AQUACULTURE

The Development of Molecular and Biochemical Tools to Assess Changes in Yellow Perch (*Perca flavescens*) Growth Hormone<

Final Report

The yellow perch is a very important food fish and ecological fish species in the midwest. The commercial supply of yellow perch has decreased as more restrictions have been made on the commercial fishery. In part, these restrictions have been necessitated by decreasing natural populations of perch in the Midwest. A perch aquaculture industry has developed, but the relative slow growth of this species has posed a problem for cost effective production. The goal of this research project is to provide tools for researchers to study the environmental and endocrine control of yellow perch growth as a means to increase the efficiency of perch aquaculture. Specifically, our research deals with growth hormone (GH), a major regulator of growth in vertebrates. In this context, the overall objective of our study is to produce an antibody to yellow perch GH that can be used in the development of assays for the levels of GH in perch. To accomplish this, we must obtain the perch GH itself. This past year, we did this indirectly using a molecular approach in which we isolated the messenger RNA that directs the synthesis of the GH protein in perch. We then used this message to produce an artificial perch GH ("recombinant" protein). This was used as an antigen to produce an antibody in rabbits. At the present time we are testing the antibody to see if it can be used to measure the natural perch GH.

To facilitate the dissemination of information from our project to other researchers, we have developed a webpage (**http://www.mbl.edu/goetz/perca.html (http://www.mbl.edu/goetz/perca.html)**). The page lists the reagents that are available for distribution and chronicles our research progress. The page is linked from several research related sites and will be continually updated as more reagents, such as proteins and antibodies, become available.

Major Goals and Objectives

1) To obtain the full-length perch growth hormone (GH) cDNA.
2) To clone and express the yellow perch GH mRNA in a protein expression system.
3) To assay the recombinant perch GH for growth promoting activity in juvenile perch.
4) To produce antibodies to the recombinant perch GH.
5) Produce a webpage that would describe our research on growth in perch and would list reagents produced during the grant that would be available to other researchers

Progress

To date, the full-length perch GH cDNA has been cloned; it has been used to produce a recombinant perch GH protein that was used to make a polyclonal antibody. The recombinant perch GH does not appear to have significant growth promoting activity. The polyclonal antibody does successfully recognize yellow perch GH protein from pituitaries. A webpage has been constructed that describes our research and lists the available reagents to date. It has been linked to a number of other pertinent websites.

Applications/Benefits

Since the antibody that was produced during this project recognizes the natural perch GH, it can then be used to assay perch GH in experiments designed to look at the regulation of growth. This will be particularly important for the commercial aquaculture industry to optimize perch growth.

Keywords
yellow perch, growth hormone, antibody, *Perca flavescens*

Narrative report

Prior to the final year of the grant, we had already obtained the full-length perch growth hormone (GH) cDNA, expressed the yellow perch GH mRNA in a protein expression system, and produced an antibody to the recombinant perch GH. Thus, the final year has been primarily spent determining the effectiveness and specificity of the antibody. The recombinant protein was also assayed for growth promoting activity in juvenile perch.

In order to assay the effectiveness of the antibody, Western analysis was used. Recombinant yellow perch GH or 20 ug of total pituitary protein was separated on a 10% reducing gel using sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). Whole pituitary samples were used because of the difficulty of purifying GH from pituitaries and the fact that almost 50% of the contents of the pituitary is GH. Following electrophoresis, proteins were transferred to Westran-PVDF membranes (Schleicher and Schuell) using a Trans-Blot SD Electrophoretic Transfer Cell (BioRad) for 30 minutes at 15V. The membranes were blocked minimally for 1 hour in TBS containing 5% non-fat dried milk and 0.1% Tween 20. Following blocking, membranes were incubated with the diluted primary YPGH antibody (1:1000), washed in buffer and then incubated with a horseradish peroxidase labeled secondary antibody. Following the secondary antibody incubation, blots were washed and incubated with a lumigen substrate for 5 minutes and then detected directly on a Storm 840 phosphoimager (Molecular Dynamics).

A single protein band of ~21kDa was detected in the whole pituitary protein sample. This is the predicted mass of yellow perch GH. To verify that the antibody was actually recognizing GH, pure recombinant sea bream GH (GroPep Limited) was used to test the antibody. Sea bream GH was chosen because of the close phylogenetic relationship and corresponding high protein sequence homology (>90%) to yellow perch GH. The GH antibody did recognize sea bream GH. Brook trout pituitary samples were also analyzed. The antibody did not recognize brook trout GH. This is expected due to the distant phylogenetic relationship of the species and is evidence that the antibody is relatively specific for perciform GHs.

To further demonstrate that the antibody actually recognizes perch GH, we tested the effects of estradiol treatment on perch pituitary GH levels as detected by the antibody. Estradiol has been reported to increase the size of yellow perch, and increased food consumption and food conversion efficiency has been shown to be the major factors attributed to the increased size of estradiol treated fish. Growth hormone has been shown to regulate these activities and therefore an increase in immunoreactive protein measured by the antibody following estradiol treatment would further confirm that the antibody is detecting GH. A group of yellow perch was treated with estradiol for 46 days in the diet and the pituitaries were sampled at the end of the treatment and assayed using our antibody. Western analysis indicated a significant increase in the level of GH immunoreactive protein in fish treated with estradiol compared with the saline injected controls, further confirming that the antibody recognizes perch GH.

In order to assay the effectiveness of the recombinant yellow perch GH protein to stimulate growth in juvenile perch we injected perch with the protein and analyzed liver and blood tissue for IGF-1. It is known that in fish, the growth promoting effects of GH are mediated by IGF-1, therefore if our recombinantly produced GH protein has growth promoting capabilities we would expect levels of IGF-1 to increase. Levels of IGF-1 were not significantly different in GH injected fish compared to saline injected fish. Additionally, we have supplied one of our collaborators, Dr. Terence Barry, with the recombinant protein so that he can assay the effects of the protein on growth of juvenile yellow perch. The results of the IGF-1 study suggest that even though the recombinantly derived protein produced an effective antibody it may not have the capability of stimulating growth in juvenile yellow perch.

As an outreach aspect of our project we have developed a webpage that chronicles our research progress, but more importantly serve as a means to disseminate reagents. The web address for the page is: **http://www.mbl.edu/goetz/perca.html (http://www.mbl.edu/goetz/perca.html)**. The full length cDNA of yellow perch growth hormone (GenBank Accession # AY007303) is already available. The perch GH antibody that we have produced will soon be available through the website. We have already distributed the antibody to other researchers to aid in the understanding of growth in yellow perch. This webpage also contains information on other research we have done on yellow perch concerning reproduction with emphasis on mRNAs regulated in the ovaries. To get our information out to those who could utilize it we have arranged to have our page linked from websites including AquaNIC (**http://aquanic.org/ (http://aquanic.org/)**), International Association for Great Lakes Research (**http://iaglr.org/ (http://iaglr.org/)**), and Great Lakes Fishery Commission (**http://glfc.org/ (http://glfc.org/)**).

Lay summary

The yellow perch is a very important food fish and ecological fish species in the midwest. The commercial supply of yellow perch has decreased as more restrictions have been made on the commercial fishery. In part, these restrictions have been necessitated by decreasing natural populations of perch in the Midwest. A perch aquaculture industry has developed, but the relative slow growth of this species has posed a problem for cost effective production. The goal of this research project is to provide tools for researchers to study the environmental and endocrine control of yellow perch growth as a means to increase the efficiency of perch aquaculture. Specifically, our research deals with growth hormone (GH), a major regulator of growth in vertebrates. In this context, the overall objective of our study is to produce an antibody to yellow perch GH that can be used in the development of assays for the levels of GH in perch. This past year, we tested the antibody we produced and found that it is accurately measures the level of GH in yellow perch. Now others can use this antibody to measure GH of yellow perch in the laboratory and hatcheries to see what environmental parameters increase levels of GH and thereby will presumably increase growth rates for aquacultured yellow perch.

To facilitate the dissemination of information from our project to other researchers, we have developed a webpage **http://www.mbl.edu/goetz/perca.html (http://www.mbl.edu/goetz/perca.html)**. The page lists the reagents that are available for distribution and chronicles our research progress. The page is linked from several research related sites and will be continually updated as more reagents, such as proteins and antibodies, become available.

Media Coverage: Chicago Tribune Article: “Looking for bigger fish to fry- scientists see a way to put more great lakes perch on diners' plates --if they can just get the tasty little guys to grow faster” By Jeff Long Tribune environment reporter
April 13, 2001

Partnerships with other institutions/individuals:

Dr. Jeff Malison (Aquaculture Program at the University of Wisconsin-Madison) Dr. Terence Barry (Aquaculture Program at the University of Wisconsin-Madison)

Publications:

Roberts, SB (2002) Characterization of growth hormone in yellow perch and myostatin in several teleost species. Ph.D dissertation. University of Notre Dame, Indiana.

Roberts, S., Malison, J., Barry, T., Goetz, F. Production of a recombinantly derived growth hormone antibody and the characterization of growth hormone levels in yellow perch (in preparation).

Undergraduate/graduate students supported by project : Steven Roberts – Ph.D. student

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