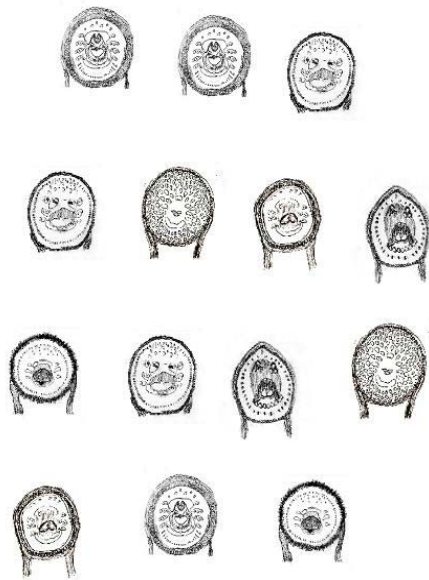


# ***The Biology of Lampreys***

SYMPOSIUM PROCEEDINGS



**Mary Moser  
Jennifer Bayer  
Don M<sup>ac</sup>Kinlay**

*International Congress on the Biology of Fish*  
*University of British Columbia, Vancouver, CANADA*



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## PREFACE

It has been thirty years since M. W. Hardisty and I. C. Potter first compiled work on the biology of lampreys. In his forward to these volumes, J. Z. Young noted that lamprey retain primitive characteristics and thereby lay claim to the interest of zoologists. He also acknowledged the wealth of study stemming from the need to understand the biology of *Petromyzon marinus* in the Great Lakes following their rapid expansion through this system. These factors continue to stimulate research on lampreys; however, current appreciation for conservation of native lampreys has generated research on a greater diversity of species. This symposium brings together researchers working on a variety of species to discuss the new information emanating from this work.

The presentations in this symposium represent research on a variety of species and many aspects of lamprey biology. Physiological and biochemical studies of lamprey physiology are juxtaposed with studies of trophic ecology and behavior to facilitate discussion of lamprey adaptations to a specialized life history. A further objective of this symposium was to stimulate discussion of similarities and differences among lamprey forms: anadromous and land-locked, parasitic and non-parasitic, indigenous and non-indigenous. Concerns about the fate of native lampreys, in addition to the need to control the sea lamprey in the Great Lakes, have highlighted the need for basic research on lamprey biology and identification of opportunities for generality among lampreys. We are fortunate in this symposium to have representatives that work on a wide variety of species and systems to allow steps in that direction.

Special thanks to Rebecca Reiche for the cover art.

Symposium Organizers:

Mary Moser  
Jennifer Bayer  
Don MacKinlay,

## **CONGRESS ACKNOWLEDGEMENTS**

This Symposium is part of the International Congress on the Biology of Fish, held at the University of British Columbia in Vancouver B.C., Canada on July 22-25, 2002.

The sponsors included:

- Fisheries and Oceans Canada (DFO)
- US Department of Agriculture
- US Geological Service
- University of British Columbia Fisheries Centre
- National Research Council Institute for Marine Biosciences
- Vancouver Aquarium Marine Science Centre

The main organizers of the Congress, on behalf of the Physiology Section of the American Fisheries Society, were Don MacKinlay of DFO (overall chair, local arrangements, program and proceedings) and Rosemary Pura of UBC Conferences and Accommodation (facility arrangements, registration and housing). Thanks to Karin Howard for assistance with Proceedings editing and word-processing; to Anne Martin for assistance with the web pages; and to Cammi MacKinlay for assistance with social events.

I would like to extend a sincere 'thank you' to the many organizers and contributors who took the time to prepare a written submission for these proceedings. Your efforts are very much appreciated.

Don MacKinlay  
Congress Chair

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**SEA LAMPREY, *Petromyzon marinus*, AMMOCOETE**

**MOVEMENTS IN THE SUBSTRATE**

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**EXTENDED ABSTRACT ONLY – DO NOT CITE**

Sea lamprey, *Petromyzon marinus*, is highly appreciated in Portugal and consequently submitted to an intense fishery during upstream reproductive migration in the rivers where natural populations still subsist.

Sea lamprey life cycle is well documented, but it is comprehensible that the knowledge about ammocoete behaviour during the burrowing phase is scarce (see Hardisty and Potter, 1971; Hardisty, 1979).

Habitat destruction (water pollution and sand extraction) is pointed out as a major factor affecting the sea lamprey populations by its effect on the ammocoete fraction of the populations.

Such threats might be specially negative for sea lamprey populations if we consider the length of the ammocoete burrowing phase. Consequently the understanding of the ammocoete behaviour during the burrowing phase is of enormous interest both in a management and in conservation perspective.

As referred by Hardisty and Potter (1971) the study of the larval behaviour is difficult except when thin section aquaria or artificial substrates are used. But even using thin section aquaria we have found that the amount of information that can be obtained by direct observation of the ammocoetes is rather reduced.

In order to obtain information on the ammocoete movement patterns during the borrowing phase we used a thin section aquarium (60x30x3 cm) with a sandy substrate very similar to the natural one (Young *et al.*, 1990).

A PIT tag was surgically implanted in the anterior part of the abdominal cavity of six ammocoetes (mean length - 12.69 cm and mean weight - 2.65 g). During two five days trials in consecutive weeks the position of each ammocoete was registered every hour. Mean temperature during the trials was 23.1 °C (22-24 °C) is similar to the registered in the river Estorãos (Lima basin), where ammocoetes were caught (Carneiro & Valente, personal communication). Water velocity in the aquarium section was 0.4 cm.s<sup>-1</sup>.

Almost all ammocoete activity (99.29 %) (Figure 1) was restricted to the upper 4.5 cm of the substrate. Even considering that the PIT tag was implanted in the anterior third of the body, this means that sea lamprey only in exceptional occasions is found deeper.

The only observed behaviour at the substrate surface was the formation of the typical conical depressions (Hardisty, 1979) that reveals the ammocoete mouth position. Such behaviour occurs during all the 24 hour period but with more intensity during the daylight period (57%).

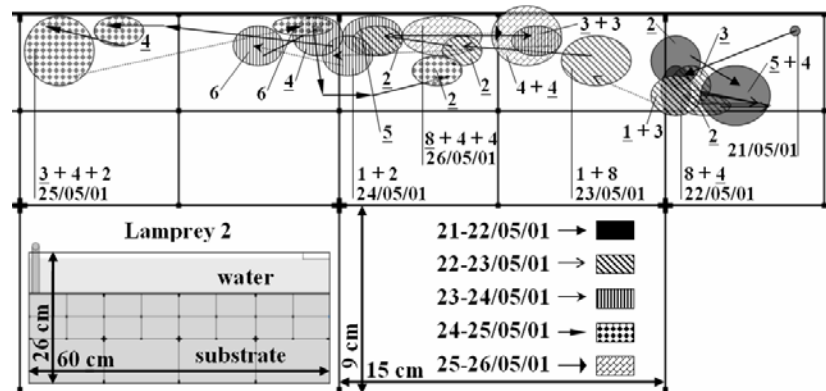


Figure 1 – Scheme with part of the frontal view of the aquarium showing the movements of one of the sea lamprey ammocoetes during a five day trial. In the left lower corner a general scheme of the aquarium. Closed areas represent sites where ammocoetes were detected in two or more consecutive occasions and represents possible detection errors. Underlined numbers indicate time (n. of hours) spent in any location during day time and the other during night time. Arrows with

continuous line represent movements during day time and arrows with discontinuous line represent movements during night.

Another important observation, which is in disagreement with the literature (Enequist, 1937 cited by Hardisty, 1979; Sterba, 1962 cited by Hardisty & Potter, 1971), is the high mobility of most of the ammocoete in this upper substrate layer. As evidenced in figure 1 most ammocoete move inside the substrate without leaving it; surface activity was a rarely observed behaviour.

The burrowing activity registered during the two trial showed that ammocoetes may move during all the 24 h period, but are more active during the illuminated period (Table 1). Only one ammocoete showed more activity during the night (ammocoete number 5 in Table 1), but this was the lamprey that exhibited less mobility during both trials.

In spite of all the work analyzing sea lamprey ammocoete behavior and preferences in the wild, none focused, as far as we know, in the burrowing behavior and movement patterns in the substrate.

Lamprey	Day	Night
1	64,44	35,56
2	55,81	44,19
3	60,00	40,00
4	60,42	39,58
5	44,83	55,17
6	58,33	41,67
Mean	58,20	41,80

Table 1 – Mean activity of each ammocoete during day and night (in percentage) during a five day trial.

Our work proves that new technologies, such as the use of PIT tags, might be used to further enlighten our knowledge on the sea lamprey ammocoete behavior.

It is evident in our results that ammocoetes use only a thin upper layer of the river bottom.

Sea lamprey ammocoetes seem to be very active in the substrate and do not assume a static or immobile feeding position in the substrate. On the

contrary the majority seem to be very active, and such activity occurs along the 24 hour period.

### **Acknowledgements**

We thank Teresa, Pedro Correia and Sá Pereira for their help during the trials.

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**THERMAL REQUIREMENTS OF EARLY LIFE HISTORY STAGES OF  
PACIFIC LAMPREYS (*LAMPETRA TRIDENTATA*) AND WESTERN  
BROOK LAMPREYS (*L. RICHARDSONI*)**

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**EXTENDED ABSTRACT ONLY – DO NOT CITE**

**Introduction**

The importance of temperature in determining the distribution, abundance, and survival of animals has been widely demonstrated. However, little is known about the specific thermal requirements of lamprey species native to the Columbia River Basin. Recent alterations in the thermal regime of the Columbia River, specifically increases in spring and summer temperatures (Quinn and Adams, 1996), have prompted interest in habitat requirements of aquatic species in the Columbia River. Understanding how temperature affects individuals is crucial to understanding the basic ecology of a species. The influence of temperature on survival during early life stages is particularly important since this period is a critical determinant of recruitment for many fish populations (Houde, 1987). Therefore, we examined the effects of temperature on development and survival of early life stage Pacific lampreys (*Lampetra tridentata*) and western brook lampreys (*L. richardsoni*).

**Materials and Methods**

Adult Pacific and western brook lampreys were collected from the wild and transported to the Columbia River Research Laboratory where they were artificially spawned. For each species, one hundred viable zygotes were placed into each of ten replicate rearing vessels per treatment (10° C, 14° C, 18° C, and 22° C). Rearing vessels were supplied with a continuous inflow of sterilized

river water at 0.05 L/min. Individuals in each rearing vessel were examined daily through embryological and larval life stages until larvae had assimilated approximately 50% of their yolk reserves. The number of live individuals and their approximate developmental stage were recorded. A temperature unit model ( $TU_a$ ) was developed to allow comparisons among treatments with animals at similar developmental stages. Analysis of variance was used to compare percent survival to 50% hatch (280  $TU_a$ ) and to 50% yolk depletion (550  $TU_a$ ) among treatments for each species and Bonferroni  $t$ -tests were used to make between treatment comparisons when overall differences were significant.

### Results and Discussion

Temperature had a significant affect on survival to 50% hatch (280  $TU_a$ ) for Pacific ( $F_{3,28}=74.10$ ,  $P<0.0001$ ) and western brook ( $F_{2,24}=66.50$ ,  $P<0.0001$ ) lampreys with significantly decreased survival at 22° C when compared to other temperatures examined for both species ( $P<0.05$ ; Figure 1).

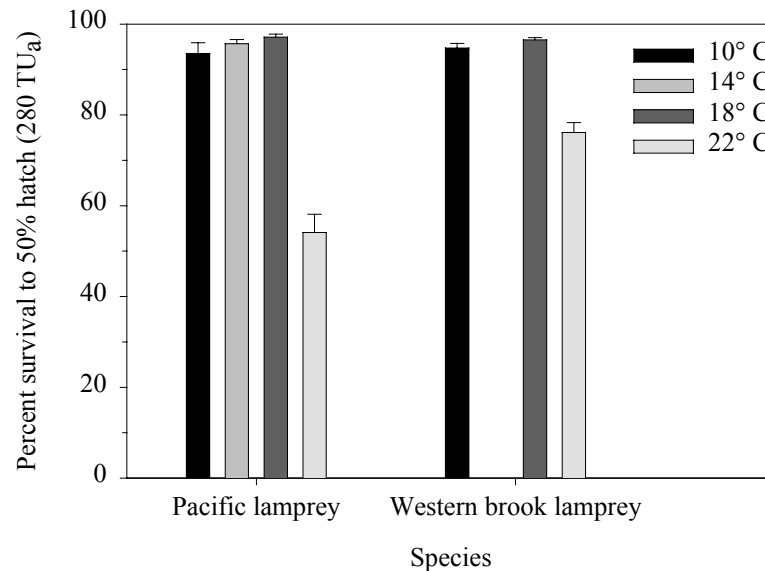


Figure 1. Mean percent survival to 50% hatch (280  $TU_a$ ) (+SE) for Pacific and western brook lampreys exposed to 10° C, 14° C, 18° C, and 22° C.

Temperature had a significant affect on survival to 50% yolk depletion (550 TU<sub>a</sub>) for Pacific ( $F_{2,21}=53.00$ ,  $P<0.0001$ ) and western brook ( $F_{2,24}=70.16$ ,  $P<0.0001$ ) lampreys with significantly decreased survival at 22° C when compared to other temperatures examined for both species ( $P<0.05$ ; Figure 2).

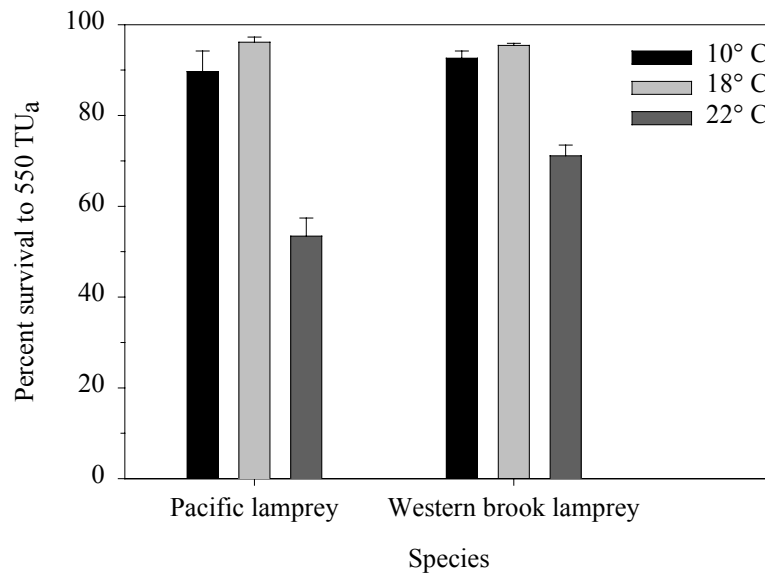


Figure 2. Mean percent survival to 50% yolk depletion (550 TU<sub>a</sub>) (+SE) for Pacific and western brook lampreys exposed to 10° C, 14° C, 18° C, and 22° C.

Lampreys held at 22° C had reduced survival during embryological development (fertilization to hatch) as compared to lampreys held at other temperatures; however, little mortality was observed from hatching until 50% yolk depletion. These data suggest thermal optima similar to that of other lamprey species (e.g. sea lamprey, *Petromyzon marinus*). Piavis (1961) and Rodriguez-Munoz et al. (2001) reported optimal survival temperatures from zygote to burrowing larva (similar to individuals at 550 TU<sub>a</sub> in this experiment) for sea lampreys to be 18.4° C and 19° C, respectively. Although similarities in optimal temperatures for survival exist among the species studied in this experiment and sea lampreys,

the range of temperatures for survival of Pacific and western brook lampreys appears to be greater than that observed for sea lampreys. Piavis (1961) observed no survival to the burrowing stage below 15.5° C or above 21.1° C and Rodriguez-Munoz et al. (2001) observed low survival from fertilization to hatching and no survival from hatching to burrowing for sea lampreys at 11° C. Results of this study indicate the sensitivity of early life stages, particularly embryological stages, to the affects of temperature and provide a means for assessing potential spawning and rearing habitats for Columbia River Basin lampreys.

### **Acknowledgements**

Funding for this project was provided by Bonneville Power Administration (Project number: 200002900). The authors wish to thank the Confederated Tribes of the Umatilla Indian Reservation, the U.S. Fish and Wildlife Service, and the U.S. Geological Survey technicians that helped with the collection of animals used in this study and resulting data.

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**DOES ADULT LIFE HISTORY ACCOUNT FOR VARIOUS  
PHYSIOLOGICAL/BIOCHEMICAL DIFFERENCES BETWEEN THE  
NONPARASITIC AMERICAN BROOK LAMPREY (*LAMPETRA*  
*APPENDIX*) AND THE PARASITIC SEA LAMPREY (*PETROMYZON*  
*MARINUS*)?**

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**EXTENDED ABSTRACT ONLY - DO NOT CITE**

The life cycle of lampreys consists of larva, juvenile, and reproductive adult periods. The larva period is comprised of a growth phase and a metamorphic phase with the latter being a time when some larval genes are suppressed and adult genes are expressed. Adult life history is species specific with juveniles of some species immediately entering an interval of feeding (parasitic species) before sexual maturation. In others, juveniles commence sexual maturation almost immediately following the completion of metamorphosis without ever feeding (nonparasitic species). Some extant parasitic and nonparasitic species show a close genetic relationship, for they are likely derivatives of the same parasitic ancestor; they are referred to as paired or satellite species. The present view is that the nonparasitic adult life history is most recent and that it may have been a consequence of natural selection for a protracted larva period (Youson, 2002a).

Juvenile sea lampreys (*Petromyzon marinus*) have parasitized native teleosts in the Great Lakes of North America and are partly responsible for the dramatic decline of sport and commercial fisheries in this watershed. As in other lamprey species, larvae of sea lampreys are filter feeders in freshwater streams and their presence in this watershed is only problematic when they complete their metamorphosis. To inhibit or to redirect the metamorphosis of larvae is one approach to sea lamprey control (Youson, 2002b). The working hypothesis of the present study is that nonparasitic lamprey species contain the clues that will

allow us to redirect sea lamprey adult life history. An investigation that compares the physiological and biochemical requirements of parasitic- with nonparasitic-adult life history could yield these clues. Since sea lampreys are not members of a paired species, the comparison was made with sea lampreys and the American brook lamprey (*Lamptera appendix*). To date, comparisons have been made of the temporal expression of the genes for the pituitary prohormone, proopiomelanocortin (POMC), and the nature and timing of appearance of a leptin-like protein and a serum protein (albumin). The data are discussed in terms of their relevance to variations in adult life history.

POMC is represented as two genes in lampreys, POC (proopiocortin) and POM (proopiomelanotropin), with the former coding primarily for adrenocorticotropin and the latter for mainly melanocyte-stimulating hormone. A 93% and 95% nucleotide identity is shared between POC and POM, respectively, of the two species (Heinig, Keeley, and Youson, unpublished data). Since the sea lamprey and *L. appendix* represent lamprey genera that diverged early, POC and POM genes have had little selective pressure during lamprey evolution. Northern blot analysis of pituitary RNA with species-specific cDNAs of both POC and POM revealed differential expression within species and between species during their life cycles. In both species, maximum levels of POC expression were observed in prespawners, but gene expression in late metamorphosis in *L. appendix* was equivalent to that in juvenile sea lampreys. In general, POM expression was higher during *L. appendix* metamorphosis than in sea lamprey metamorphosis, however, the last stage of *L. appendix* metamorphosis had equivalent POM expression to that of the juvenile sea lamprey. The data from POC and POM expression indicate that elevation coincides with the time of sexual maturation, a process that begins earlier in *L. appendix*.

Metamorphosis in sea lampreys occurs in larvae when they reach a critical size and physiological state (Youson, 2002a,b). Adequate fat stores are an essential physiological requirement and when they are attained, metamorphosis takes place. To see whether the adipose tissue yields a signal (hormone) that is a cue to metamorphosis, a search was made for the existence of a leptin-like protein in the sea lamprey using a leptin antibody (Ob Sc) and Western blotting (Yaghoubian et al., 2001). Comparisons were made with *L. appendix*. A 65 kD protein in the sera of larvae and metamorphic stages of sea lampreys was immunoreactive with an Ob Sc antibody, but sera of spawning-phase sea lampreys and both larva and adult brook lampreys were negative. These data, coupled with the fact that adipose tissue from early metamorphic sea lampreys contains a 16-17 kD protein immunoreactive to the Ob Sc antibody

(mammalian leptin is 16 kD), imply that a leptin-like protein may be involved in sea lamprey metamorphosis, but not metamorphosis of *L. appendix*.

Sea lampreys have two different albumin-like serum proteins (AS and SDS-1) during the course of their life cycle. AS is the predominant protein in larvae and metamorphosing individuals but it is eventually replaced by SDS-1 in adult life, with the latter becoming the predominant serum protein in spawning-phase adults. Larvae of *L. appendix* have a serum protein (LAS) that is antigenically similar to AS (Danis et al., 2000). Unlike AS, however, LAS disappears in the serum before the completion of metamorphosis and there is no serum protein in adult *L. appendix* that is antigenically similar to SDS-1 of adult sea lampreys. In fact, there is no major serum protein in adult *L. appendix*. Since albumins are important in most vertebrates for the maintenance of colloid osmotic pressure and the transport of various ligands, this raises questions on how the adult *L. appendix* compensates for the loss of these functions. The relationship between the absence of an albumin-like protein in adults and nontrophism during metamorphosis and adult life in *L. appendix* needs further investigation.

The present report describes differences in three physiological/biochemical parameters during the life cycle of a nonparasitic and a parasitic species. These differences may be related to differences in their adult life histories. There is a need to examine these same parameters in more closely related species, such as in a paired species that have genetic links to a common ancestor.

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**THE PHYSIOLOGY OF REPRODUCTION AND MOLECULAR  
EVOLUTION OF GnRH AND GnRH RECEPTORS IN LAMPREYS**

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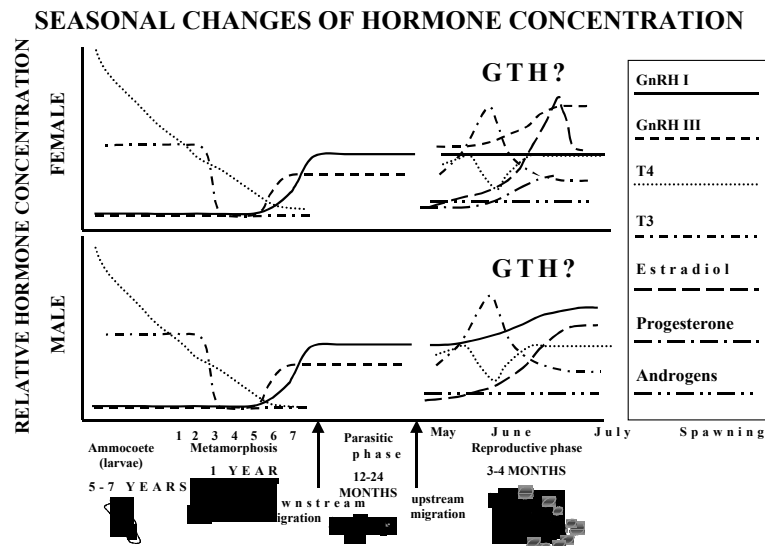
**Lamprey Reproductive Physiology in Sea Lamprey**

A key neuroendocrine function of the hypothalamus in the control of reproduction is the timed release of the decapeptide, GnRH, which acts on the pituitary to regulate the pituitary-gonadal axis for all vertebrates. Gonadotropins, secreted in response to GnRH, are released from the pituitary gland and are the major hormones influencing steroidogenesis and gametogenesis.

Until about 18 years ago, there had been little evidence for brain control of reproduction in lampreys. However, we have made substantial progress in this area (See Reviews: Sower, 1998, 2002; Sower and Kawauchi, 2001). We now have established a well-defined neuroendocrine axis in lampreys. We identified and sequenced two molecular forms of GnRH in the sea lamprey: lamprey GnRH-I and lamprey GnRH-III. In addition, we have identified the cDNA of lamprey GnRH-I (Suzuki et al., 2000) and lamprey GnRH-III (Silver et al. 2001). Lampreys are the most primitive vertebrates for which there are demonstrated functional roles for multiple GnRH neurohormones involved in pituitary-reproductive activity. Both lamprey GnRH-I and -III have been shown to induce steroidogenesis and spermiation/ovulation in adult sea lampreys. In lampreys undergoing metamorphosis, there is an increase of brain lamprey GnRH-I and -III that coincides with the acceleration of gonadal maturation (Fig 1). In immunocytochemical studies, both ir-lamprey GnRH-I and -III can be found in the cell bodies of the rostral hypothalamus and preoptic area in larval and adult sea lamprey. Most of the ir-GnRH in the brain of larval stage

lampreys has been shown to be lamprey GnRH-III. Thus, lamprey GnRH-III may be the more active form during gonadal maturation. Such information suggests that the structure and function of the GnRHs in vertebrates are highly conserved throughout vertebrate evolution.

**Fig 1** - Schematic diagram of the relative circulating hormone concentration during the life cycle of the female sea lamprey. T4, thyroxine; T3, triiodothyronine; GnRH-I, lamprey gonadotropin-releasing hormone-I; lamprey GnRH-III, lamprey gonadotropin-releasing hormone-III; E2, estradiol; and prog, progesterone.



### Molecular Cloning of the GnRH Receptor cDNA in the Sea Lamprey

Previous studies from our laboratory have shown two distinct GnRH binding sites in the pituitary of the sea lamprey. Concentration of these binding sites were shown to increase in correlation with increased gonadal maturation and brain GnRH concentration to peak near and at ovulation (Sower, 2002). GnRH's action is modulated through a 7-transmembrane G-protein coupled receptor. The question that arises is whether there are two binding sites or two different

receptors. The cDNA of one GnRH receptor has been cloned in the sea lamprey (Nucci et al., 2002). This receptor has significant identity to several other GnRH receptor cDNAs including 61.2% with the aquatic caecilian *Typhlonectes natans*, 60.7% and 59.8% with the bullfrog receptors 1 and 3, respectively, and 59% with the striped bass *Morone saxatilis*.

### **Molecular Phylogenetic Analysis Within the Petromyzontiforme Lineage Using The cDNA For Lamprey Gonadotropin Releasing Hormone-III**

In addition to our research on the reproductive hormones in the sea lampreys, we have extended these studies to isolate and sequence the cDNA precursor of lamprey GnRH-III in representative species of each of the three families of lamprey in order to assess their phylogenetic relationship. The lamprey lineage is generally considered to be divided into three families, the Petromyzonidae, or holarctic family, found in the northern hemisphere, and the two southern hemisphere families, the Geotriidae and Mordaciidae. The phylogeny of these families has been primarily based on size, shape, and distribution of dentition. The cDNA encoding prepro-lamprey GnRH-III was isolated from two holarctic species, *Petromyzon marinus* and *Lampetra tridentatus*, and one from each southern hemisphere species, *Geotria australis* and *Mordacia mordax* (Silver et al., 2001). Initial segments of the transcript were isolated using PCR on cDNA constructed from total RNA that were extracted from each respective species hypothalami. 5'RACE was used to amplify the rest of the template using the Marathon cDNA Amplification Kit (CLONTECH) and full-length transcripts were isolated. Using PAUP v4.0, the molecular phylogenetic relationship of lamprey GnRH-III between the three families of lamprey was investigated. These data suggest that the lamprey GnRH-III cDNAs of the two southern hemisphere species are highly divergent from the lamprey GnRH-III cDNA of the holarctic species.

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### **Acknowledgements**

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**ANNUAL PHYSIOLOGICAL PROFILES OF PACIFIC LAMPREYS:  
IMPLICATIONS FOR MIGRATIONS PAST DAMS ?**

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**EXTENDED ABSTRACT ONLY – DO NOT CITE**

**Introduction**

Recent research by the University of Idaho and the National Marine Fisheries Service (NMFS) indicates that a large percentage of upstream migrating adult Pacific lampreys (*Lampetra tridentata*) have difficulty negotiating fishways at Bonneville Dam, the first dam on the Columbia River. Although such poor performance can be attributed in part to design constraints in the fishways and the relatively poor swimming ability of lampreys, questions remain as to whether there may be any physiological basis for this behavior. For the third year of our research on the swimming performance and energetics of Pacific lampreys, we sampled individuals repeatedly every 3 weeks in a laboratory experiment to document some sex steroid and other physiological profiles on an annual basis. In addition, we sampled blood from lampreys migrating through fishways at Bonneville Dam to assess the possibility of an underlying physiological basis for their migration behavior.

## Methods

For our laboratory study, adult lampreys were collected from Bonneville Dam during their upstream migration and held from August, 2000 through May, 2001 in two artificial streams. Alternate groups of lampreys ( $N = 20$  per group) were sampled repeatedly for blood and measured for length, weight, and girth every 3 weeks until peak reproductive development and spawning. Plasma samples were assayed for levels of estradiol, progesterone, thyroxine, protein, triglycerides, and glucose. For our study at Bonneville Dam, we sampled lampreys that were used for radio tracking studies conducted by the NMFS. The NMFS captured fish from a trap at the dam and, prior to implanting a radio tag in them, sampled blood from each fish. We assayed the plasma for levels of the constituents mentioned above plus melatonin. During their radio tracking study, the NMFS monitored the tagged fish to determine which fish successfully passed the dam and which fish did not. We assessed the plasma constituents from these fish to explore the possibility of a physiological basis for their disparate migratory behavior.

## Results

*Laboratory study.*—We sampled fish from September 2000 through May 2001, with fish undergoing final sexual maturation during late April through May. Briefly, we observed the following morphological and physiological changes: (1) total length of male and female lampreys gradually decreased from about 60-65 cm to about 47-52 cm; (2) the weight of male lampreys decreased only during May, whereas weight in females first gradually decreased before increasing slightly in late May; (3) the maximum girth in males was generally stable, whereas in females girth increased substantially during May as the gonads developed; (4) levels of estradiol decreased in both sexes from ca. 2 ng/mL in September to ca. 1 ng/mL in March, peaked at about 2-4 ng/mL in late April, and dropped again in May, with levels consistently higher in males than in females; (5) levels of progesterone in both sexes were almost undetectable from September through early April, but then increased substantially from late April through May (peak ca. 2-4 ng/mL), with levels again higher in males than in females; (6) thyroxine levels in males more than doubled from September (0.75 ng/mL) through February (2.5 ng/mL) and decreased thereafter, whereas levels in females showed no distinct trend; and (7) levels of nutritional factors plasma protein, triglycerides, and glucose generally decreased during the fall and winter with triglycerides and glucose showing distinct peaks in late April to early May.

*Field study.*—We sampled blood from 101 females and 72 males that were captured at Bonneville Dam, implanted with a radio tag, and released below the dam. Of these fish, 47 females and 27 males passed the dam. Length and weight of females that passed the dam were significantly greater than those that did not pass. There was no difference in these metrics between males from the two groups. Plasma levels of estradiol were significantly higher in males that passed the dam compared with those that did not pass. Levels of estradiol in females that passed the dam were also higher than those that did not pass, but not significantly so. There were no differences in progesterone levels between any groups. We are currently assaying and analyzing data on thyroxine and melatonin levels.

### **Summary**

Our study has documented some physiological changes in adult Pacific lampreys during the periods of overwintering and final sexual maturation. Such basic information on their reproductive biology, along with information on the physiological status of wild fish, may help increase our understanding of Pacific lamprey life history and migratory behavior.

### **Acknowledgements**

This study was funded by U. S. Army Corps of Engineers, Portland District. We thank Mary Moser of the National Marine Fisheries Service for her assistance, Stacia Sower and personnel at the University of New Hampshire for the sex steroid assays, Dick Ewing of Biotech, Inc. for the thyroxine and melatonin assays, and personnel at the Columbia River Research Laboratory for their excellent help in the laboratory.



**STEROIDS PRODUCED BY PREGNENOLONE METABOLISM,  
IN VITRO, BY ADULT OVARY AND TESTIS OF SEA LAMPREYS**

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**EXTENDED ABSTRACT ONLY - DO NOT CITE**

**Introduction**

In comparison with other vertebrate classes, the reproductive endocrinology of lampreys is poorly understood. This study is part of an ongoing program to develop a better understanding of the sex steroids of lampreys, an essential step in furthering knowledge of the basic biology of these species, and developing technologies for reproductive control of lamprey populations in regions where they impact on commercial fish species, such as the Great Lakes of North America.

Low levels of estrogens, androgens and progestogens have been measured by RIA in the blood of several lamprey species, either during natural reproductive cycles, or following experimental manipulation (e.g., Fukayama and Takahashi, 1985; Sower and Larsen, 1991). However, hormone changes with season, or following experimental intervention have not been fully consistent with these hormones playing a major role in reproduction. The question remains as to whether the “classical” steroids are the principal factors regulating lamprey and hagfish reproduction; indeed, some evidence suggests that 15 $\alpha$ -hydroxylated (15 $\alpha$ -OH) steroid derivatives may be the major factors.

We used HPLC to separate the steroids synthesized from the metabolism of tritiated pregnenolone (7 - [<sup>3</sup>H]P<sub>5</sub>) to investigate the steroidogenic pathways present in vitellogenic ovarian follicles and mature testis fragments of sea lampreys, *Petromyzon marinus*; we also investigated the ability of the gonadal tissues to conjugate steroid metabolites, and examined serum steroid profiles.

### Materials and Methods

The in vitro methods, extraction and HPLC methods used for the study have been described previously (Reddy et al., 1999). Tissues were incubated for 6 or 18 hours at 10°C in the presence 19 nmol ml<sup>-1</sup> of (7 - [<sup>3</sup>H]P<sub>5</sub>) (0.73 Tbq mmol<sup>-1</sup>). Elution times of steroids from two solvent gradients were compared with 25 authentic steroids, including the 15 $\alpha$ -OH forms of 17 $\beta$ -estradiol (E<sub>2</sub>), estrone (E<sub>1</sub>), testosterone (T), progesterone (P<sub>4</sub>) and androstenedione (A<sub>4</sub>).

### Results and Discussion

#### Serum

The major HPLC peaks for males and females were extremely polar compounds, eluting between 2.0 and 7.0 minutes, consistent with 15 $\alpha$ -OHT for males, and 15 $\alpha$ -OHT and 15 $\alpha$ -OHE<sub>2</sub> for females; a smaller peak, possibly dihydroepiandrosterone (DHEA), was seen for both sexes. Peaks consistent with the presence of T, E<sub>2</sub> or E<sub>1</sub> were not seen.

#### Incubation medium (Table 1)

For both testis and ovary preparations, the conversion of the P<sub>5</sub> precursor was progressive, resulting in the formation of polar metabolites (free and conjugated), probably 15 $\alpha$ -OH steroids, together with a few minor peaks. There was no evidence

of T formation by either testis or ovary tissue, but traces of free and conjugated E<sub>2</sub> were produced by ovary preparations.

Table 1. Radioactive Free and Conjugated Steroid Peaks Produced Following the Incubation of Testis and Ovarian Fragments with [<sup>3</sup>H]P<sub>5</sub>

	Incubation: 6 hours		Incubation: 18 hours	
	Free	Conjugated	Free	Conjugated
<b>Testis</b>				
[P <sub>5</sub> ]	**	** (S/G)	nf	** (S/G)
15 $\alpha$ -OHP <sub>4</sub>	**	tr (S)	**	nf
norT	tr	tr (S)	nf	tr (S)
Polar 15 $\alpha$ -OH steroids <sup>#</sup>	tr	tr (G)	**	** (S/G)
DHEA	tr	tr (S)	nf	nf
Unk (34 min)	tr	** (S)	nf	nf
Unk (18 min)	nf	nf	nf	tr (S)
<b>Ovary</b>				
[P <sub>5</sub> ]	**	** (S/G)	nf	nf
Polar 15 $\alpha$ -OH steroids <sup>#</sup>	**	tr (S/G)	**	** (S/G)
15 $\alpha$ -OHP <sub>4</sub>	+	+ (S)	nf	nf
DHEA	+	+ (S/G)	nf	tr (S)
E <sub>2</sub>	tr	tr (S/G)	nf	+ (S/G)
norT	tr	tr (S/G)	nf	+ (S)
<i>Abbreviations:</i> A <sub>4</sub> - Androstenedione; E <sub>2</sub> - 17 $\beta$ -Estradiol; DHEA - Dihydroepiandrosterone; 15 $\alpha$ -OHP <sub>4</sub> - 15 $\alpha$ -Hydroxyprogesterone; norT - Nor-testosterone; P <sub>5</sub> - Pregnenolone; Unk - Unknown peak (elution time); G - Glucuronide conjugate; S - Sulfate conjugate; ** Major steroid peak(s); + Present as distinct peak; tr - Trace only; nf - Not found; <sup>#</sup> See text for explanation.				

After 18 hours incubation, the very polar steroid peaks had run together, but the 6 hour incubation provided a good separation of the peaks: only one 15 $\alpha$ -OH steroids peak was found for the testis (consistent with 15 $\alpha$ -OHP<sub>4</sub>), and three the ovary

(consistent with 15 $\alpha$ -OHE<sub>1</sub>, 15 $\alpha$ -OHE<sub>2</sub>, and 15 $\alpha$ -OHP<sub>4</sub>). These findings suggest that the principal androgen is likely to be 15 $\alpha$ -OHT (consistent with previous reports by Kime and Rafter (1981); Kime and Callard (1982)), and the principal estrogens are probably 15 $\alpha$ -OHE<sub>2</sub> and 15 $\alpha$ -OHE<sub>1</sub>.

We could not differentiate between P<sub>5</sub> and P<sub>4</sub> using our system, but we do have indirect evidence (not reported here) of significant P<sub>4</sub> formation from [<sup>3</sup>H]P<sub>5</sub>. How much (if any) P<sub>4</sub> enters the peripheral circulation is still not known.

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**15 $\alpha$ -HYDROXYLATED STEROIDS PRODUCED *IN VITRO* AND *IN VIVO* IN THE SEA LAMPREY, *PETROMYZON MARINUS***

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**EXTENDED ABSTRACT ONLY – DO NOT CITE**

The sea lamprey (*Petromyzon marinus*) is an useful model species in studies of comparative and evolutionary reproductive endocrinology. As a member of superclass Agnatha, the lamprey is one of the earliest evolving vertebrates still alive today. Considerable research has been performed to establish that, similar to teleosts and higher vertebrates, the sea lamprey has a hypothalamus-pituitary-gonadal axis that controls reproduction. Previous research has indicated that sea lamprey may use unusual steroid hormones that are the result of a specific pathway. These studies demonstrated that the major products of exogenous androsteindione in sea lamprey testes are 15 $\alpha$ -hydroxyandrosteinedione and

15 $\alpha$ -hydroxytestosterone (15 $\alpha$ -T). Another study established that the ovaries and testes of another lamprey species, *Lampetra fluviatilis*, produce 15 $\alpha$ -hydroxyprogesterone (15 $\alpha$ -P) and 15 $\beta$ -hydroxytestosterone (15 $\beta$ -T), respectively.

Our research was conducted to confirm that 15 $\alpha$ -testosterone is produced in sea lamprey testes, and to determine if it is circulated in the plasma and used as a functional hormone. We have demonstrated that 15 $\alpha$ -hydroxytestosterone is produced by sea lamprey testes through incubation experiments using tritiated testosterone and subsequent analysis using HPLC and TLC. Additionally, we have confirmed the presence of 15 $\alpha$ -T in plasma by developing enzyme-linked immunosorbent assays (ELISAs) for both 15 $\alpha$ -T and 15 $\beta$ -T to measure small amounts of these steroids, and found only 15 $\alpha$ -T to be present. To further substantiate these results, sea lamprey plasma was extracted, fractionated via HPLC, and ELISAs were performed on the fractions. 15 $\alpha$ -T was present only in the expected fraction, and 15 $\beta$ -T was not detected. Plasma was collected and assayed from sea lamprey at several life stages, and a trend toward elevated 15 $\alpha$ -T levels were found only in fully mature spermiating males. Thus, there is chemical and immunoreactive evidence that 15 $\alpha$ -T is produced both *in vitro* and *in vivo*. Additional experiments are being performed to further elucidate the role of 15 $\alpha$ -T in sea lamprey reproduction, and a project similar to the current one has been initiated to study the occurrence of 15 $\alpha$ -hydroxyprogesterone in sea lamprey.

This work is supported by the Great Lakes Fishery Commission.

**OXYGEN CONSUMPTION OF ADULT PACIFIC LAMPREYS**  
**(*LAMPETRA TRIDENTATA*) IN**  
**RELATION TO SWIMMING SPEED AND TEMPERATURE**

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**EXTENDED ABSTRACT ONLY – DO NOT CITE**

**Introduction**

Pacific lamprey (*Lampetra tridentata*) populations in the Columbia River Basin (CRB) are believed to be in decline. One factor potentially limiting lamprey production is the amount of energy they expend negotiating upstream passage facilities at dams. Results provided by the National Marine Fishery Service and the University of Idaho suggest that Pacific lampreys have difficulty negotiating the fishways at Bonneville Dam. To compliment this work, we conducted laboratory studies to determine swimming performance and the metabolic costs of activity in Pacific lampreys. We estimated resting and active rates of oxygen consumption ( $\dot{V}O_2$ ) for lampreys swimming at defined speeds at 10, 15, 20, and 23°C. Our results provide a foundation towards estimating metabolic costs associated with swimming for Pacific lampreys.

**Materials and Methods**

Adult Pacific lampreys were collected from the wild and held at the Columbia River Research Laboratory. Oxygen consumption during forced activity was measured on individual lamprey in a Blazka respirometer at four water temperatures (10, 15, 20, and 23  $\pm$  1°C). Each trial consisted of a progression of steps where water velocity increased from acclimation (0.25 BL·s<sup>-1</sup>) to 0.7 BL·s<sup>-1</sup> and by 10 cm s<sup>-1</sup> each increment thereafter. For each increment, the respirometer was sealed and oxygen concentration measured every 15 s.

Oxygen consumption rate ( $\dot{V}O_2$ ; mg O<sub>2</sub> kg<sup>-1</sup> h<sup>-1</sup>) for an individual at each speed was determined using the following equation:  $\dot{V}O_2 = A \times V \times \frac{1}{W}$  where V was the

volume of the respirometer (L); W was the weight of the fish (kg); and A was the amount of oxygen consumed (mg L<sup>-1</sup> O<sub>2</sub>) during the velocity increment as determined by linear regression analysis of the reduction in O<sub>2</sub> over time (t):  $A = b_0 + (t \times b_1)$ . Regression was used to determine the best-fit model for predicting  $\dot{V}O_2$  as a function of swim speed. The effects of temperature and swim speed on oxygen consumption were evaluated using a multivariate ANOVA model predicting oxygen consumption as a function of swim speed, temperature, and interactions between these parameters.

## Results and Discussion

For all temperatures, we documented a similar pattern of increase in  $\dot{V}O_2$  with increasing swim speeds up to approximately 90 cm/s, followed by a decline in  $\dot{V}O_2$  at higher swim speeds. Oxygen consumption increased in a curvilinear fashion with swimming speed at each temperature (Figure 1). Although the relation between swimming speed and  $\dot{V}O_2$  was similar at each temperature,  $\dot{V}O_2$  at each speed was generally higher at 20°C than at the lower temperatures (Table 1). It was difficult to achieve sustained swimming at 23°C and these data are more variable than other temperatures. Oxygen consumption decreased and variability in  $\dot{V}O_2$  tended to be higher at the fastest speeds. Our estimates of active  $\dot{V}O_2$  of adult Pacific lampreys are the first reported for this species and represent estimates of metabolic costs over a range of activity and temperatures. Although active  $\dot{V}O_2$  has been determined for many teleosts, we found reference to only one study that examined this in lampreys. Beamish (1974) reported a mean active  $\dot{V}O_2$  of 475.5 mg O<sub>2</sub> kg<sup>-1</sup> h<sup>-1</sup> for adult sea lampreys (*Petromyzon marinus*) swum at 30-40 cm s<sup>-1</sup> at 10°C, which is lower than the mean  $\dot{V}O_2$  we determined for Pacific lampreys under similar conditions. The relatively high  $\dot{V}O_2$  from our lampreys may be partly due to the forced nature of our swim tests. In nature, lampreys use a combination of holdfasting and swimming during their upstream migration to help them conserve energy. Because our data indicate that swimming in lampreys is energetically quite costly, particularly at high water velocities, a greater understanding of lamprey bioenergetics in the wild is necessary before the energetic costs of migration can be evaluated as a factor in the decline of Pacific lampreys in the CRB.

Figure 1.

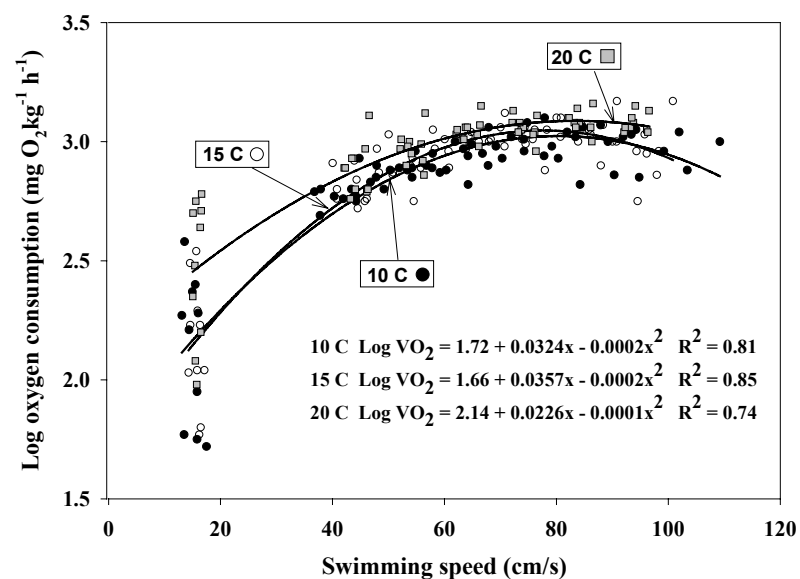


Table 1.

Mean active oxygen consumption in $\text{mg O}_2 \text{ kg}^{-1} \text{ h}^{-1}$ (SD)								
TEMP (C)	SWIM SPEED (CM/S)							
	Rest	13-17	40-45	50-55	60-65	70-75	80-85	90-95
10	34 (6)	166 (34)	634 (46)	783 (29)	866 (54)	1023 (52)	994 (80)	939 (91)
15	66 (9)	164 (31)	681 (52)	794 (69)	966 (40)	1098 (56)	1153 (44)	993 (148)
20	98 (27)	353 (61)	747 (47)	935 (34)	1090 (25)	1181 (36)	1236 (31)	1219 (47)
23	124 (54)	165 (84)	736 (68)	843 (132)	942 (106)	791 (N=1)	NA	NA

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**THE RENIN-ANGIOTENSIN SYSTEM AND VOLUME  
REGULATION IN LAMPREYS**

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**EXTENDED ABSTRACT ONLY- DO NOT CITE**

Some species of lampreys are anadromous migrants between (FW) and seawater (SW) and adjust ionic and osmoregulatory mechanisms in a similar way to teleost fish (Rankin, 1997). Hyper-osmoregulation in FW demands excretion of large volumes of urine, whilst hypo-osmotic regulation in SW depends on increasing drinking and drastically reducing urine output. Endocrine control of body fluid volume and composition in lampreys is poorly understood and existence of a lamprey renin-angiotensin system (RAS) has until recently been in doubt (Henderson *et al.*, 1993). This endocrine system plays a key role in regulation of cardiovascular function and blood volume homeostasis in many vertebrates and is involved in survival of teleost fish in high external salinities.

Recent isolation and sequencing of endogenous angiotensin I (Ang I) in *Lampetra fluviatilis* and *Petromyzon marinus* (Rankin, Watanabe, Nakajima and Takei, unpublished) led to our measurement of the physiologically active angiotensins (angiotensin II and III: Ang II & III) (Rankin *et al* 2001). In these studies, *Lampetra fluviatilis* showed higher levels angiotensins in SW than in FW. Our more recent studies have aimed to investigate the activation of the RAS and its possible role in regulation of renal function.

Adult lampreys (*Lampetra fluviatilis* L.) were caught in Ringkøbing Fjord, Denmark at the start of their migration into FW and held either in FW or

gradually-acclimated to hyperosmotic environments (Brown and Rankin, 1999) where they were held for ~ 3-5 weeks.

### **Investigation of the activation of the renin-angiotensin system**

A range of approaches were employed including blood volume depletion of lampreys acclimated to 21ppt (~40% blood volume removal), isotonic volume loading of FW-acclimated lampreys (1% body weight ip), salt loading of FW-acclimated lampreys (4M NaCl, 1% body weight ip,) and rapid changes in environmental salinity (FW to 605 mOsm/kg; 758 mOsm/kg to FW). Circulating angiotensins were determined by radioimmunoassay after collection of blood samples from the caudal vasculature of MS222-anaesthetised fish.

Measurement of circulating angiotensins showed a rapid activation of the RAS after volume depletion (Fig 1). In agreement with the activation of the RAS by hypovolaemia (or the resultant hypotension), ip injection of isotonic saline (1% body wt) resulted in rapid decline of plasma angiotensins (within 15 min post-injection,  $P<0.01$ ) followed by restoration to control levels 30 and 60 min post-injection. However, regulation of the RAS appears to involve interaction of volume receptors and osmoreceptors since injection of hypertonic saline, (again at 1% body wt) which raised plasma osmolality compared to injection of isotonic saline within 15 min ( $P<0.01$ ), did not affect plasma angiotensins.

Transfer of lampreys from 70% SW to FW which significantly lowered plasma osmolality and might be predicted to cause acute volume expansion was associated with a significant decline in plasma angiotensins ( $P<0.05$ ) after 24h, although plasma angiotensins returned to a concentration not dissimilar from those in 70% SW after 7 days. After acute transfer from FW to 60% SW, the significant rise in plasma osmolality and predicted volume depletion was associated with rising plasma angiotensins. These results imply that volume receptors exert dominant control of the RAS

### **Renal Effects of Angiotensin II infusion**

The renal effects of [Asp<sup>1</sup> Val<sup>5</sup>]Ang II were determined by intravenous infusion of anaesthetised *Lampetra fluviatilis* with  $10^{-10}$  or  $10^{-9}$  moles min<sup>-1</sup> kg body wt<sup>-1</sup> (n=4 and 5 respectively) and serial collection of urine samples



(as described by Brown & Rankin, 1999). Both doses of Ang II led to a decline in urine flow rate with a significant decline in urine output after 40 min of  $10^{-9}$  moles  $\text{min}^{-1}$   $\text{kg body}^{-1}$  (Fig 2). Preliminary studies also indicated that Asn<sup>1</sup>-Val<sup>5</sup>-Ang II at 1 to  $1.6 \times 10^{-9}$  moles  $\text{min}^{-1}$   $\text{kg body wt}^{-1}$  exerts an antidiuretic action in the sea lamprey, *Petromyzon marinus*.

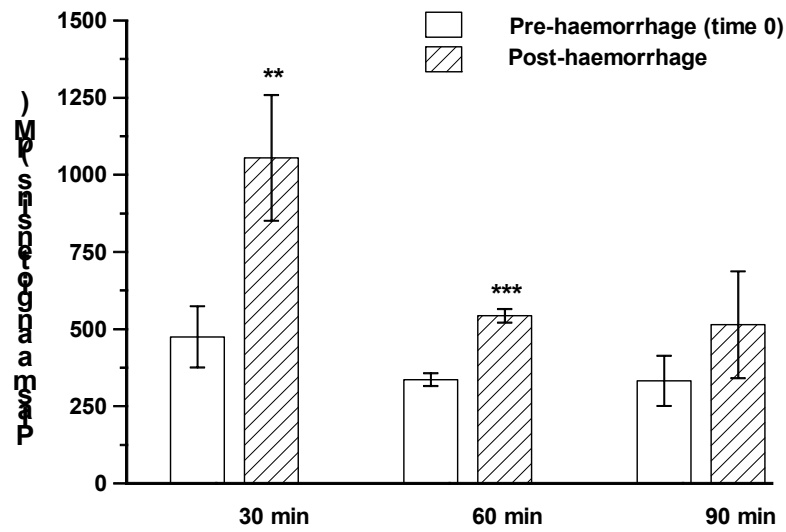


Fig 1. Plasma angiotensins (pM) in lampreys acclimated to 21ppt, 576 mOsm  $\text{kg}^{-1}$  prior to a haemorrhage and 30min (n=10), 60min (n=11) or 90 min (n=7) after removal of 40% blood volume. Data are means  $\pm$  SE; \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$ , paired t tests.

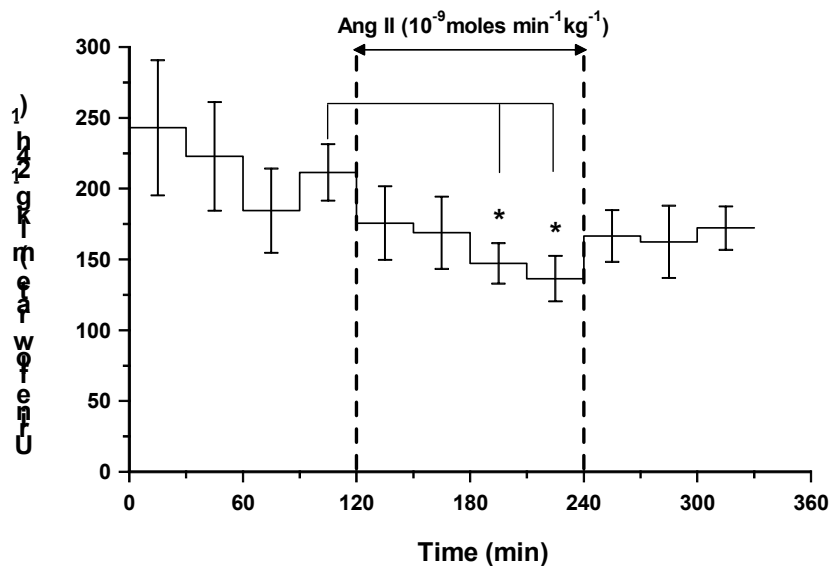


Figure 2. Effect of Ang II on urine flow rate of the river lamprey, *Lampetra fluviatilis*, (n=5) infused with saline via the posterior cardinal vein ( $2\mu\text{l min}^{-1}\text{ kg body wt}^{-1}$ ) for 2h control period, saline containing Ang II at  $10^{-9}\text{ moles min}^{-1}\text{ kg body wt}^{-1}$  for 2h and then returned to a saline infusion.

In summary, the newly-discovered renin-angiotensin system of the river lamprey appears to be activated by volume receptors. This is in keeping with the vasoconstrictor action and antidiuretic actions of Ang II.

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### **Acknowledgments**

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## EFFECTS OF LIGHT ON MIGRATING ADULT PACIFIC LAMPREY

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### EXTENDED ABSTRACT ONLY – DO NOT CITE

#### Introduction

Abundance of anadromous Pacific lamprey (*Lampetra tridentata*) in the Columbia River drainage has declined dramatically in the past two decades (Close, 2001). Hydropower dams on the lower Columbia River may have contributed to this decline by preventing some upstream migrating adults from reaching historical spawning grounds. Recent radiotelemetry data indicate that adult Pacific lamprey have relatively poor passage efficiency at the lower Columbia River hydropower dams (Moser et al., In Press). At Bonneville Dam (the first dam they encounter at Rkm 235) adult lamprey were delayed and/or obstructed at specific parts of the fishways, including fish count stations (Moser et al., In Press). Unlike adult salmonids, adult lamprey are most active at night and some species exhibit negative phototaxis (Ullen et al., 1997). We tested the hypothesis that bright lighting at the fishway count stations elicits an avoidance response in migrating adult Pacific lamprey.

#### Methods

We trapped lamprey from a fishway at Bonneville Dam (Figure 1). Uniquely-coded transmitters were surgically implanted in the body cavity of selected fish following the methods of Moser et al. (In Press). The radio-tagged lamprey were released below the dam and their approach to and progress through the two count stations (Figure 1) was monitored via an array of underwater antennas.

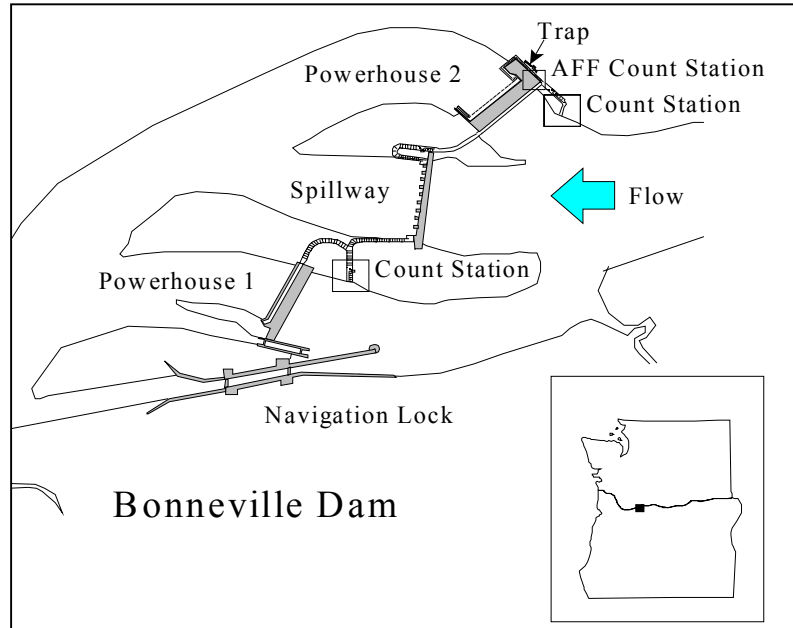


Figure 1. Study area at Bonneville Dam and locations of the count stations, experimental count station at the Adult Fish Facility (AFF), and lamprey trap.

We also conducted controlled experiments of lamprey responses to lighting in a  $1.2 \text{ m} \times 13.4 \text{ m} \times 3 \text{ m}$  chamber with flowing water ( $0.8 \text{ m s}^{-1}$ ). The chamber was partitioned along its axis so that lamprey could choose to move up either a brightly lighted side (simulated count station lighting) or a dark side. For each test, we introduced 10 lamprey into the downstream end of the chamber and recorded the number that had passed through each side after 1 hr. Each group of fish was tested in during day and night.

We also manipulated lighting at an experimental count station in the fishway to test the effects of white lighting (simulated count station lighting), red lighting, and no lighting on catch per unit effort (CPUE) at a trap located approximately 20 m upstream from the light treatment. On consecutive nights we alternated the dark treatment with each of the two light treatments and compared CPUE between treatments using a paired t-test (Zar, 1984).

## Results

We radio tagged 299 lamprey in 2000 and 298 in 2001. Of these fish, 141 in 2000 and 147 in 2001 entered Bonneville Dam fishways and approached the count station areas at the top of the fish ladders. In 2000, 106 (75%) of these fish passed the brightly lit count window, negotiated the serpentine weir section, and exited out the top of the ladder and into the forebay of the dam (Figure 2). Most of the remaining fish either passed into the makeup water channel adjacent to the count station or fell back downstream after entering the serpentine weir section (Figure 2). Only 5 fish did not pass upstream from the count window. Similarly, 77% of the lamprey passed successfully through the count station in 2001, but only 2 did not move upstream from the count window (Figure 2).

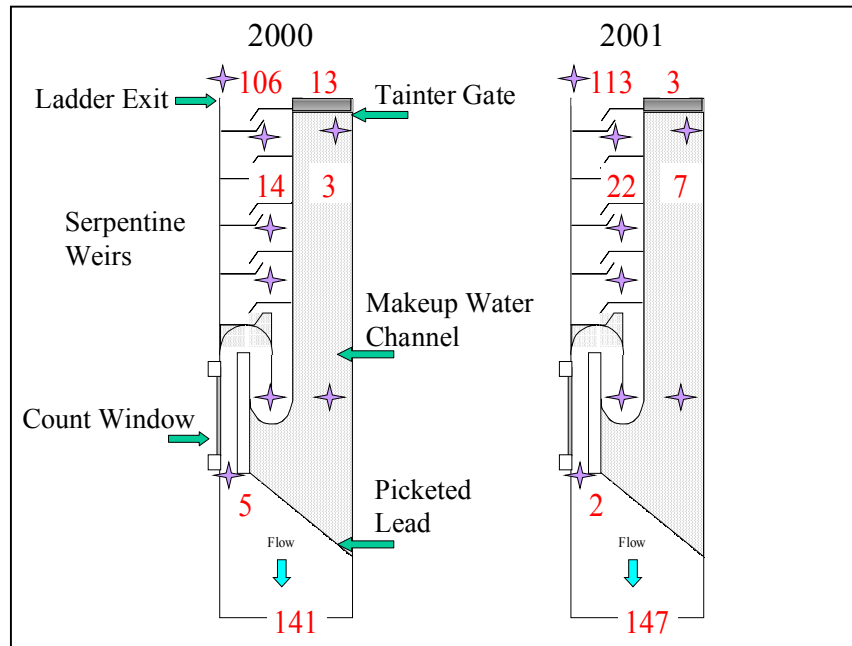


Figure 2. Count stations at Bonneville Dam and the number of lamprey that failed to pass through each area in 2000 and 2001. Stars indicate antenna sites.

We conducted 7 replicates of the laboratory experiments. Lamprey did not avoid the lighted side of the chamber during either day or night ( $P > 0.05$ ). Moreover, there was no significant difference in mean CPUE (lamprey night<sup>-1</sup>) during white light

(5.2) or red light (4.6) treatments and dark (5.0) treatments in the fishway ( $P > 0.05$ ).

### **Discussion**

Radiotelemetry and laboratory studies indicated that adult Pacific lamprey do not avoid the lighting (1 – 3 lux) at Bonneville Dam count stations. Experiments in the fishway further indicated that light quality had no effect on lamprey passage. We recommend future investigation of adult lamprey passage through the serpentine weir sections of the fishways, as radiotelemetry indicated that more lamprey were obstructed in this area than by the brightly-lit count windows.

### **Acknowledgements**

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**DIRECT EVIDENCE THAT 7 $\alpha$ ,12 $\alpha$ ,24-TRIHYDORXY-5 $\alpha$ -CHOLAN-3-  
ONE 24-SULFATE FUNCTIONS AS A MALE ATTRACTANT IN THE  
SEA LAMPREY *PETROMYZON MARINUS***

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**EXTENDED ABSTRACT ONLY – DO NOT CITE**

The sea lamprey (*Petromyzon marinus*) is a member of superclass Agnatha, and one of the earliest evolving vertebrates still alive today. Previous research has established that sexually mature sea lampreys rely on sex pheromones to communicate among individuals of opposite sex. Recently we have found that male sea lampreys release a potent sex pheromone, 7 $\alpha$ ,12 $\alpha$ ,24-trihydroxy-5 $\alpha$ -cholan-3-one 24-sulfate (3 keto-petromyzonol sulfate; 3KPZS) upon spermiation. This compound appears to induce preference behavior and locomotion in ovulating females under controlled laboratory conditions. It has yet to be demonstrated that a synthetic copy of 3 KPZS stimulates the olfactory organ and induces characteristic behaviors in ovulating females. To provide direct evidence that synthetic 3-keto petromyzonol sulfate functions as a sex pheromone in a natural spawning environment, we first used electro-olfactograms (EOG) to determine the detection thresholds and dose response curves of the synthetic pheromone. Then in a section of spawning stream we observed the behavioral response of ovulating females to the synthetic pheromone (at concentrations determined by EOG experiments). EOG results showed that the synthetic pheromone is detected at approximately 10<sup>-12</sup> M. When introduced into the spawning stream section, ovulating females swam to and stayed at the source of the synthetic pheromone. We conclude that synthetic

3 keto-petromyzonol sulfate functions as a sex pheromone in a natural environment at concentrations that are likely to be encountered in the wild.

The Great Lakes Fishery Commission supported this study.

**DYNAMICS OF PHEROMONE-BASED STREAM FINDING BY  
MIGRATORY ADULT SEA LAMPREYS**

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**EXTENDED ABSTRACT ONLY – DO NOT CITE**

The sea lamprey (*Petromyzon marinus*), an anadromous parasitic fish that is now landlocked in the Great Lakes, must locate spawning streams after maturing and being transported by hosts into unfamiliar areas. We have found that unlike salmon that recognize the odor of their natal streams, migratory adult sea lampreys select streams with appropriate habitat for spawning using an innately recognized pheromone released by stream-resident larvae. Because this pheromone is extremely potent, it has potential to be used for sea lamprey control in the Great Lakes and in the rehabilitation of threatened lamprey populations elsewhere. Herein, we review evidence for this pheromone, focusing on recent studies which demonstrate the importance of the pheromone for stream selection and an ongoing telemetry study which is demonstrating how sea lamprey locate pheromone plumes in lakes.

Initial indications that sea lamprey might employ a pheromone to locate spawning streams arose from observations that the number of migratory adults

entering tributaries often dramatically declines if larvae are removed by lampricide treatment. Strong supporting evidence comes from our recent laboratory experiments which have used a two-choice maze to assess the preferences of adult lampreys offered different stream waters and larval odor (Vrieze and Sorensen, 2001). We find that lampreys are strongly attracted into stream water from lake water at very low, relevant concentrations (1,000 time dilutions) and that larval odor alone is extremely attractive: a single larva activates over 4,000L of water in 12 hours. Further, water from streams that contain larval lamprey are more attractive than those that do not, and this preference can be reversed by adding larval odor to the previously less-attractive stream water. Preferences are innate because animals do not show any inherent preference for waters collected from streams they were caught entering.

A variety of evidence demonstrates that the migratory pheromone is comprised of a unique bile acid, petromyzonol sulfate (PS), and at least one other as yet unknown compound released by larval sea lamprey. As measured by electrophysiological recording, PS is a potent and specific odorant with a sub-picomolar detection threshold (Li et al., 1995). Picomolar concentrations of PS have been measured in stream waters using mass spectrometry. Further, PS possesses behavioral activity (Bjerselius et al., 2000), albeit less than unpurified larval odor (Vrieze and Sorensen, 2001). Larval *Lampetra* and *Ichthyomyzon* also release PS and are attractive to adult sea lampreys, suggesting that the pheromone may not be specific to sea lamprey (Sorensen and Vrieze, in press).

Recently we have examined how migratory sea lampreys use pheromone plumes to locate spawning streams. In one study we tested the role of olfaction in stream-finding by occluding the nasopores of migratory lamprey with inert plastic or rinsing them with gelatin as a control. Multiple releases of these animals outside three different rivers emptying into Lake Huron consistently demonstrated that lampreys with functional olfactory systems locate streams five to ten times more successfully than lampreys with occluded olfactory systems. For example, in 1997, 585 lampreys were released 3.6 km from the mouth of the Cheboygan River, and while only 4% of the occluded lampreys were later caught entering the river, 47% of the control animals were.

In another ongoing study we are using telemetry to describe the behavior of migratory sea lampreys in the Great Lakes as they search for, and then orient within river plumes. We are especially interested in vertical movement patterns within stratified stream waters, the possibility that lampreys might follow shorelines, and lamprey daily activity cycles. To date, over 40 lampreys have

been implanted with acoustic tags (some of which transmit depth and temperature) and released into Lake Huron outside a river that contains many larvae. Animal location is determined from a GPS-equipped boat while the concentration and location of river water is monitored by measuring water conductivity. Initial results suggest that lampreys move only at night, but then very actively. They frequently exhibit high swimming speeds (median speed of 1.6 km/h ranging to over 3 km/h) while swimming in surprisingly straight courses that appear to reflect an orientation into local currents (Fig. 1). Upon approaching the shoreline, they generally follow it. In addition, we find that lacustrine sea lampreys exhibit extensive vertical movements throughout the water column (Fig. 2). This pattern of swimming is strikingly similar to that observed in migratory salmon (Doving et al., 1985) and may have evolved to maximize the animals' chances of encountering odorous river waters. As predicted from this search strategy, we find that relatively more sea lampreys are able to find our study river in early spring when its plume is colder and thicker. Together these data suggest that for the purposes of lamprey control, the migratory pheromone should be added at night and into rivers with thick plumes. (Funded by the Great Lakes Fisheries Commission)

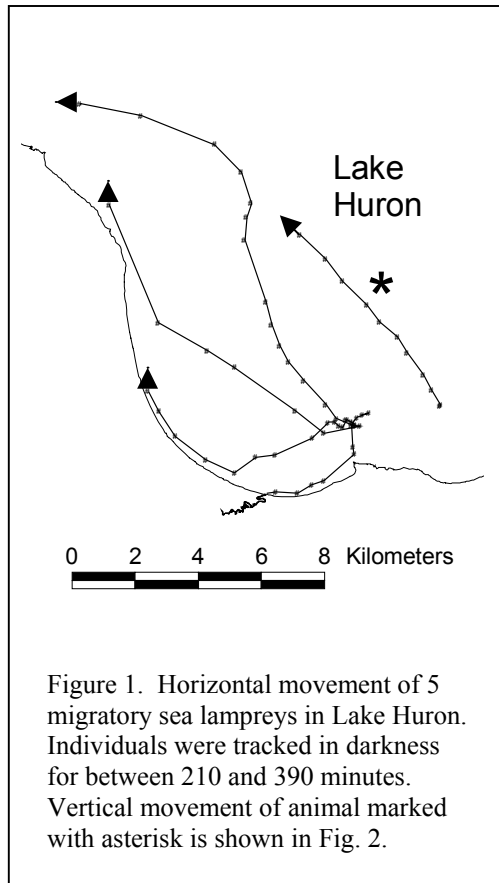
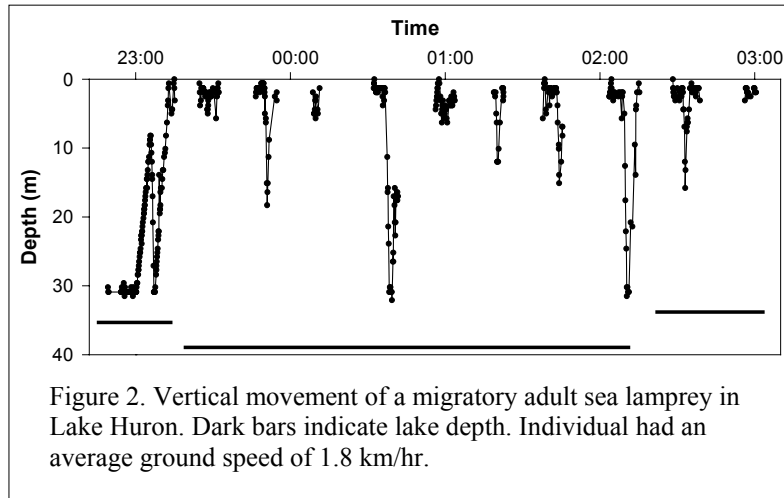


Figure 1. Horizontal movement of 5 migratory sea lampreys in Lake Huron. Individuals were tracked in darkness for between 210 and 390 minutes. Vertical movement of animal marked with asterisk is shown in Fig. 2.



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**THE ANADROMOUS SEA LAMPREY IN PORTUGAL:  
BIOLOGY AND CONSERVATION PERSPECTIVES**

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**Abstract**

The sea lamprey is a species with high economic value in Portugal. During the spawning migration they are most active at night, and attain a ground speed that varies between 0.84 km h<sup>-1</sup> and 1.5 km h<sup>-1</sup>. The duration of the larval stage in Portuguese rivers is between four and nine years and metamorphosis occurs from autumn to winter, with a peak in October-November. Larval distribution is strongly dependent upon sediment, especially particle size composition. Smaller individuals (20 mm < TL ≤ 60 mm) are commonly found on silty sand bottoms. Ammocoetes with a total length of 60 mm to 140 mm prefer a more heterogeneous substrate, where gravel and silt seem to make an identical contribution to sediment composition (gravel-silty-sand). The larger ammocoetes (140 mm < TL ≤ 200 mm) clearly prefer more coarse sediments, substrates composed of sand or gravelly sand. The major constituents of the microphagous diet of the ammocoetes are microalgae belonging to the class Bacillariophyceae. Overfishing and habitat destruction are the two major threats to the conservation of the sea lamprey in Portugal.

## **Introduction**

The sea lamprey (*Petromyzon marinus* L.) is an anadromous species, which supports commercial fisheries in most of the major Portuguese river systems, particularly in the central and northern regions of the country (Figure 1).

Sea lampreys are considered a delicacy in Portugal and, due to the high economic value of this fishery (one animal can cost as much as € 45), the main Portuguese estuaries and rivers are crowded with fishermen and poachers during the annual sea lamprey spawning migration. These intense fisheries along with the reduction of suitable habitat, due to the construction of impassable dams, are the major threats to the survival of this species in Portuguese river basins (Figure 1) (Almeida et al., 2002).

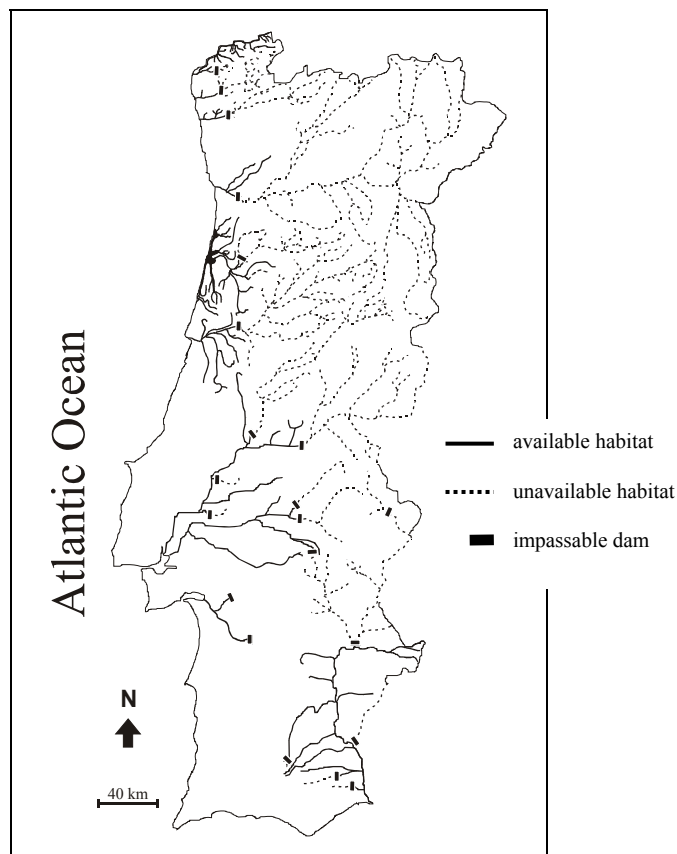
This paper is a synopsis of work on the general biology of the sea lamprey in Portuguese rivers. The information presented in this paper is mainly based on data obtained by the authors in recent research projects and summarizes previously published papers. The major threats to the survival of this species in Portuguese river basins are identified and discussed.

## **The spawning migration**

Sea lampreys are usually captured when entering the estuaries to initiate their upstream reproductive migration, which, in Portuguese rivers, begins in December. Peak spawning migration occurs between February and April and spawning usually takes place between May and June, depending on the meteorological conditions (Guimarães, 1988; Machado-Cruz et al., 1990; Almeida et al., 2000; in press).

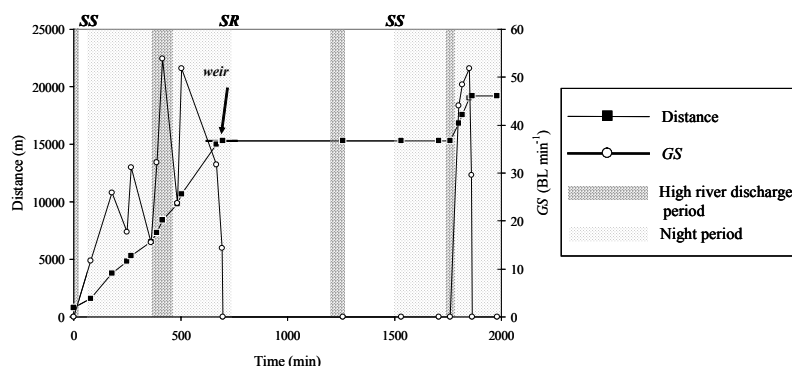
During this period, the lampreys exhibit a strong diel pattern of migratory activity. They are active during the hours of darkness and avoid light during the day, seeking out resting places on rocky substrates. Migratory activity begins at dusk and, under normal conditions, ends at dawn (Almeida et al., 2000; in press).

During periods of continuous movement, sea lampreys attain ground speeds (*GS*) of between  $16.5 \text{ BL min}^{-1}$  and  $29.3 \text{ BL min}^{-1}$  ( $0.84 \text{ km h}^{-1}$  to  $1.5 \text{ km h}^{-1}$ , for an 85 cm sea lamprey) shortly after starting their movement (Almeida et al., 2000; in press) (Figure 2).



**Figure 1.** Habitat available to sea lamprey populations in Portuguese river basins where the species is known to occur.

The alternation between high and low flow regimes, resulting from the operation of dams, can act simultaneously as a stimulus and as a constraint to migration. When the discharge reached the sea lampreys it stimulated the animals to move, or to increase their swimming activity (Figure 2), however if the flow was too high ( $>72 \text{ m}^3\text{s}^{-1}$ ) it caused a delay in their upstream migration (Almeida et al., in press; Quintella et al., in press). Similar conclusions were made by Machado-Cruz et al. (1990) for the same species in River Tagus, in that the high flow released from dams can have a negative impact on sea lamprey migrations.



**Figure 2.** Distance moved by a sea lamprey during its upstream migration in a Portuguese river. The ground speed (*GS*) values were registered using radiotelemetry techniques. *SS* – sunset; *SR* – sunrise (adapted from Almeida et al., in press).

### General ecology of larval sea lampreys in Portuguese rivers

#### *Duration of the larval phase*

Due to the absence of bony structures, such as otoliths, scales or spines, investigations on the larval growth of lampreys have usually relied on the analysis of length-frequency data (Hardisty and Potter, 1971). Recent studies provided reliable age estimates when comparing ages determined from statoliths and length-frequency distribution (Barker et al., 1997). Based on theoretical growth from length-frequency distribution analysis, Quintella et al. (in review) found that the sea lamprey larval stage in a Portuguese river (River Mondego) lasted four years. Sousa (1992) suggested that the ammocoetes from the River Lima (Northern Portugal) remained in the river for a period of 7 to 9 years before metamorphosing. Compared with colder, more northern rivers, the larval phase in River Mondego lasts a shorter duration. This is probably the result of higher productivity associated with the high water temperature, which may enhance ammocoete feeding efficiency and growth (Quintella et al., in review).

On average, the ammocoetes attained 31.4% of the total length (TL) in the first year (i.e. 72 mm) and 72.1% of the TL by the end of the second year (i.e. 165 mm). In the size class corresponding to the second year of life, in spite of reaching the minimum length to initiate metamorphosis, only 18.7% of the lampreys captured had already begun this process. In the third year the growth rate showed a slight reduction; the ammocoetes attained 88.6%

of the TL (i.e. 203 mm), and about 29% of the individuals belonging to this size class showed signs of having begun metamorphosis. In the fourth year of the larval phase the growth rate was also quite low, with ammocoetes attaining 95.2% of the TL (i.e. 218 mm), and all larvae having initiated metamorphosis. It is possible that a small number of ammocoetes could remain in the river for a few more months, until the onset of the feeding migration, at the beginning of the autumn, not completing five years in freshwater (Quintella et al., in review).

The metamorphosis season for *P. marinus* in Portuguese rivers extends from autumn to winter, with a peak in October-November. Even though the ammocoetes from Portuguese rivers initiate metamorphosis at earlier ages than in most other studies, the total length required to initiate metamorphosis coincides with the average length presented by other authors (i.e. approximately 140 mm) (Quintella et al., in review).

#### *Habitat selection*

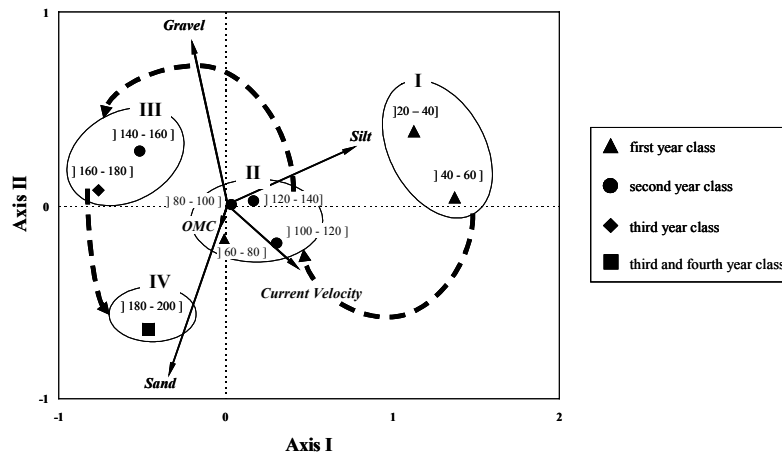
Although the location of larval lamprey populations within a river system can usually be predicted with some accuracy by an experienced observer, it is more difficult to specify in precise physico-chemical terms the essential characteristics of ammocoete habitat (Hardisty and Potter, 1971).

It is widely recognized that the availability of optimal river substrate particle size is one of the most important factors limiting the distribution of ammocoetes. Almeida and Quintella's (2002) work confirmed this observation and showed that distinct ammocoete length-classes prefer sediments with different particle size composition (Figure 3).

According to Almeida and Quintella (2002), ammocoetes with a total length between 20 and 60 mm prefer silty-sand substrates, i.e. sediments with a comparatively high percentage of sand, but also with a relatively large portion of silt (Figure 3). Considering the larval burrowing habit, it is understandable that smaller ammocoetes are usually associated with fine-grained sediments. Soft sediments allow younger larvae with a reduced swimming capacity to propel the head and branchial region below the surface.

A gravel-silty-sand substrate was the selection of the 60-140 mm ammocoete length-class (Figure 3). In this substrate gravel and silt seem to have an identical contribution to the composition of this more heterogeneous sediment. The larger body of the ammocoetes in this class gives them the opportunity to colonize a wider range of sediment types (Almeida and Quintella, 2002).

The larger ammocoetes ( $140 \text{ mm} < \text{TL} \leq 200 \text{ mm}$ ) sampled by Almeida and Quintella (2002) preferred coarse-grained sediments (gravely sand and sand) (Figure 3).



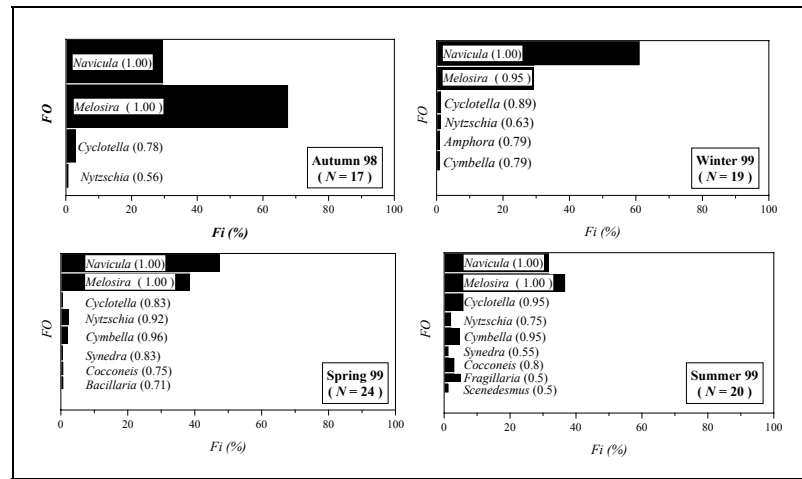
**Figure 3.** Sea lamprey ammocoete length-class distribution according to the sediment particle size, organic matter content (OMC) and current velocity. Detrended canonical correspondence analysis ordination diagram, with symbols corresponding to the nine length-classes, and arrows representing the environmental variables. The Roman numerals identify length classes with similar preferences and correspond to theoretical age classes. The dashed arrows represent the colonization sequence of the different sediment types (adapted from Almeida and Quintella, 2002).

Since the selection of the burrowing sediment is size-dependent, the differences observed in the preferences for distinct sediment types within the same age group probably resulted from a reorganization of the ammocoete distribution at the end of each annual growing season. This behavior could be a strategy developed by this species to avoid high densities in areas colonized by younger individuals, thereby reducing intraspecific competition for space and food (Almeida and Quintella, 2002).

## Diet

Based on an analysis of the gut contents of sea lamprey larvae, Quintella (2000) found that microalgae belonging to the class Bacillariophyceae were the major constituents of the diet of the ammocoetes.

Among them, the genera *Melosira* and *Navicula* are the two most important food items, occurring in more than 95% of the observed gut contents, and corresponding to 86% of the total identified food items. The genera *Cyclotella*, *Cymbella*, *Nitzschia*, *Cocconeis*, *Bacillaria*, *Synedra* and *Rhizosolenia* were also classified as preferred food items (Figure 4).



**Figure 4.** Ammocoete nutrition during the four seasons of the year. Numerical frequency ( $F_i$ ) is given for each of the food organisms and the width of the bars is proportional to the frequency of occurrence ( $FO$ ), which is indicated between brackets (adapted from Quintella, 2000).

During the spring and summer periods, as expected, the diversity of food items present in the analyzed gut contents was much higher than during autumn and winter (Figure 4). The diversity of food items was low throughout the year mainly due to the almost absolute dominance of the genera *Melosira* and *Navicula* (Quintella, 2000).

Quintella (2000) did not find any food items in the gut contents of the macrophthalmic lampreys.

### **Species conservation**

In the last decades several authors have pointed out a reduction in sea lamprey population abundance in Portuguese rivers (Guimarães, 1988; Almaça, 1990; Assis, 1990; Assis et al., 1992; Ferreira and Oliveira, 1996; Almeida et al., 2000; Almeida et al., 2002). According to the Portuguese Red Book of endangered species, the Portuguese sea lamprey populations are considered “Vulnerable” (Vários, 1991). Habitat destruction resulting from dam construction, dredging, gravel extraction, channelization and pollution have contributed decisively to the decline of the sea lamprey populations in Portugal.

Freshwater use for agricultural, industrial and domestic purposes is also responsible for a considerable reduction in the river flow. As a consequence, migratory clues have been eliminated, resulting in a decrease in the number of adult sea lampreys that enter the rivers to spawn (Almeida et al., 2000).

As stated before, intense fishing pressure is one of the major threats to the conservation of this species in Portuguese river basins (Almeida et al., 2002). Professional fisheries regulations define the fishing season between December and April, and capture is allowed in both estuarine and freshwater environments.

Conservation measures can be divided into those concerning the habitat rehabilitation and those promoting the management of the commercial exploitation of sea lamprey populations in Portuguese rivers.

The rehabilitation of Portuguese river systems for sea lampreys should guarantee: (i) access of the adults to upstream spawning grounds by installing adequate and effective fish passages at impassable dams; (ii) the use of a flow regime in regulated river basins that would minimize the negative impacts resulting from high fluctuations in river discharge; (iii) limitations on dredging in river stretches considered irreplaceable for this species, namely spawning grounds and ammocoetes beds; and (iv) maintenance of water and sediment quality that are compatible with the ecological needs of sea lamprey.

Finally, the sustainable commercial exploitation of sea lampreys should be managed to ensure the existence of professional fishing areas, a five to eight day hiatus in fishing activity during the peak of spawning migrations, a reduction in the fishing effort and/or the establishment of annual quotas for each river basin, and eradication of poaching.



### Acknowledgements

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**THREE PROPOSED NEW SPECIES OF LAMPREY ON THE PACIFIC  
COAST OF CANADA**

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**EXTENDED ABSTRACT ONLY- DO NOT CITE**

We report three proposed new species of lamprey in British Columbia, Canada. Two undescribed and possibly rare species are from the Nass River. The third undescribed species is from Skidegate Lake located in the Queen Charlotte Islands. Standard characters used in lamprey systematics as well as a new character are used to distinguish the new forms. We also propose tentative names, *Lampetra nisga*, *Lampetra lisims* and the Skidegate Lake Lamprey, for these three species.

*L. nisga* was found in fresh water and the prominence of the teeth and the size are indications that it is parasitic. *L. nisga* is distinguished from *Lampetra tridentata* by its dentition, velar tentacles, body properties and a new character, the morphology of the gill pore papilla. *L. nisga* is distinguished from *Lampetra ayresi* by having a smaller eye, a shorter prebranchial region, a shorter preorbital length, a larger disc and a distinct pattern of gill pore papilla. *L. nisga* also has fewer cusps on the longitudinal lingual lamina. We have only

one adult specimen of *L. nysga*, a maturing female that has no evidence of secondary sexual characters (upturned tail, shortened caudal area with a swollen tissue around the cloaca and an increased height of caudal and dorsal fins). This suggests that the specimen was migrating up the river to spawn in the following year. The well-developed, but empty gut, the sharp and well-developed teeth, the large size of 24.7 cm and the large number of eggs in the gonad relative to *Lampetra richardsoni* indicate that this species has a parasitic life history. The size is similar to smaller adult *L. ayresi*. Thus it is probable that the lamprey fed only during the late spring and summer, but it is unknown if it feeds in fresh water or the ocean.

The new character is the number and morphology of the gill pore papilla. There are a series of papilla on the posterior margin of each branchiopore. There also may be a single, larger papilla deeper on the posterior wall of the pore. Preliminary studies of a number of species indicate that the number and morphology of the gill-pore papilla are distinctive at both a species and genus level. The number, morphology and the placement of these papilla appear to be particularly useful when comparing populations of the nonparasitic form of paired species.

The second proposed new species, also from the Nass River, *L. lisims*, was represented by 6 specimens and is a non-parasitic species that differs from the only other non-parasitic species in British Columbia *L. richardsoni*, and from a single but smaller specimen in the collection. There is some variation in the appearance of the 6 larger specimens but collectively and individually they were distinct from the one smaller specimen in the sample, and from type specimens of *L. richardsoni*. We grouped all 6 as representing one, new species. The teeth in all 6 were obsolete; some were so degenerate that no cusps were visible, indicating that the specimens were non-parasitic. The digestive tract was thin in all specimens, another indication that the lamprey were non-parasitic. All specimens had spawned or were spawning when captured. *L. lisims* was distinguished from the single smaller specimen by having a larger disc, a longer preorbital length, a larger branchial, and possibly a larger prebranchial length. The velar tentacles of *L. lisims* appeared reduced in number (range of 2-3) compared to the small specimen (4). However, since both the number of tentacles and number of specimens were small, the velar tentacle number would not be a distinguishing character. The large and small forms had a posterior margin of the branchiopore that formed a lip or ridge with a central notch that gives the lip a bilobed appearance. Each lobe had two rows of papilla. The papilla in the smaller form were smaller than the larger specimens while the

deeper single papilla was larger. As this is a new character, it has not been possible to compare our measurements with the holotype and paratypes of *L. richardsoni* as no published descriptions exist.

The proposed third new species was represented by 2 specimens. The fish collection at the University of British Columbia contained two lamprey specimens collected from Skidegate Lake on the Queen Charlotte Islands in British Columbia in 1963 and identified as *L. ayresi*. Each specimen was a mature male that was in spawning condition. These mature lamprey had prominent teeth that were not obsolete as is characteristic of maturing non-parasitic lamprey. Thus, it appeared that these two lamprey were parasitic, but their relatively small size (176 mm, 178 mm) indicated that if they were parasitic, the duration of the feeding period was quite short. Another possibility was that they were non-parasitic, but different from *L. richardsoni* because they retained prominent teeth at maturity.

Extensive measurements of these two specimens were made in 1984. Unfortunately the specimens are no longer in the fish collection at the University of British Columbia. However, recent surveys in November 2001 were able to capture ammocoetes that may be the Skidegate Lake lamprey as they are the largest recorded in British Columbia, growing to lengths of 20 to 24 cm. The Skidegate Lake lamprey is not *L. ayresi* because the eye to total length ratio is smaller than the bottom of the range reported for the type specimens of *L. ayresi*. The prebranchial length to total length ratio is also smaller than the lowest value reported for *L. ayresi*. The two specimens have 5 and 6 velar tentacles which is similar to the number characteristics of *L. ayresi* and *L. richardsoni* but each velar apparatus had a “feathery” appearance as projections were apparent along the longitudinal axis of some tentacles. The Skidegate Lake lamprey is similar to *L. richardsoni*, but has a smaller prebranchial length, that when averaged for the two specimens, is less than the lowest length for *L. richardsoni*. It is important to note that a major reason the *L. richardsoni* was originally described as a distinct species was that the branchial region was longer than the branchial region of *Lampetra planeri* by 12%. Thus the smaller prebranchial region of the Skidegate Lake lamprey is a significant difference of taxonomic importance at the species level. The Skidegate Lake Lamprey also exists in an area that may have not been covered with ice during the last glaciation.



## SEA LAMPREY TROPHIC ECOLOGY IN LAKE SUPERIOR:

### RESULTS FROM STABLE ISOTOPE ANALYSIS

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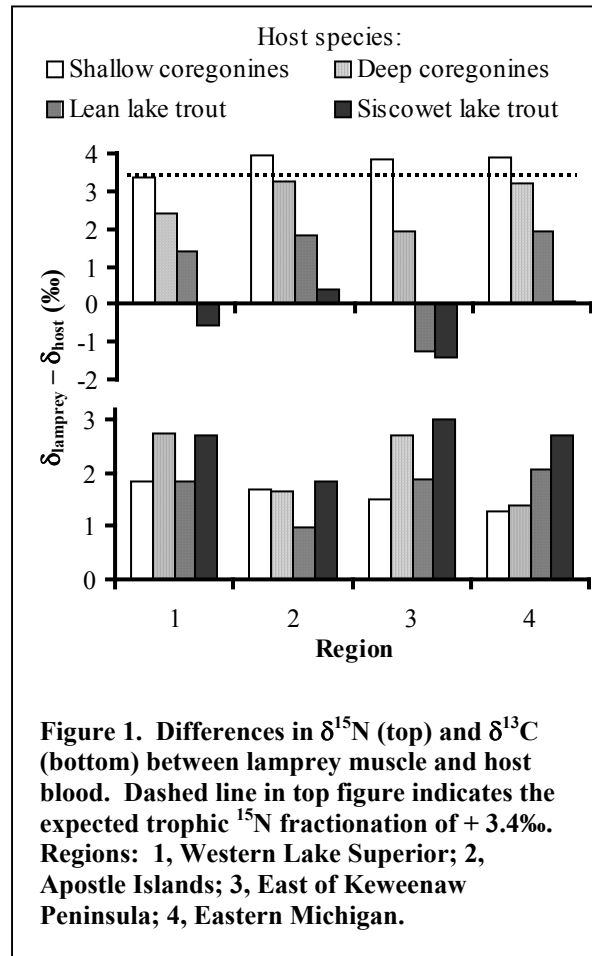
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### EXTENDED ABSTRACT ONLY - DO NOT CITE

The exotic sea lamprey (*Petromyzon marinus*) contributed greatly to collapses of fish populations in the Laurentian Great Lakes in the mid 1900s (Christie 1974). Despite stringent population control actions in the 1950s and 1960s, sea lamprey persist and continue to affect populations of host fishes. The relative importance of different host species to sea lamprey production is unknown. Therefore, we used stable isotope analysis to ask whether sea lamprey in Lake Superior derive most of their production from lake trout (*Salvelinus namaycush*), as is often assumed, or from alternative hosts. The stable isotope ratio of nitrogen ( $\delta^{15}\text{N}$ ) in a consumer's tissues indicates its trophic level, whereas carbon ( $\delta^{13}\text{C}$ ) indicates its base of production (Harvey et al., 2002).

We collected transforming, parasitic, and spawning Lake Superior sea lamprey. Transformers and parasites came from eastern U.S. waters. Spawners as well as four potential hosts (lake herring, *Coregonus artedii*; bloater, *C. hoyi*; lean lake trout; siscowet lake trout) were captured throughout U.S. waters. We measured

$\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  in sea lamprey muscle and host blood. We then compared empirical  $\delta^{15}\text{N}$  of sea lamprey in eastern Lake Superior to values predicted by a model, developed by Harvey et al. (2002), that estimates stable isotope ratios as functions of growth, based on bioenergetics. Simulations ran one year, during which sea lamprey fed exclusively on one of four hosts (shallow-water coregonines, lean lake trout, siscowet lake trout, or deepwater coregonines).

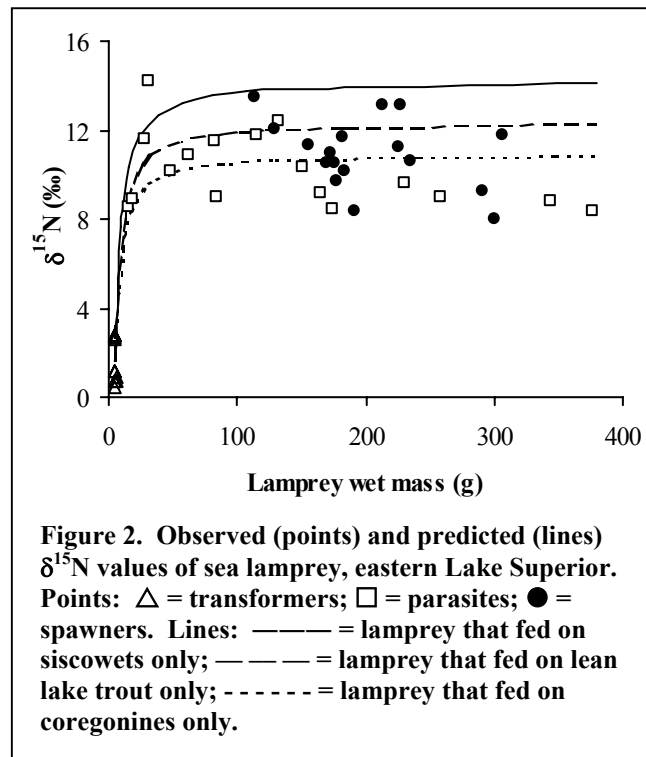




Relationships between stable isotope ratios of spawning sea lamprey and host fishes varied spatially, and implied sea lamprey diets dominated by coregonines (Fig. 1). Average spawning sea lamprey  $\delta^{15}\text{N}$  was 3.75‰ greater than lake herring blood at all sites, which is similar to the generally accepted  $^{15}\text{N}$  trophic fractionation of 3.4‰ (Harvey et al., 2002). Spawning sea lamprey  $\delta^{13}\text{C}$  was 1 to 3‰ greater than host blood, the smallest mean difference being with lake herring (1.6‰). Because carbon does not fractionate strongly between consumer and diet (Harvey et al., 2002), this again suggests that coregonines were the main component of sea lamprey diets.

We examined  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  from transformers, parasites and spawners in eastern Lake Superior. There was no change in  $\delta^{13}\text{C}$  as sea lamprey grew (data not shown). However,  $\delta^{15}\text{N}$  increased from a mean of 1.6‰ for transformers to between 8‰ and 14‰ for parasites and spawners (Fig. 2). This wide range implies that sea lamprey fed on hosts spanning 2 to 3 trophic levels. When empirical data were compared to model projections, empirical  $\delta^{15}\text{N}$  most closely resembled simulated sea lamprey that fed only on coregonines.

Thus, based on empirical and modeling results, Lake Superior sea lamprey appear most dependent on coregonines, followed by lean lake trout and finally siscowet lake trout. The primary nearshore coregonines upon which sea lamprey feed are probably lake whitefish (*Coregonus clupeaformis*), which are large, abundant, benthic, and isotopically similar to lake herring (Harvey, unpublished data). Many large parasitic sea lamprey exhibited very low  $\delta^{15}\text{N}$  values (Fig. 2). There are two explanations for this. First, sea lamprey may not fractionate  $^{15}\text{N}$  in the same manner as other fishes. Second, sea lamprey may parasitize other hosts that have relatively low  $\delta^{15}\text{N}$  values. For example, suckers have been shown to have very low  $\delta^{15}\text{N}$  in other lakes (Kidd et al., 1995).



Lake Superior lake trout remain targets of sea lamprey, and sea lamprey-induced lake trout mortality estimates currently resemble or exceed fishing mortality in regions of Lake Superior (Hansen et al., 1994). However, we must recognize that sea lamprey predation occurs in a complex ecological context and affects many species. The techniques described here can yield major advances in our understanding of those dynamics and contribute to estimates of sea lamprey-derived ecological and economic impacts.

#### Acknowledgements

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**UPSTREAM MIGRATION OF PACIFIC LAMPREYS IN  
THE JOHN DAY RIVER:  
BEHAVIOR, TIMING, AND HABITAT USE**

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**EXTENDED ABSTRACT ONLY – DO NOT CITE**

**Introduction**

Pacific lamprey (*Lampetra tridentata*) populations in the Columbia River Basin (CRB) are believed to have declined dramatically compared to their populations prior to human development (Close et al., 1995). Little is known about the biology and life history of this Agnathan in the CRB. Identification of the biological and ecological factors that may limit lamprey production is critical to population assessment and recovery efforts. The USGS is using radio telemetry to study Pacific lamprey migration behavior, timing, and habitat usage in the John Day River Basin (JDRB). Assessment of over-wintering and spawning habitat usage in the JDRB will be helpful in establishing goals for recovery projects in other sub-basins of the CRB. Specifically, this study will support the lamprey restoration efforts of the Confederated Tribes of the Umatilla Indian Reservation (CTUIR) in the Umatilla River Basin (Jackson et al., 2000), a drainage with similar geomorphology to the JDRB. Data on adult habitat usage will complement ongoing larval habitat studies by the CTUIR and provide a complete picture of habitat needs for all of the fluvial life stages of the Pacific lamprey in the JDRB.

**Materials and Methods**

Pacific lampreys were captured after sunset in the John Day River, OR at Tumwater Falls (riverkilometer (RKM) 16.9) using dip nets. Forty-two

lampreys were surgically implanted with radio transmitters and released at the capture site. Movements of lampreys tagged by USGS at Tumwater Falls, as well as some tagged by National Marine Fisheries Service (NMFS) at Bonneville Dam in July, 2000, were followed in the John Day River by three methods: 1) Five fixed-site receivers were used to observe timing of movements past key points and to limit the aerial search area, 2) Aerial telemetry was used to find general positions of tagged lampreys over large portions of the basin and to observe whether over-wintering behavior had been initiated, and 3) Terrestrial telemetry was used to find accurate locations of lampreys for determination of over-wintering habitat use. Preliminary habitat data were recorded for each over-wintering position at the time of location. Temporal habitat characteristics (water depth, flow, and temperature) were measured at each lamprey position and substrate characteristics of the immediate area surrounding the location were qualitatively described.

## Results and Discussion

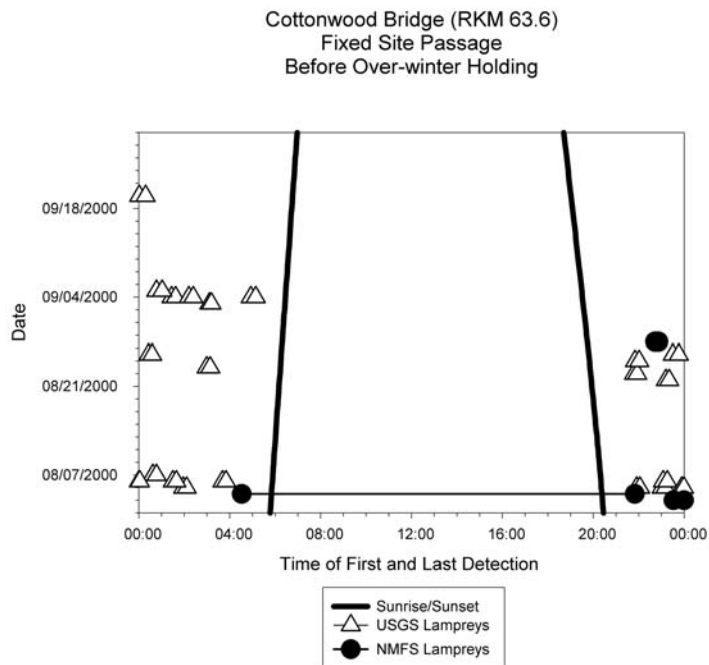


Figure 1

Movement past fixed receivers was exclusively between sunset and sunrise, with one lamprey holding position in front of a receiver during daylight hours as shown in a representative figure (Figure 1). Most over-winter holding was initiated by mid-September, 2000 and continued until mid-March, 2001, when 19 lampreys resumed upstream migration as shown in a representative figure (Figure 2).

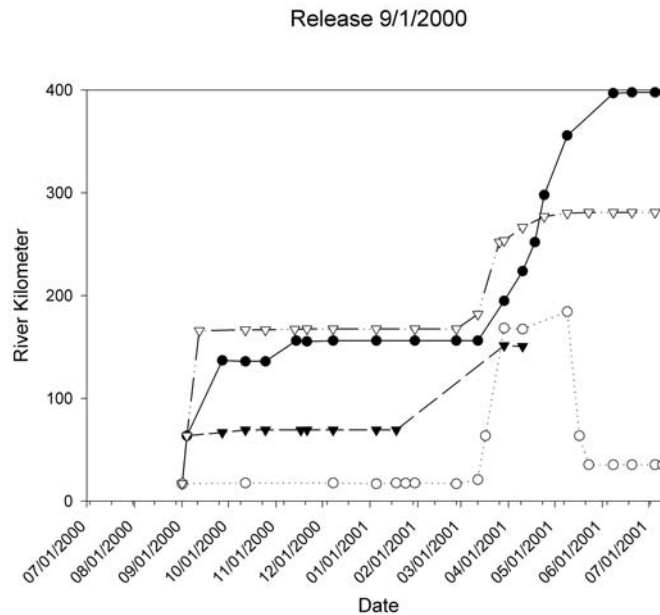


Figure 2

Thirty-five over-wintering lampreys were chosen for more accurate locations from the ground or by boat. Individuals over-wintered under boulders in riffles/glides. Substrate was dominantly boulders (>25.4 cm) at 30 locations and dominantly cobbles (5.1 to 25.4 cm) at 1 location. Four locations were too deep to observe substrate. Upstream migration ceased in May, 2001 (Figure 2), perhaps indicating spawning activity when river temperatures reached levels associated with spawning activity in laboratory studies. Five lampreys tagged by NMFS were found in proximity to USGS-tagged lampreys and behaved similarly. Future tasks for this project will be to verify over-wintering behavior observed in 2000-2001 by collecting migration behavior data over multiple

seasons, and to describe over-wintering and spawning habitat usage by adult Pacific lampreys.

### **Acknowledgements**

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**ASSESSING STATUS OF PACIFIC LAMPREY (*LAMPETRA*  
*TRIDENTATA*) IN THE COLUMBIA RIVER BASIN, U.S.A.**

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**EXTENDED ABSTRACT ONLY – DO NOT CITE**

**Introduction**

Methods for assessing abundance and distribution of Pacific lamprey in the Columbia River Basin are inadequate. Adult lamprey are enumerated as they pass count stations in fishways at hydropower dams (Starke and Dalen, 1995). However, the counting protocols were designed to assess salmonids and do not conform to lamprey migration patterns. We used lamprey-specific assessment methods (trapping, radiotelemetry, and electrofishing for larvae) to examine lamprey distribution and abundance. The results of this work were then used to identify research and monitoring needed to establish Pacific lamprey status in the Columbia River and other drainages in the northwestern United States.

**Methods**

We trapped lamprey in a fishway at Bonneville Dam (the first dam adult lamprey encounter during their upstream migration in the Columbia River) in 1998 – 2000 and computed catch per unit effort (CPUE, lamprey h<sup>-1</sup>). CPUE was compared to visual adult lamprey counts made at the same time and in the same fishway. In each year we also surgically implanted uniquely-coded radio transmitters in selected lamprey (as in Moser et al., In Press). Passage of radio-tagged lamprey at the three lower Columbia River hydropower dams (Bonneville Rkm 235, The Dalles Rkm 308, and John Day Rkm 347) was monitored via an extensive network of fixed-site

receivers (Moser et al., In Press). We compared radiotelemetry results to lamprey counts made at the same dams during traditional count periods. For both counts and radiotelemetry data, we determined the proportion of lamprey lost in each reservoir by subtracting the total number of lamprey that passed the count windows at each successive upriver dam from the number that passed the count windows at the previous dam.

In 1999, we electrofished for ammocoetes (larval lamprey) in July – September at stations in nine Columbia River tributaries in northeastern Oregon and southeastern Washington where Pacific lamprey historically occurred (Figure 1). We anaesthetized and measured the ammocoetes and computed length frequencies and densities for each tributary.

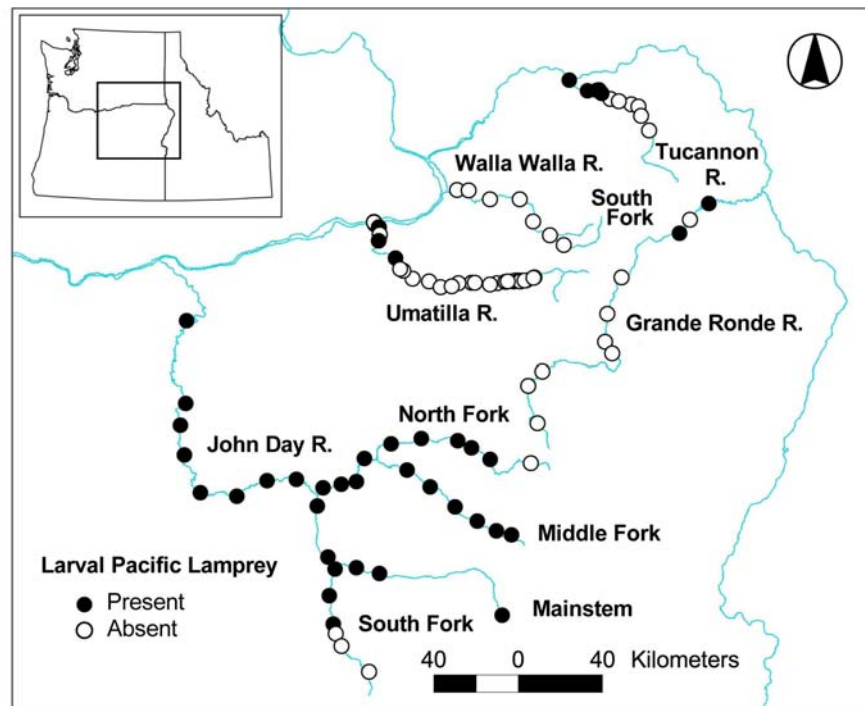


Figure 1. Presence (closed dots) or absence (open dots) of Pacific lamprey ammocoetes at electrofishing stations in tributaries of the Columbia River.

## Results

In the fishway where our trap was deployed, lamprey passage (lamprey  $\text{h}^{-1}$ ) based on visual counts was 9.4 in 1998, 12.7 in 1999, and 4.5 in 2000. Trap CPUE (lamprey  $\text{h}^{-1}$ ) in those years was 1.0 in 1998, 0.7 in 1999, and 0.5 in 2000. We found no significant correlation between the mean weekly lamprey abundance obtained using the two methods in 2000 ( $P > 0.05$ ).

In 1998, 1999, and 2000 we released 205, 199, and 299 radio-tagged lamprey below Bonneville Dam. Annual losses for the area from Bonneville Dam to The Dalles Dam were similar for radiotelemetry and count data (Figure 2). However, for the area between The Dalles Dam and John Day Dam, the two methods produced very different results. Intensive tracking of lamprey in 2000 indicated that 67% of the lamprey that passed counting windows at Bonneville Dam would not have been detected because they passed during the night. In addition, 6% of the fish passed over the dam via routes without count stations.

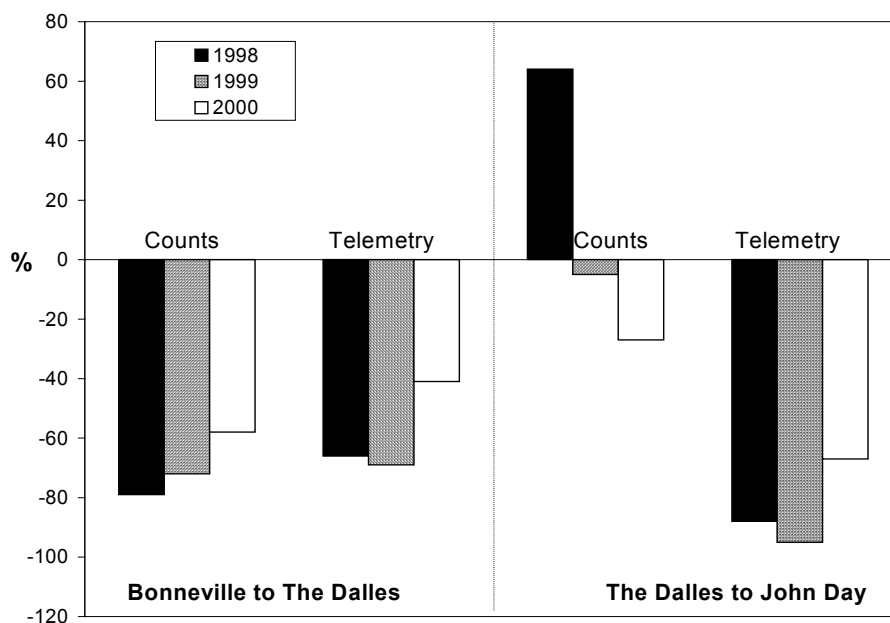


Figure 2. The percentage of lamprey lost in each reach as determined by lamprey counts and radiotelemetry in 1998 – 2000.

Ammocoetes were not found in the upper reaches of tributaries we sampled, nor were they in any of the Walla Walla River samples (Figure 1). Density was highest in the John Day River and its tributaries and lowest in the Grande Ronde River. Mean ammocoete lengths were lowest in the John Day River drainage and highest in the Umatilla and Grande Ronde rivers.

## Discussion

Our data indicated that adult lamprey counts at hydropower dams are unreliable and can be misleading. This is not surprising in light of the fact that lamprey are nocturnal and capable of passing the dams via unmonitored routes. While dam counts are problematic, they represent the only historical index of lamprey abundance and should be continued. However, other methods should be used to correct count data or to obtain absolute adult abundance estimates.

Ammocoete abundance was highly variable, but indicated a lack of recent recruitment in the upper reaches of most rivers we sampled. Truncated size frequencies also indicated poor recruitment in recent years. Standardized, basin-wide monitoring of ammocoetes, coupled with habitat delineation is needed to adequately assess ammocoete distribution (Pajos and Weise, 1994).

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**ORGANOGENESIS IN A NORMAL SERIES OF EMBRYOS OF  
THE SEA LAMPREY.**

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**EXTENDED ABSTRACT ONLY-DO NOT CITE**

**Introduction**

Some features shared by lampreys and gnathostomes may represent primitive conditions at the level of the vertebrates. This makes lampreys of considerable interest to vertebrate evolutionary biologists.

Studies of lamprey embryology have recently been used to make inferences about primitive developmental patterns and the evolution of vertebrates (Kuratani et al., 2001). Lampreys were of great interest to nineteenth century embryology, which had a strong phylogenetic perspective. For this and other reasons, the classical literature contains an outstanding series of descriptions of development in *Lampetra* and *Petromyzon* species.

There is a growing interest in molecular genetics of lampreys as well as in aspects of histogenesis. Lampreys have been used to study hypotheses about gene duplication events in vertebrate evolution and there is evidence that lampreys have 3 *Hox* gene clusters. A recent study suggests that loss of *Hox* gene expression in the lamprey mandibular arch was a key event facilitating the evolution of jaws in gnathostomes (Cohn, 2002).

In view of the importance of lampreys to biology, it is unfortunate that there is no

general, well illustrated survey of lamprey organogenesis before metamorphosis. Most studies have concentrated on the earliest stages of development up to and including neurulation. Many classical studies of lamprey development are in relatively inaccessible publications. The most recent overview of *Petromyzon* development is given in the Piavis staging series which describe development from fertilization to formation of the larva (Piavis, 1971). In addition, stages for *Lampetra reissneri*, correlated with Piavis stages have been described by Tahara (1988). We are supplementing Piavis's valuable account of *Petromyzon* development by providing descriptions and clear illustrations of some key external and internal developmental events in embryos of different chronological ages. In addition to providing an overview and literature survey, this study will form the basis of future study of lamprey developmental characters within a phylogenetic framework.

## **Materials and Methods**

Adult sea lamprey were captured during upstream spring migration in late May/early June from a fish ladder in New Germany, Nova Scotia. Eggs and sperm were extracted manually from ripe, unanesthetized lamprey. Eggs were artificially fertilized and resultant zygotes were raised *in vitro* at  $18.4 \pm 0.5^{\circ}\text{C}$ . Ten to twenty embryos were collected daily between days 5-70 post fertilization, anesthetized and fixed *in toto* in either Bouin's fluid or 10% neutral buffered formalin. Embryos were staged by days post fertilization and the criteria of external appearance described by Piavis. We examined more than seventy fixed embryos spanning Piavis stages 11 to 18+ (5- 70d post fertilization) by gross observation and histology. This period begins at late neurulation stages, and ends with the formation of the larva.

## **Results and Discussion**

In the first section of our results we present an overview of some aspects of the regional anatomy of the embryo including fins, pericardial region, pharynx and intestine. We explain our anatomical nomenclature particularly concerning the numbering of pharyngeal structures and their axial relations, which is a potential source of confusion. The second section of our results present a set of some key transformations during seven age groups (5d, 6d, 7-9d, 10-12d, 13-17d, 18-23d and 36-70d) which include development of the head and pharynx, organs of the pericardial cavity, and postpericardial region. We were unable to confirm reports by Piavis that the anus develops directly from a persistent blastopore. In our material,



the anus develops in the former position of the blastopore by secondary canalization. A major issue for clarification is the identity of eosinophilic cells in the dorsal coelomic wall. One possibility is that they are germ cells, although specific markers are required to confirm this.

We note that chronological age does not correlate completely with morphological maturity. Thus our division of lamprey embryos into age groups does not provide a developmental sequence of high resolution. We are currently planning to prepare a highly resolved developmental sequence from this material by making a detailed, quantitative character analysis using the method of event-pair cracking as described by Jeffery et al. (2002). Further, we plan to analyze the characters, described and illustrated here, in a phylogenetic context; and to correlate them with the Piavis stages.

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