GREAT LAKES FISHERY COMMISSION

2003 Project Completion Report[1]

Regulation and manipulation of metamorphosis in sea lampreys: the relevance of a sea lamprey obesity factor and brook lamprey life history

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INTRODUCTION

Present day lampreys have a complex life cycle that involves a protracted larval period with growth and metamorphic phases, a juvenile period, a period of sexual maturation, and a period of spawning followed by death. There has been some question as to the time of appearance of metamorphosis in the evolutionary history of lampreys (Youson, 1999; Youson and Sower, 2001; Youson, 2003a), but undoubtedly this phase has been important to the survival of a group that has had a rather conserved evolution for nearly 350 million years. Metamorphosis of lampreys involves a sophisticated series of developmental events that result in immense changes to tissues and organs that prepare the organism for adult life. During this phase the animals are vulnerable to insults that may alter the developmental sequence and result in an incomplete metamorphosis; such is the case during development in all other vertebrates.

The sea lamprey (*Petromyzon marinus*) is a non-native predator in the Great Lakes and there is a control program to reduce the population. This program primarily relies on chemical treatment of streams to control the harmless larvae before and during their metamorphosis. The Great Lakes Fishery Commission (GLFC), that oversees the control program, has been looking for alternate means for controlling sea lamprey populations to meet a goal of reducing the use of larvicide. Although the larvicide is quite specific to lampreys, continued treatment is not "environmentally friendly" in the modern context and the chemical also has targeted native lamprey species in the Great Lakes. These native lamprey species are either parasitic or nonparasitic (nonfeeding) as adults and they have been a component of the ecosystem of their natal stream at least since the last ice age.

We have proposed that metamorphosis is an important period of the life cycle to lampreys and that it might be possible to redirect the outcome of the event by manipulation of the process. We have provided proof in our studies that sea lamprey metamorphosis can be manipulated (Holmes and Youson, 1993; Youson et al., 1997) and there is a certain degree of plasticity in the event in other species in nature that results in varying phenotypes (Youson and Beamish, 1991). A study needs to be undertaken to compare metamorphosis in lamprey species that have different adult life histories. What events of metamorphosis in a nonparasitic species dictate that the postmetamorphic animal will not feed but go directly into sexual maturation? Is there any way that this event can be induced in metamorphosing sea lamprey larvae to result in the inhibition of adult feeding? Are there physiological or biochemical differences in the parasitic and nonparasitic larvae and adults that we can relate to adult life history differences? These types of comparisons are

a major undertaking, if all systems are under study. We have chosen to compare expression of adenohypophysial hormones and the profiles of two physiologically significant proteins (leptin-like protein and albumin) in sea lampreys with other species. The completion report describes the progress to date on these three investigations.

Endocrine study

Lampreys are among only a few groups of vertebrates that have a metamorphosis in their life cycle. The metamorphoses of anuran amphibians and flatfishes (Pleuronectiformes) are similar in that they can be triggered by an exposure to thyroid hormones and inhibited by exposure to antihyperthyroid substances such as goitrogens. In contrast, now there is unequivocal evidence that lamprey metamorphosis can be induced by the goitrogens and inhibited by exposure to thyroid hormones. Whereas spontaneous (natural) metamorphosis in anurans and flatfishes is characterized by elevated serum levels of thyroid hormones, a sharp decrease in serum thyroid hormones marks the onset of lamprey metamorphosis (Youson, 1997). The cascade of endocrine events that control metamorphosis in lampreys is of great interest to comparative endocrinologists and to developmental biologists. There is also potential to exploit the differences in endocrine control of metamorphosis for use in control of lampreys that are pest species (Youson, 2003b). However, before this potential can be realized there is need to know more about the endocrinology of the higher centres in the endocrine axes. Fortunately, there has been a great deal of effort in the past 15 years by the laboratories of Drs. Stacia Sower (New Hampshire) and Hiroshi Kawauchi (Sanriku, Japan) to identify hormones of the adenohypophysis (anterior pituitary) of the adult sea lamprey, *Petromyzon marinus*. Stimulation of the adenohypophysis would normally be the second step in a cascade of events that would begin in the hypothalamus of the brain and result in the stimulation of a target endocrine organ/tissue. The identity of which hypothalamic and adenohypophyseal hormones are upregulated during lamprey metamorphosis would be a valuable asset to understanding of its endocrine control but also might lead to a novel method of altering the process.

Biochemical studies

Besides the hormonal requirements for lamprey metamorphosis, there are both metabolic and environmental criteria that must be in place before spontaneous metamorphosis can occur in sea lampreys (Youson, 1994; 2003b). In addition, there are some distinct differences in important physiological and biochemical parameters between larvae and adult sea lampreys that might be unique to this species and related to their parasitic adult life history. The present research contract focused on two of these physiological/ biochemical/metabolic parameters. The first of these revolves around the fact that sea lampreys must have extensive fat reserves before they enter metamorphosis (Youson et al., 1979). The second physiological feature of metamorphosis that was exploited during this study is that it is the developmental event when a major change in the profile of serum proteins between larvae and adult sea lampreys is initiated (Filosa et al., 1992).

Leptin

Several laboratory-based collaborative studies have yielded data that illustrate the importance of fat deposition in sea lamprey larvae in the year prior to the onset of their metamorphosis. This fat deposition is manifested in increased weight of immediately premetamorphic larvae compared to nonmetamorphic larvae of the same length; this difference can be guaged through the calculation of a condition factor (see, Youson et al., 1993; Holmes and Youson 1994, 1997, 1998; Holmes et al., 1994). The question of energy storage and utilization is intimately related to that of reproduction since it is known that in many animals the reserves of adipose tissue can affect reproductive capability. Since sea lamprey larvae require fat deposits for metamorphosis, and reproductive maturation only follows this metamorphosis, preventing animals from either accumulating or using these fat reserves would be an obvious direction for inhibiting metamorphosis and, therefore, reproduction. Furthermore, we propose that the stimulus for the initiation of spontaneous metamorphosis in sea lampreys may come through a signal released by fat-storing tissues that have accumulated sufficient fat reserves. In mammals, the protein leptin has been identified as playing an important role as an "adipostat". Leptin released from adipose tissue signals the brain about the body's energy reserves. Increased leptin levels in mammals can cause a cascade of events, but important to the present study on lampreys is the reduction of food intake and the stimulus to the reproductive system. Lamprey metamorphosis is characterized by a cessation of feeding and an elevation of gonadotropin-releasing hormone in the hypothalamus of the brain (Youson and Sower, 1991).

The primary objectives of our study were two-fold: 1. to explore whether sea lampreys possess a leptin-like molecule and if so, whether it is differentially expressed during the life cycle; 2. to look for a leptin-like molecule in a lamprey species that does not appear to require the fat reserves prior to metamorphosis and one that has a different adult life history. A secondary objective was to isolate a lamprey leptin or to clone a lamprey leptin cDNA in order to describe its primary structure. Identification of a leptin receptor was also suggested. This research was considered to be ambitious given the funding available and the knowledge of failure of other investigators searching for a fish leptin.

Serum Protein

As in many other vertebrates, the developmental intervals in lampreys are marked by changes in the profiles of proteins in the serum. For the past 20 years our laboratory has been the primary centre for research on serum proteins in sea lampreys; this research was driven by the belief that, as in other vertebrates, certain serum proteins are critical to various physiological and metabolic processes (Filosa et al, 1982). Among our findings are that the nature of the important serum protein, albumin, changes during the life cycle of sea lampreys (Filosa et al., 1992). There is a larval type of albumin that we called AS and adult type of albumin referred to as SDS-1. The primary structures of these two albumins were deduced from cDNA clones and their differing structure was consistent with our results that showed their different antigenicity based on immunological studies. Their structures were also of interest from the point of view of evolution of the albumin molecule in vertebrates, for both AS and SDS-1 were comprised of 7 domains compared

to only 3 in most other vertebrates. Although the two molecules are similar, they are encoded by two different genes.

An important focus to our research on sea lamprey albumins is whether there is anything about this molecule that can be exploited for use in the control program for this species in the Great Lakes. Since SDS-1 seems to be associated with adult life and AS is characteristic of larval life and early metamorphosis, is there anything that can be done to either enhance the expression of AS or to inhibit the expression of SDS-1 in adults? Before trying to answer this question, we decided to examine the albumin profile in another lamprey species with a different adult life history than the sea lamprey. At the outset of the present contract, we were studying albumin in the American brook lamprey, *Lampetra appendix*. We noted that, although there was an AS-like protein in larvae and early metamorphosing individuals, there was no equivalent to SDS-1 in adults of *L. appendix*. Because this suggested that SDS-1 could be an adult-only, sea lamprey specific, serum protein, we proposed to explore further the albumin profiles in *L. appendix*, and perhaps study those in other lamprey species, compared to that in sea lampreys. The fundamental question was, is the gene for SDS-1 one of a whole complex of genes associated with a parasitic way of life, all of which have been turned off in nonparasitic species such as *L. appendix*?

RESULTS

1. Study of expression of pituitary GH-like peptide and liver IGF in lamprey

Growth hormone (GH), produced in the anterior pituitary, is important to both growth and metabolic processes in most vertebrates. Knowledge of the involvement of this hormone during the various developmental intervals of sea lamprey is of great potential value to the program that is involved in the management of this species. A pituitary GH-like peptide has been both cloned and isolated from the pituitary of adult sea lampreys (Kawauchi et al., 2002).

In our first annual report we described a Northern blot analysis of the expression of mRNA for pituitary GH-like peptide in larvae and pre-spawning adult sea lamprey (both female and male) pituitaries. Northern analysis detected a 2.5 kb mRNA in both female

and male adult pituitary but no hybridization signal was apparent in the larvae pituitary. The size of the mRNA (2.5 kb) was reasonable considering the size of cDNA (2.1 kb) and the poly-A tail. These preliminary results were confirmed over the second year and attempts were made to examine expression of mRNA for this peptide in other periods of the life cycle, particularly metamorphosis. This project required large numbers of immediately premetamorphic larvae which will go through metamorphosis and we were not successful in obtaining sufficient numbers. The pituitary of larvae and metamorphosing animals is tiny, as are those of juveniles. A good-working protocol for Northern analysis must be included to ensure the limited samples we have are not wasted when the Northern blot was carried out. We needed pituitaries from a minimum of 20 animals for each stage; approximately 50 µg of total RNA can be extracted from this number of pituitaries. However, this amount of total RNA is enough for only 5 blots for Northern analysis. This year we collected only 30 immediately premetamorphic animals from Covert Creek that went through metamorphosis. We have just completed sampling of pituitaries from 4 stages of metamophosis and immediately postmetamorphic adults and are now prepared to do the Northern blot analysis with our GH cDNA probe. We also have some feeding adults we will use in our analysis. This latter analysis will be very important to show the expression of GH-like peptide during the critical period of rapid somatic growth in adults.

Recently, Kawauchi et al. (2002) cloned and sequenced a cDNA obtained from the liver of sea lamprey that encodes a protein showing a deduced amino acid sequence of significant identity with mammal and fish insulin-like growth factor I (IGF-I) Results from this study indicate that, as in mammals, the GH-IGF-I axis may play an important role in the regulation of development and somatic growth in fish. In the present contract we characterized expression of lamprey IGF-I to understand the role of the peptide in regulating metamorphosis and subsequent development.

Lampreys were collected from a variety of sources in Ontario in previous years (Oshawa Creek, Humber River) and Michigan (Lake Huron Biological Station). Larvae were of a nonmetamorphic size group (99-120 mm and 1.3-2.5 g) and an immediately pre-metamorphic group (121-143 mm and 2.7-4.7 g) based on criteria previously described by our laboratory and were termed L and LL, respectively. Staging of lampreys through metamorphosis (stage 2, 4, 6, 7) used criteria that was previously described by Youson and Potter (1979). Tissues were taken from post metamorphic feeding-phase adults (juveniles) separately collected by size and were termed P1 (125-195 mm and 1.96-9.79 g) and P2 (230-290 mm and 15.00-39.10 g). Tissues from adults in their upstream spawning migration (pre-spawners) were separately collected by gender (male and female). Lampreys were anaesthetized in 0.05% tricaine methanesulphonate (MS-222) and killed by decapitation. Pituitaries and livers were removed from 20 lampreys at each period of the life cycle. Tissues were immediately frozen in liquid nitrogen and stored at -80° C until used. Total RNA was extracted from the frozen tissues with TRIzol Reagent (Gibco) using the procedure specified by the manufacturer. The total RNA (10 µg) was electrophoresed on 1% agarose/6.6% formamide gel containing 1X MOPS buffer. The RNA was transferred to a nylon membrane and was fixed by microwave. The lamprey IGF cDNA was labeled with [32P]dCTP by random priming. The membrane was hybridized

for 18h at 50° C and washed twice at room temperature in 2X SSPE containing 0.1% SDS for 10 min followed by two further washes at 68° C in 0.1X SSPE containing 0.1% SDS for 20 min. After the final washing, the membrane was exposed to X-ray film at -80° C for 5 days.

a. Expression of pituitary GH-like peptide mRNA during the life cycle of the lamprey

Northern analysis verified the result from the previous year that there is no expression of lamprey larvae GH (**Fig 1**, L). However, in both the adult male (M) and female (F), there is significant expression of GH, yet the amount of expression differed, with the male having a much greater signal than the female.

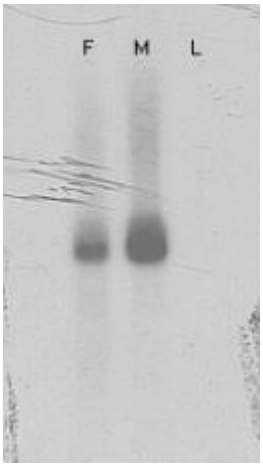


Figure 1. Northern blot using total RNA from pituitaries of adult male (M), female (F) and larva (L) of P. marinus. Blot was probed with P^{32} - labeled lamprey IGF- cDNA.

b. Expression of liver IGF mRNA during the life cycle of the lamprey

Northern analysis detected expression of a 1.0 kb mRNA. The size of mRNA is a reasonable value, considering the size of cDNA (800bp) and the addition of the poly-A tail. Northern blotting (**Fig. 2**) showed low but detectable expression levels of IGF in the liver in the two larval groups (L and LL) and in metamorphic animal stage 2. In these stages, IGF expression decreased markedly in the LL compared to the L and the stage 2. Relative to these earlier intervals, IGF expression appeared to have increased in metamorphic animals (stage 4, 6 and 7), juveniles and pre-spawning adults (both female and male). IGF-I has been shown to stimulate DNA synthesis, cartilage sulfation, protein synthesis, spermatogenesis and induce final oocyte maturation in fish. These results suggested that lamprey IGF also possesses functions similar to those seen in other fish. However, we are reluctant to provide definitive relevance of these data until bioassay analyses are performed.

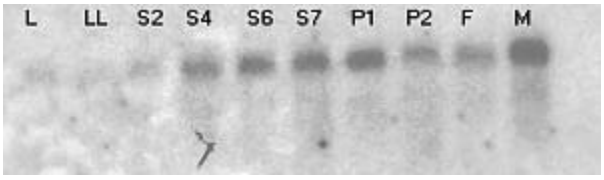


Figure 2. Northern blot of RNA from liver of two larval groups (L and LL), metamorphic stages 2, 4, 6 and 7 (S2, S4, S6, S7) , adult male (M) and female (F). Blot was probed with P^{32} - labeled lamprey IGF-cDNA

In summary the present data on both pituitary-GH-like peptide and lamprey IGF indicate that the genes for these two hormones are most highly expressed at times in the life cycle when there are key developmental and metabolic processes taking place in sea lampreys.any down-regulation of this expression could have adverse effects on sea lamprey development and hence reduce recruitment of feeding juveniles to the Great Lakes.

2. Search for a lamprey leptin or its receptor

Previous work led to the conclusion that an accumulation of lipid in the ammocoete of *P. marinus* is required before metamorphosis can take place (Youson et al., 1993; Holmes and Youson, 1994). This observation suggested that the ammocoete must have a mechanism for detecting the level of lipid in order to proceed with metamorphosis. In recent years, studies on mammals resulted in the discovery of a 16 kDa protein synthesized by adipose tissue, leptin, that appears to have the properties of a "lipostat". Leptin levels increase as body fat levels increase and this increase in leptin regulates metabolism by inhibiting food intake and increasing thermogenesis.

Our initial investigations were directed towards determining whether *P. marinus* had a leptin-like protein. Such a protein could be involved in a mechanism for estimating ammocoete lipid levels to signal the go-ahead for metamorphosis. The possibility that a lamprey would have this protein was not far-fetched since the original report describing the cloning of the leptin gene in the mouse (Zhang et al 1994) also reported that a probe of the gene hybridized on a Southern blot with DNA from non-mammalian animals: a teleost, the eel, and an invertebrate, *Drosophila*.

In order to search for leptin in the lamprey, we first used antisera made against mammalian leptin. The leptin protein is highly conserved in mammals and the chicken. Using Western blot analysis we were able to identify a number of proteins that gave a strong reaction with the antiserum. Of these proteins, only one had the same molecular mass (16 kDa) of mammalian leptin while the others had masses of 50, 65, and 100 kDa. The 16 kDa and 65 kDa proteins were found in the adipose tissue of the nephric fold, Yaghoubian et al., 2001). While these results from Western blots were suggestive, they do not constitute proof that the immunoreactive molecules are the lamprey counterparts of mammalian leptin, either structurally or functionally.

To obtain such proof , it was decided to clone the lamprey version of leptin using RT-PCR with probes based on the highly conserved regions of mammalian leptin. Despite using various combinations of probes and different tissues, and despite using a PCR method that was sensitive to very low amounts of transcript, no clones with a sequence match to mammalian leptin were found. This result is consistent with other laboratories attempting to clone a fish leptin (R. Londraville, personal communication)

At this point we thought that if a protein with the same function as the leptin of higher vertebrates exists in lamprey, it could have a very different structure but possibly have at least one domain that is very similar to leptin. This domain would likely be the one that binds to the leptin receptor. If we found in the lamprey a protein homologous to the leptin receptor of higher vertebrates, this would suggest that the lamprey has a leptin-like ligand. In higher vertebrates, leptin binds to a receptor located in the plasma membrane of cells,especially cells in the hypothalamus . The binding region of the receptor is a domain of about 300 amino acids. Using primers for conserved regions of the binding domain and lamprey brain RNA, we used PCR in an attempt to clone a putative leptin receptor in the lamprey. Although a number of clones were isolated, none showed significant similarity to the leptin receptor of higher vertebrates.

This past year our work on leptin was suspended due to lack of funding for sequencing costs, but there are still approaches that should be tried before abandoning the search for a leptin-like molecule in the lamprey. In addition, we now have our own sequencing facility and a new NSERC-supported postdoctoral student to carry out the study. The following are approaches that we are now going to take:

a. Using the cloned mouse leptin gene as a probe, Southern blots of lamprey genomic DNA will be done to see if there is hybridization. The fact that Zhang et al. (1994) found hybridization with eel DNA suggests that this approach might yield some positive result. It would be possible to isolate and sequence the hybridized DNA.

b. We will atttempt Northern blots of RNA from lamprey adipose tissue using a mouse probe.

c. The PCR search for the receptor will be continued using RNA ammocoete brain; our previous attempt used adult brain because of limited resources of ammocoetes.

d. If adipose tissue of ammocoetes does in fact make a “lipostat” type of molecule that functions as we proposed, it might be possible to detect it by using extracts of the tissue to induce premature metamorphosis in ammocoetes that have not reached condition factor status. This approach tests directly the idea that such a molecule exists and presumes nothing about whether the molecule is leptin. In fact, if a leptin-like molecule does exist in lamprey, there is no certainty that it functions as does the leptin of higher vertebrates.

3. Serum Albumin

Prior to the tenure of this grant, work from our laboratory (Filosa et al., 1982; Filosa et al., 1986) had demonstrated that the sea lamprey, *Petromyzon marinus*, had two distinct serum glycoproteins with physical and chemical properties that suggested they were the lamprey equivalent of serum albumin of higher vertebrates. One of these proteins, which we designated SDS-1, has a molecular mass of about 170k Da, is the predominant protein in the spawner and is found in lower amounts at earlier phases of the life cycle (**see Fig. 3**). The other albumin type protein was named AS; it is the predominant protein in the ammocoete serum, has a molecular mass of about 160 kDa and decreases in amount during the life cycle so that it is virtually absent in spawners. (Ito et al., 1988; Filosa et al., 1992) [**see Fig. 3**].

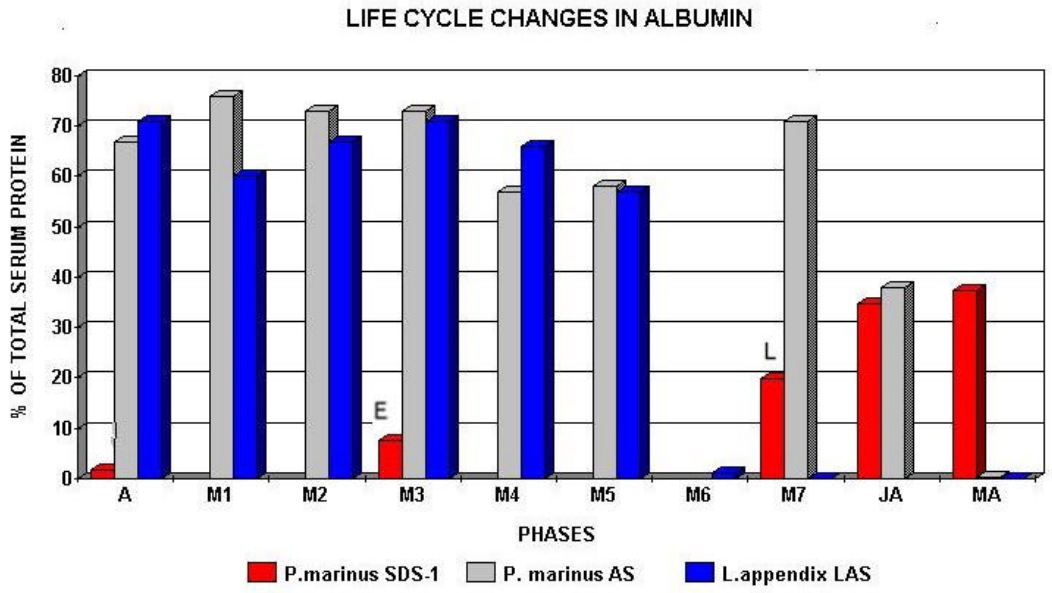


Figure 3 Changes in albumin during life cycle. A: ammocoete; M1-M7: metamorphic stages 1 through 7; JA: juvenile adult; MA: mature adult. E: average of stages 1,2 and 3; L: average of stages 4,5,6,7. Data for *P. marinus* is from Filosa et al., 1982, 1988; data for *L. appendix* is from Danis et al., 2000.

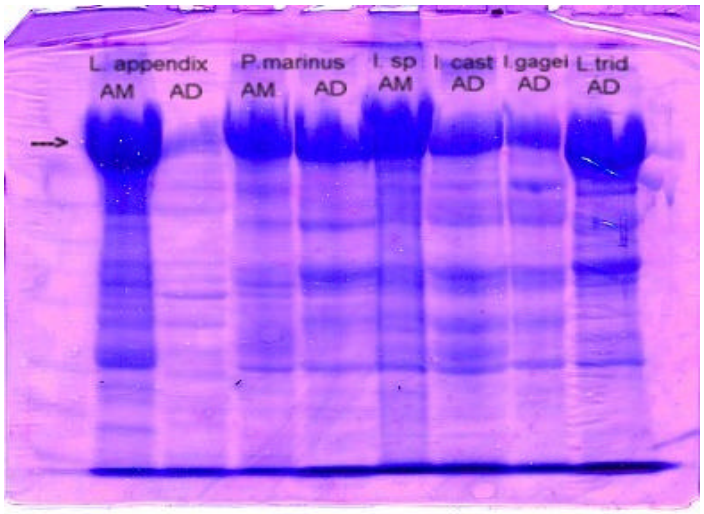
Later, Gray and Doolittle (1991) cloned the cDNA for the SDS-1 protein and from the derived amino acid sequence this protein was definitively identified as a member of the albumin family. Later, Filosa et al. (1998) isolated a partial clone of the gene for the AS protein; its derived amino acid sequence placed it in the albumin gene family and showed that AS was about 40% identical to SDS-1. During the tenure of this grant we extended the study of lamprey serum albumin to the freshwater, non-parasitic *Lampetra appendix* (Danis et al., 2000). In this species, we found that the ammocoete and the first 6 stages in larval metamorphosis had a predominant serum protein (designated LAS) that reacted

with antiserum made against the *P. marinus* AS protein. Polyacrylamide gel electrophoresis showed that the adults of this species have no predominant serum protein (**See Fig. 4**) and no protein that crossreacted with antiserum made against the *P. marinus* adult albumin, SDS-1. It was concluded that *L. appendix* has only a single type of serum albumin and this is limited to pre-adult phases; the *L. appendix* adult has no serum protein comparable to the SDS-1 albumin of *P. marinus* adult. We suggested that because the *L. appendix* adult does not feed, the failure to synthesize a high molecular mass albumin could be an adaptation for sparing amino acids and energy for use in gamete production.

Because the adult of *P. marinus*, which has an albumin, has a lifestyle that includes a marine environment and parasitism while the *L. appendix* adult , which has no albumin, lives in freshwater and does not feed, the question arises as to whether the presence of an albumin in adult lampreys is associated either with adult feeding or a marine environment. To pursue this inquiry , we decided to examine the albumin profiles of as many lamprey species as available to answer the questions: Is there an association between life in a marine environment and albumin in the adult? Is there an association between parasitism and presence of albumin in the adult? Of special interest would be paired species whose members are supposed to be genetically related by descent from a common ancestor and which inhabit the same environment but which differ in their adult feeding behaviour. Does only the parasitic member of the pair have albumin in the adult phase?

Our initial attempts to obtain different species were frustrating but with the help of a number of investigators we finally obtained animals. Dr Philip Cochran of St Mary's University of Winona, Minnesota, provided us with adult specimens of the paired species *Ichthyomyzon castaneus* (parasitic) and *Ichthyomyzon gagei* (non-parasitic). He also provided *Ichthyomyzon* ammocoetes from the same locale but it was not possible to identify them with regard to species. Two investigators with the US Geological Survey in Washington state, Drs Mary Moser and Michael Meeuwig, provided blood serum and tissues from *Lampetra tridentata,* a parasitic, anadromous species.

When sera from adults of the species we obtained were electrophoresed on polyacrylamide gels, all the sera were seen to have a predominant serum protein with approximately the same molecular mass as that of the *P. marinus* adult. The sera of *Ichthyomyzon* ammocoetes (species unknown) had a predominant protein and it had approximately the same molecular mass as that of the *P. marinus* ammocoete.
(**see Fig. 4**)



Figue 4 Four microliters of serum from each animal were electrophoresed on 10% polyacrylamide gels; gels stained with Coomassie Blue. AM: ammocoete; AD: adult, I.sp: ammocoetes of *Ichthyomyzon* not identified as *I. gagei* or *I. castaneus*; L. trid : *Lampetra tridentata*. Arrow at left indicates the location of albumin . As described in the text, our previous work showed that the bands for *P. marinus* AD and AM are different albumins.

In order to relate the albumin in the *Ichthyomyzon* species and *Lampetra tridentata* to those of *P. marinus* and *L. appendix,* we employed the crossed-immunoelectrophoresis technique (CIE) using antisera made against albumin of these two latter species to see if there was any crosssreactivity. The serum of the *Ichthyomyzon* ammocoetes and adults of both *Ichthyomyzon* species gave an immunoprecipitate with the following three antisera: one made against the AS protein , one against the LAS protein and one against the SDS-1 protein. The same result was obtained for *L. tridentata*.

These results by themselves do not tell us whether the crossreactivity is due to a single albumin species that has antigenic determinants found in the two albumins of *P. marinus* and that of *L. appendix* or whether there are two different albumins, as in *P. marinus*. In our study of *P. marinus*, we had produced monospecific antisera for both adult and ammocoete albumins and found that antiserum against the SDS-1 protein did not crossreact with the AS protein and vice-versa, suggesting two different molecules. This was confirmed by cloning of the SDS-1 gene (Gray and Doolittle, 1992) and the AS gene (Filosa et al., 1998). It is necessary to use similar procedures with the *Ichthyomyzon* ammocoete and adult albumins to see whether the albumins of these two phases are indeed different. At the present time we are preparing the appropriate antisera to examine this question. For *L. tridentata*, we have thus far had only adult specimens to work with and it is important to obtain ammocoetes for study. We hope to contact investigators who can provide these animals.

The question of whether the presence of an adult albumin is related to feeding is seen in a another light by our observations on the *Ichthyomyzon* paired species. Since the adults of non-parasitic *I. gagei*, and parasitic *I. castaneous* both live in freshwater and both have

serum albumin, it is suggested that there is no necessary association between lack of feeding and absence of albumin, as we found for the non-parasitic *L. appendix*. The difference between adult *I. gagei* and *L. appendix* with regard to albumin might represent adaptations to different challenges associated with the non-parasitic lifestyle.
Another way to look at this situation is from the perspective of evolutionary time. Being paired species, *I. castaneous* and *I. gagei* are (presumably) separated by less evolutionary time than are *L. appendix* and the other *Lampetra* species we examined, *L. tridentata*, a parasitic species that does have an adult albumin. The longer evolutionary time period between the two *Lampetra* species could have allowed for changes that resulted in adaptation to the non-parasitic lifestyle by elimination of adult albumin.

None of our studies reported here have addressed the physiological significance of lamprey albumin. Serum albumin in higher vertebrates is known to function as a carrier for hormones, fatty acids and other ligands, and also plays a role in maintaining osmotic pressure of the blood. If similar functions are handled by the lamprey albumins, the absence of albumin in the *L. appendix* adult sets it off from the other 4 species examined in our study and would indicate that in this species transport of various ligands is not essential or is carried on by a different molecule. (It is known that serum of *L. tridentata*, contains a 19 kDa non-albumin-like protein that binds palmitate (Peters and Davidson, 1991)).

Our findings with regard to lamprey albumins raise interesting questions about the regulation of the genes for these molecules. In *P. marinus*, what factors are resposible for turning off the AS gene in adults and likewise what factors enhance the activity of the SDS-1 gene after metamorphosis is underway? A complete sequence of the two genes may show differences in the promoter region that can account for regulation by transcription factors.

L. appendix adults have no albumin but do they have a gene homologous to the SDS-1 gene of *P. marinus* that is silent in the adult?. Our preliminary results with RT-PCR using primers derived from SDS-1 suggest that such a gene is present in the L. appendix genome. But more work must be done to confirm these findings. Sequencing of the entire gene would indicate whether the gene is a pseudogene and thus non-functional or whether it has a normal coding sequence but is non-functional due to a modified promoter region.

Summary of serum albumin studies

1. *P. marinus* has two types of albumin molecules, one (AS) predominant at pre-adult phases and absent in adults; the other (SDS-1) predominates at the adult phase. *L. appendix* has albumin (LAS) only in the ammocoete and most of the metamorphic stages. The adult lacks any albumin.

2. The adults of *I. gagei*, *I. castaneou*s and *L. tridentata* all have an albumin serum protein that has a molecular weight around 170 kDa, similar to that of *P. marinus* adults.

3. The albumin of *L. appendix* ammocoetes, LAS is antgenically similar to the AS albumin of *P. ma*rinus.

4. The albumins of *I. gagei*, *I. castaneous* and *L. tridentata* adults share antigenic determinants with the AS, LAS and SDS-1, as determined by crossed-immunoelectrophoresis with the antisera made against these proteins. The albumin of *Ichthyomyzon* ammocoetes also crossreacts with these antisera but the ammocoetes used were not identified as members of a particular species.

5. There is preliminary evidence that *L. appendix* has a gene for a SDS-1 type albumin. This observation may have significant implications for lamprey control, for the gene encoding the albumin protein is not expressed during the non-parasitic adult life history. If the expression of the gene is important for parasitic adult life is it possible that inhibition of its expression can be used for control of sea lampreys?

SUMMARY OF THE YEAR-3 STUDY

There was some progress made in the projects undertaken in year 3 of the research contract (hormone studies, leptin-like protein, and serum proteins). However, due to financial limitations we directed most of our resources to the project that was showing the greatest promise for immediate results towards meeting our objective. This objective was to show the utility of comparison of specific physiological/biochemical parameters between lampreys with parasitic and nonparasitic adult life histories. The serum protein study provided useful data in this context.

1. Hormone study.

We confirmed data from a previous year of the differential expression of Growth Hormone-like peptide between larval and adult lampreys. We collected more pituitaries from metamorphosing and immediately postmetamorphic adults to permit a Northern blot analysis of the expression of this peptide during this important development stage. This analysis is presently in progress.

2. Leptin-like protein

We have not been successful in cloning a cDNA of lamprey leptin. We have developed a new strategy of research that is directed towards the identification of a leptin receptor. This project was limited this past year by our lack of funds for outside sequencing of putative clones, however, we now have an in-house DNA sequencing facility and the personnel to undertake the project. An award to a postdoctoral student was made by NSERC for this project. NSERC funding has been requested for the project.

3. Serum albumin

With a new graduate student, we confirmed the results of our year 1 (and earlier) studies that the nonparastic lamprey *L. appendix* possesses a larval albumin (LAS) that it is equivalent to AS in larval sea lampreys, but there is no equivalent to sea lamprey adult albumin (SDS-1) in adult *L. appendix*. We speculated that this difference might be associated with differences in adult life histories between the two lamprey species. To test this point we sought samples of other lamprey species of different genera and subgenera and two closely related species with different adult life histories, i.e, members of a paired species. We are still analyzing the albumin profiles in *Ichthyomyzon gage*i (non-parasitic),

Ichthyomyzon castaneus (parasitic) and *Lampetra tridentata* (parasitic but a different subgenus from *L. appendix*). The broad crossreactivity of the sera of the adults of these species with the antisera against AS, LAS, and SDS-1 leads us to believe that there is a good possiblity of a differing albumin profile in their life cycles compared to sea lampreys. However, we need to know more about their specific albumin proteins. Future studies will involve isolation of the albumin proteins and production of monospecific antisera against them. We would also like to examine the primary structure of albumin from these species; the amino acid sequences can be deduced from cDNA clones.

DELIVERABLES

The third year of the 3-yr research plan did not meet all our expectations but we had highly ambitious objectives. At the outset we were asked to provide a proposal on lamprey leptin with a low cost budget. It was agreed that with the same budget we could expand into three projects with the combined funding from the GLFC and NSERC.

1. We will be delivering very shortly a published result on the profile of Growth hormone expression during metamorphosis and adult feeding of sea lampreys. This is an important hormone for lamprey growth and metabolism and knowledge of its expression at metamorphosis and during parasitic life (the rapid growth period) is very important.
2. The leptin project has not been rewarding in the past year but data from sea lampreys that we published during year 1 of the contract, still represents the only evidence of a leptin-like protein in fat tissue of a fish. We are encouraged by this result and feel confident that we have the best system in which to find a leptin receptor in fish. The fact that lamprey larvae have to be fat before entering metamorphosis cannot be ignored in sea lamprey management.
3. The comparison of physiological/biochemical profiles between parasitic and non-parasitic lamprey species is proving to be a valuable research for understanding the biology of sea lampreys. Serum albumin is showing interesting species variation that might be related to adult life history. It was a primary objective of our contract to show differences between sea lampreys and brook lampreys (namely *Lampetra appendix*). Our success has initiated a broader comparison of species that will no doubt yield data that will help us better understand the unique physiology of sea lampreys. This physiology, relative to other species, may provide a partial explanation of the successful invasion of the sea lamprey in the Great Lakes watershed. Albumin

with its multiple functions may be one of these remarkable features of sea lamprey physiology; our present data indicate that albumin profile during the life cycle of sea lampreys is unique to t he species.

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