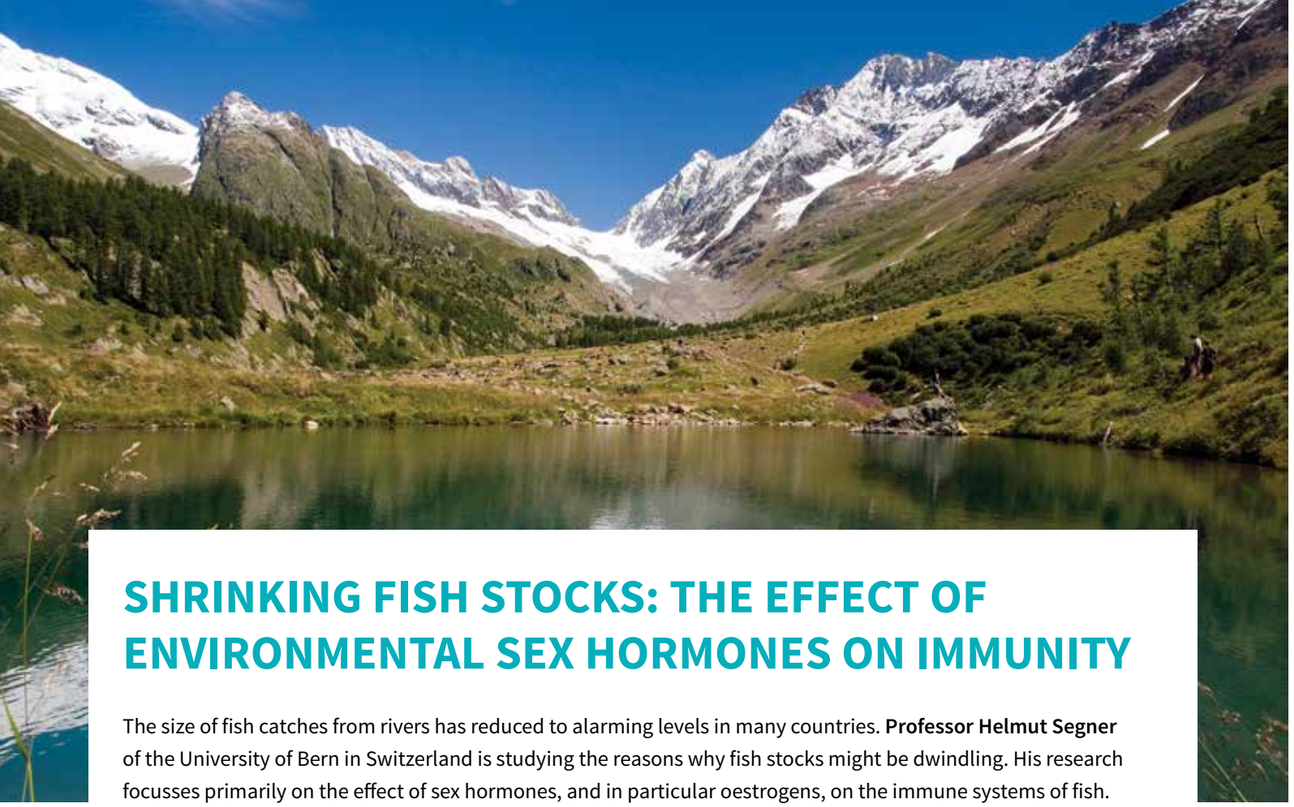

SHRINKING FISH STOCKS: THE EFFECT OF ENVIRONMENTAL SEX HORMONES ON IMMUNITY

The size of fish catches from rivers has reduced to alarming levels in many countries. **Professor Helmut Segner** of the University of Bern in Switzerland is studying the reasons why fish stocks might be dwindling. His research focusses primarily on the effect of sex hormones, and in particular oestrogens, on the immune systems of fish. These biomolecules are on the rise in water systems, for instance, due to the use of oral contraceptives and may be partially responsible for reduced fish populations.

Where have all the fish gone?

The UK, Denmark, France, Norway and Switzerland are among the countries that have reported a recent decline in fish populations. In the 1970s, anglers in Switzerland reported over a thousand fish catches a year, and this reduced to annual numbers of under 600 in the late 1990s. There have also been many reports of fish malformations and reduced fish health. This reduction in fish numbers, including salmonoids (such as trout and salmon) and eels, which are regularly eaten by humans, is of huge concern. For this reason, researchers are on a mission to investigate what factors could be causing this significant drop.

Oestrogens have widespread effects on physiology

Professor Helmut Segner and his team at the University of Bern explore the effect that sex hormones, such as oestrogens, can have on fish immune systems and their ability to modulate immune response. 'Originally, we were looking at the effects of endocrine disruptors on fish reproduction. These are environmental compounds with hormone-like activities. Our focus was on compounds with oestrogenic activity, like ethinylestradiol which is the active ingredient in many contraceptives. We wanted to know how these compounds alter sexual development and impair reproduction of fish,' he explains when asked what led him towards this line of investigation.

These initial findings led the team to become interested in how these compounds might influence other aspects of fish physiology, aside from reproductive development. 'We observed that these oestrogenic compounds had a strong effect, not only on sexual development but also on fish growth, and this effect was mediated via interference with the growth hormone/insulin-like growth factor system,' Professor Segner tells us. At this time the team also discovered that the immune parameters of fish could be changed by exposing them to oestrogenic compounds. 'As my research team was also researching the immune response of fish to infectious pathogens, we became interested in the question of whether exposure of fish to oestrogenic compounds may compromise their immunocapacity and thereby enhance susceptibility to pathogens,' Professor Segner recalls.

Females exhibit a stronger immune response

Research has shown that resistance to infectious pathogens exhibits gender differences in vertebrates. In the same way that the outward appearance opposite sexes may differ, so too does the way in which they combat infection. As Professor Segner explains: 'Generally, resistance, as well as tolerance towards viral, bacterial and parasitic pathogens, tends to be higher in female vertebrates than in male vertebrates. This is called the "female host supremacy paradigm".'

This sexual dimorphism, which has been well documented in mammals and birds, is believed to be caused by females being more immune-competent than males. 'There exists good knowledge on this in mammals, but virtually nothing on lower vertebrates including fish,' says Professor Segner. In humans for example, women show

'We became more and more interested into the "non-canonical" effect of environmental oestrogens, that is, their interference with physiological target systems other than the reproductive system'

stronger pro-inflammatory responses against pathogens than their male counterparts, but at the same women also have a better ability than men to dampen an overexaggerated inflammatory response to the pathogen. As a result, women have a much better chance of resisting infection. Professor Segner tells us about two of the factors that may contribute to this: 'Women generally have a larger relative spleen weight than males. They also tend to have higher white blood cell counts, higher immunoglobulin levels and higher CD4+ T-cell numbers.'

Despite the obvious advantages that this stronger immune system brings, there are downsides. For example, women reject transplanted tissues much faster than men and have a greater predisposition towards autoimmune disease. 'The female/male ratio of systemic lupus erythematosus SLE is 10:1, and of Grave's disease is 7:1,' Professor Segner adds.

The immune system responds to sex hormones

So, what are the physiological drivers behind these differences in immunity between males and females? The fundamental factors responsible for the differentiation of the sexes are the sex hormones. Previous molecular and physiological studies have uncovered the effects that these hormones have on both the differentiation and function of immune cells. Androgens, such as testosterone (which exists in high levels in males), prinicpally act as immunosuppressants, while oestrogens have been shown to enhance the immune system. Since females produce much higher levels of oestrogens, this is thought to be key to their higher immune-competency. 'This suggestion is corroborated by the fact that postmenopausal women, in parallel to the decline of oestrogen levels, also lose their immunological advantage against infections, but at the same time suffer less from autoimmune diseases.' Professor Segner explains. 'In addition, immunity and disease expression of women correlates with their reproductive status (i.e., oestrogen levels), for instance, SLE can undergo remission during pregnancy.'

Evolution of the immune system

Professor Segner and his colleagues want to find out if these immune actions of sex hormones are relevant in fish as well as mammals. They are also interested in understanding what lies behind this immune cell response to sex hormones. He asks the question: 'What is the evolutionary sense of this?' One hypothesis is that the sex hormones have immunomodulating activity because the organism has to deal with resource limitations for both the immune and reproductive systems – two highly valuable fitness components of animals. 'Life history

theory proposes that organisms are not selected for optimisation of either reproduction or immunity, but that evolution selects for the optimal allocation of the limited resources among fitness-associated traits,' Professor Segner explains. 'Nutrient and energy resources are limited and cannot sustain all life history traits at maximum levels. This implies that organisms have to make compromises in the allocation of resources among competing traits, and this implicates that they correlate negatively or "trade-off".'

Life history theory also explains why the effects of sex hormones may differ between males and females. 'As proposed in Bateman's principle, females gain fitness through increased survival and longevity, while males gain fitness by mating with many females,' Professor Segner explains. 'As a consequence, males invest less in immune defence and thus have lower life expectancy.' Additionally, males of most vertebrate species are less invested in parenting their young to increase the offspring's chance of survival. Instead, they devote their time to courting females and competing with other males to increase their mating success. In contrast, females cannot increase their reproductive success by mating with more than one male. 'They are choosy in mating and invest heavily in their offspring and, thus, future reproduction. In line with this, females show higher investment in the immune system in order to support survival and longevity and to increase the lifetime reproductive success,' says Professor Segner.

Understanding the effect of oestrogens on fish immunity

When embarking on their initial investigations, Professor Segner and his team found that very little was known about the effect of oestrogens on the immune systems of fish. First, the team needed to determine if fish immune cells are indeed influenced by oestrogens. To do this, they analysed the cells of rainbow trout to see if oestrogen receptors are present using Reverse Transcription Polymerase Chain Reaction (RT-PCR). Since this technique is used to detect RNA expression, the team used it to find RNA sequences that code for the creation of oestrogen receptors. Remarkably, all four nuclear oestrogen receptors (ERα1, ERα2, ERß1 and ERß2) were discovered in the studied immune cell populations. The presence of nuclear oestrogen receptors in other fish species has now also been identified, as have membrane-bound oestrogen receptors.

The team then investigated how exposure to oestrogens can modulate immune gene expression and immune functions in fish. This was done both in isolation and in the presence of pathogens. Professor Segner tells us about the importance of performing these experiments in the presence of a pathogen: 'It is often the case that an immunomodulatory effect becomes evident only when the immune system is activated, and not in the resting immune system.' They exposed rainbow trout either to oestrogens alone, or to a combination of oestrogens and a pathogenic parasite. Using a microarray approach, they discovered that when exposed to both stressors, parasite and high oestrogen levels, the fish response differed to then exposed to a single stressor alone. For example, some genes were downregulated (less expressed) when exposed to oestrogens, but in the presence of the pathogen they were upregulated (more expressed), while others remained downregulated.

The challenge experiment

The team needed to prove that oestrogenic substances had an impact, not only on immune parameters but also on the immune capacity of fish. In particular, they needed evidence to show that

'Fish are complicated because they possess up to four nuclear oestrogen receptors (ERα1, ERα2, ERß1 and ERß2), and we found them all to be present in the various immune cell populations of rainbow trout'

oestrogenic substances, endogenous or exogenous toxicants, can have a compromising effect on fish immune systems, making them more susceptible to succumbing to disease. They devised a challenge experiment, using rainbow trout that they exposed to a pathogen called Yersinia ruckeri, which causes the enteric red mouth diseases of salmonids. The development of the disease and survival rates were then compared between a control group and an oestrogen-treated group. Unlike us humans, the fish exposed to oestrogen were significantly more susceptible to the parasite than the control fish.

Future work

Professor Segner's work is ongoing as he seeks to further understand evolutionary factors that give rise to an immune-reproductive trade-off. This interaction has relevance for both animal and human health, and sheds light on the immunological risks associated with exposure to endocrine disruptors. 'In the case of high fish mortality in rivers, the observer may blame pathogens as being responsible. But, the real cause would be the combined effect of the oestrogenic and the pathogenic stressor. Given the fact that, despite improved water quality that we now observe in many river systems, we still have a decline in fish populations, it is likely that these effects are not monocausal but arise from the cumulative impact of multiple stressors,' he concludes.

Meet the researcher

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Professor Helmut Segner is Head of the Centre for Fish and Wildlife at the University of Bern, Switzerland. He completed his Master's degree in Chemistry and Biology and a PhD in Biology at the University of Heidelberg in Germany. He then went on to carry out postdoctoral research at the same university before taking a post as a research scientist at the Tetra Fish Food Company. Before arriving at his current position, Professor Segner was Assistant Professor at the University of Karlsruhe and then Head of the Aquatic Ecotoxicology Research Group at the Helmholtz Centre for Environmental Research in Leipzig. He also acts as an Associate Editor for the journals Aquatic Biology and Fish Physiology and Biochemistry, has published over 200 peer reviewed articles, and serves on a number of national and international advisory committees.

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