



Institutionen för Systemekologi

Department of Systems Ecology
Stockholm University
S- 106 91 Stockholm, Sweden

Phone Int +46 8 164258
Fax Int +46 8 158417

NFR
Box 7142
103 87 Stockholm

12 May 1997

Enclosed please find 18 copies of a project proposal titled "Mapping the Relationship of Otolith Microchemistry to Water Masses: Effects of Salinity, Geology, and Species Observed at Different Scales." This proposal is submitted to Biology (marine and freshwater ecology, also perhaps zoophysiology).

Please do not hesitate to contact me in the event that further clarification of any points are required. Thank-you for considering this proposal.

Sincerely,

Karin E. Limburg, Ph.D.

Reg nr 650-	
Appl. id.	
Date received	Official responsible

Personal details - Mailing address (place of work)

Main applicant's last name Limburg	Main applicant's first name Karin
Co-applicant's last name, first name Westin, Lars	University affiliation Systems Ecology, Stockholm Univ.
Wickström, Håkan	Inst. Freshwater Res., Drottningholm
Svedäng, Henrik	Inst. Coastal Res., Öregrund
Grant to be administered by	

Title Forskarass.	Sex (M/F) F	National Swedish reg no. (personnr) 540607-2685
Institution (motsvarande) Department of Systems Ecology		
Universitet (motsvarande) Stockholm University		
Gata, Box, etc. Svante Arrheniusv. 21A		
Postnummer S-106 91	Ort Stockholm	
Telefon 08-16 49 20	Fax 08- 15 84 17	
E-mail address Karin_L@system.ecology.su.se		

Posts/Equipment

Application includes funding for

<input type="checkbox"/> New postgraduate fellowship	<input type="checkbox"/> Adjunct professorship	<input type="checkbox"/> Junior individual grant
<input type="checkbox"/> Junior researcher	<input type="checkbox"/> Postdoctoral exchange fellowship	
<input type="checkbox"/> Lecturer grant	<input type="checkbox"/> Guest researcher grant	<input type="checkbox"/> Equipment (SEK 0,5-2,0 million)

New/Continuation

New project If continuation, project reg. nr

Classification codes	Priority area (Instatsområde)
Nd1c	<input type="checkbox"/> Energy - related basic research
Nd1b	<input type="checkbox"/> Global biogeosphere dynamics
Nd2e	<input type="checkbox"/> Mathematics & natural sciences /technology
	<input type="checkbox"/> Solid state physics and chemistry

Research area

<input type="checkbox"/> Interdisciplinary research
<input type="checkbox"/> International cooperation
<input type="checkbox"/> Climate research
<input type="checkbox"/> Environmental research

Project

Title of project:
Mapping the Relationship of Otolith Microchemistry to Water Masses: Effects of Salinity, Geology, and Species Observed at Different Scales

Summary of research programme:
Otoliths, calcareous structures that form part of the hearing/balance system in fish, are generally faithful recorders of age and growth histories of individual organisms. Recent breakthroughs in microchemical techniques are being used to assay trace elemental "fingerprints" i.e., suites of trace elements entrained in otoliths that reflect environmental conditions for the fish. To date few such studies exist and none for the Baltic. We propose to conduct a fundamental study of the relationship of otolith microchemistry to environmental conditions and intend to exploit the major gradient in salinity around the Swedish coastline (full-strength seawater to approx. 2 ‰) as well as a major gradient in bedrock geology. For this study we will use two species of fish that have small home ranges: viviparous blenny (*Zoarces viviparus*) and threespine stickleback (*Gasterosteus aculeatus*) that should be good integrators of their local environment. We will also collect water samples several times during the year to characterize the aquatic environment at the same sites and analyze bulk chemistry in addition to salinity. Samples (otoliths and water) will be analyzed with either inductively-coupled plasma mass spectrometry (ICP-MS) or atomic emission spectroscopy (ICP-AES).
Our statistical analysis will address the degree to which otolith chemistry varies as a function of spatial scale. Specifically, we will test whether or not variability can be seen at the "macro" (whole salinity gradient), "meso" (different major bedrock types: Gotland vs. mainland), and "micro" (heterogeneity of bedrock types on Gotland) scales and whether or not variation is a function of water chemistry at those scales. Further, by using two fish species, we will be able to address to some extent the degree to which species differences affect otolith uptake of trace elements, and whether robust patterns appear despite species differences. This has important consequences for the application of microchemistry techniques in future studies of Baltic species, particularly those with large migratory lifestyles. The careful analysis of how chemistry of otoliths relates to the aqueous environment will lay the groundwork for new approaches to fish life history and ecology in Swedish coastal waters.

Key words:
Otoliths, microchemistry, ICP-MS, ICP-AES, spatial scale, resident fish, coastal waters

Budget Request

Amounts in SEK	1998	1999	2000
Salaries and fellowships	0	0	0
Materials, technical pers., travel costs, etc	321 380	214 460	0
Equipment	0	0	0
Total costs	321 380	214 460	0

E-mail/Computer

Also submitted by
 Email Discette

Appendices

Application submitted together with:

Main applicant's CV	<input checked="" type="checkbox"/>	} original form
Project description	<input checked="" type="checkbox"/>	
Energy appendix	<input type="checkbox"/>	} +
Specs for equipment	<input type="checkbox"/>	
List of publications	<input checked="" type="checkbox"/>	} identical copies
Other	<input type="checkbox"/>	

different publications (max 3).
2 copies of each

Signatures

Applicant's signature

Department chair, signature

Department chair, full name (please print or type)

Department chair, telephone

Other sources of funding

This project is identical similar to a project also applied for to:

Cf MFR SJFR

Nv TFR NUTEK

FV	ÅO	ÅO	ÅO	ÅO	BG	BG	BG	F/R	F/R	Other authority or grant institution
BG	F/R	F/R	BG	FR	F/R	BG	F/R	F/R		

Mapping the Relationship of Otolith Microchemistry to Water Masses – Effects of Salinity, Geology, and Species Observed at Different Scales

Karin E. Limburg

Department of Systems Ecology, Univ. of Stockholm, S-106 91 Stockholm, Sweden
Telephone +46-(0)8 164920, Fax +46-(0)8 158417, Email Karin_L@system.ecology.su.se

Introduction

Otoliths are small, biogenic structures that form part of the hearing and balance system of teleost fishes (Moyle and Cech 1988). These are largely aragonitic, with the CaCO₃ precipitated on a protein matrix. Because their accretion is daily (Pannella 1971), critical information on age and growth rate can be acquired for individual fish with high resolution. This capability is now heavily exploited, and not only for fisheries applications. The ability to retrieve information from otoliths, as from an airplane flight recorder, adds an exciting new dimension to basic research in life history, evolution, and general ecology as well (e.g., Limburg 1994).

Microanalysis of otolith chemistry is one of the most important and rapidly developing fields in otolith research today (Secor et al. 1995a). For instance, temporal changes of strontium concentration in otoliths, as determined with conventional microprobe technology, have been used to infer past thermal and salinity environments of fishes (Limburg 1995, Secor et al. 1995b, Townsend et al. 1995). The development of inductively coupled plasma spectrometry techniques (ICP) (Jarvis and Jarvis 1992) has permitted rapid, high precision measurement of elements and their stable isotopes at trace concentrations. Recent applications of ICP spectrometry to otoliths has permitted the construction of “elemental fingerprints” which have been used to distinguish between stocks of different origins (e.g., Edmonds 1991; Campana et al. 1995; Bronte et al. 1996; Dove et al. 1996).

Although we are confident that strontium entrained in otoliths will allow us to examine differences between freshwater and seawater residencies of fish, we wish to determine more precisely this relationship at different salinities, e.g., those characteristically observed in the Baltic Sea. Further, geological differences within sub-drainage basins within the Baltic add a second layer of elemental variation in water masses (Löfvendahl et al. 1990; Andersson et al. 1994). Whereas the concentrations of some dissolved trace elements can vary widely over fairly short periods (e.g., Stumm and Morgan 1996), otoliths have been demonstrated to be reliable integrators of the chemical environment experienced by fish (Radtke 1984; Secor et al. 1995b).

Swedish coastal waters range from full-strength seawater to nearly fresh, and Baltic basin geology divides roughly along an older, Precambrian shield to the north and a younger set of Phanerozoic rocks to the south (including Gotland, Öland, and extreme southern Sweden: Winterhalter et al. 1981). Finer-scale geological heterogeneity is evident on the island of Gotland (Svantesson 1978). A natural gradient of scales exists near at hand, with which to explore the uptake of trace elements by otoliths.

Research Questions: Thus we may pose the following fundamental questions:

1. To what extent does salinity affect otolith uptake of elements around the Swedish coast?
2. At what spatial scales can uptake variations be detected? (i.e., down to the local scale, or only at the scale of the whole Baltic)
3. Do otoliths of different sympatric fish species exhibit different uptake patterns? If so, are there any robust patterns relating to environment?

Goals and Objectives

Our goal in this project is to map the variability in otolith elemental uptake at three different spatial scales:

- a synoptic “macro-scale”: variation related to salinity;
- “meso-scale”: detecting differences between mainland Sweden (Precambrian Shield bedrock) and Gotland (Phanerozoic bedrock);
- “micro-scale”: variation around Gotland itself, in proximity to the mouths of streams draining different reef formations, and bedrock types (limestone vs. sandstone).

The ideal test organism for this study would be a completely euryhaline, nonmigratory teleost. There are at least two candidate species: viviparous blenny (*Zoarces viviparus*) and threespine stickleback (*Gasterosteus aculeatus*). Both species are coastal and have small home ranges, so elemental variation in otoliths should reflect the environment in the place of capture. *Z. viviparus* ranges around the entire Swedish coast but not in freshwater; *G. aculeatus* occurs along coastlines and in fresh water as well (Pethon and Svedberg 1996). Because of their small home ranges, both species have been identified as suitable for bioindicator studies (Jacobsson et al. 1986, Kowaleska and Korzeniewski 1991). Inter-specific comparisons of otolith elemental composition can be made where the species co-occur.

We will collect fish, extract their otoliths, and analyze their elemental composition by means of ICP-mass spectrometry (ICPMS) and ICP-atomic emission spectrometry (ICPAES). Additionally we will collect samples of water in proximity to where the fish are caught and analyze bulk chemistry in addition to salinity. Details follow below.

Methods

Fish will be collected at approximately the sites illustrated in Fig. 1. Approximately 20 fish of each species will be taken at each site. Fewer sites will be used for *G. aculeatus* (to keep expenses down) but will include a freshwater “end-member” site. Around Gotland, we will collect fish near the mouths of three streams that drain different bedrock types (determined by overlaying a map of drainage basins on top of a geological map). Sagittal otoliths will be removed, cleaned, and weighed. Care will be taken to avoid contamination (see Fowler et al. 1995). Preparation for ICP spectrometry will be done at Stockholm University following procedures outlined in Fowler et al. (1995). Samples will be analyzed by a contracted laboratory.

Water will be collected at the same sites, 3 times during the year, using acid-washed containers. Water should be collected in the vicinity of the fishes to approximate as much as possible the aqueous environment experienced by them. A

subsample of water will be filtered to eliminate particulates (> 45 µm; Andersson et al. 1994). In addition to salinity and temperature, we will measure Ca, Na, K, Cl, and S. Samples will also be analyzed by a contracted laboratory.

A fairly large number of elements have been found to occur in trace amounts in otoliths, and it is difficult *a priori* to say which elements will be found at informative levels in *Z. viviparus* and *G. aculeatus*. In addition to Ca and Sr, it will most likely be interesting to measure Ba, Cu, Zn, B, Li, and Ni (S. Campana, Department of Fisheries and Oceans, Nova Scotia, Canada, personal communication). The isotopes ⁸⁷Sr and ⁸⁶Sr will also yield useful information as a north-south gradient in ⁸⁷Sr/⁸⁶Sr exists in the Baltic (Löfvendal et al. 1990; Andersson et al. 1992). A pilot study on otoliths will be carried out in 1997 to determine which elements look promising.

Multivariate statistical techniques will be used to analyze the data. The independent variables will include locale, salinity, bulk chemical concentrations in water, and species. Size-at-age (determined by otolith aging) will be included as a covariate. Discriminant function analysis will be employed to test the degree of separation of otoliths by salinity, geographic location, and species.

Schedule

Year 1 - Collection of fish and water samples; sample preparation; analysis

Year 2 - More analysis; statistical analyses; preparation of manuscripts

Relevance of the Proposed Research

This study will be the first, and therefore groundbreaking, analysis of the relationship of fish otolith microchemistry to water masses around Sweden and in the Baltic as a whole. It is well established that otolith incorporation of elements is mediated through physiology (Kalish 1989, 1991; Sadovy and Severin 1992) and this can present a confounding influence. However, we are exploiting a strong salinity gradient and a second potentially strong gradient in drainage basin geology. It should also be possible to correct to some extent (e.g., by analysis of covariance) for growth rate effects.

By providing this basic “map” of otolith microchemistry in resident species, we will be able to judge the degree to which we can discriminate movement and recruitment of diadromous fishes in Baltic habitats at different geographic scales of resolution, as we are planning to do in a separate proposal to SJFR (Limburg et al. 1997). Furthermore, this study will lay a critical foundation for future work, as techniques, e.g., high resolution mass spectroscopy, become available to measure trace elements and isotopes at the spatial resolution of microns, as is currently possible with conventional microprobes. This new capability will greatly enhance the application of otolith chemistry to ecological and life history problems in Swedish coastal waters.

Notes on the Budget

Most of the expenses (ca. 70%) are for chemical analyses, which must be contracted out. Otolith analytic costs (estimated 310 otolith analyses) are based on approximate North American prices, which are about 40% lower than Swedish price estimates. Water analyses (estim. 66 analyses) are based on price quote from Svensk Grundämnesanalys AB, Luleå. Other major expenses include labor (laboratory

assistance (4 mo in Year 1, 2 mo in Year 2) to help with sampling and processing) and fees to fishermen for collecting fish. Principal investigator time is provided at no cost.

Literature Cited

- Andersson, P.S., G.J. Wasserberg, and J. Ingri. 1992. The sources and transport of Sr and Nd isotopes in the Baltic Sea. *Earth and Planetary Science Letters* 113: 459-472.
- Andersson, P.S., G.J. Wasserberg, Ingri, J., and M.C. Stordal. 1994. Strontium, dissolved and particulate loads in fresh and brackish waters: the Baltic Sea and Mississippi Delta. *Earth and Planetary Science Letters* 125: 195-210.
- Bronte, C.R., R.J. Hesselberg, J.A. Shoesmith, and M.H. Hoff. 1996. Discrimination among spawning populations of Lake Superior lake herring based on trace element profiles in sagittae. *Transactions of the American Fisheries Society* 125: 852-859.
- Campana, S.E., A.J. Fowler, and C.M. Jones. 1994. Otolith elemental fingerprinting for stock identification of Atlantic cod (*Gadus morhua*) using laser ablation ICPMS. *Canadian Journal of Fisheries and Aquatic Sciences* 51: 1942-1950.
- Campana, S.E., J.A. Gagne, and J.W. McLaren. 1995. Elemental fingerprinting of fish otoliths using ID-ICPMS. *Marine Ecology Progress Series* 122: 115-120.
- Dove, S.G., B.M. Gillanders, and M.J. Kingsford. 1996. An investigation of chronological differences in the deposition of trace elements in the otoliths of two temperate reef fishes. *Journal of Experimental Marine Biology and Ecology* 205: 15-33.
- Fowler, A.J., S.E. Campana, C.M. Jones, and S.R. Thorrold. 1995. Experimental assessment of the effect of temperature and salinity on elemental composition of otoliths using solution-based ICPMS. *Canadian Journal of Fisheries and Aquatic Sciences* 52: 1421-1430.
- Jacobsson, A., E. Neuman, and G. Thoresson. 1986. The viviparous blenny as an indicator of environmental effects of harmful substances. *Ambio* 15: 236-238.
- Jarvis, I. And K.E. Jarvis. 1992. Plasma spectrometry in the earth sciences: techniques, applications, and future trends. *Chemical Geology* 95: 1-33.
- Kalish, J.M. 1989. Otolith microchemistry: validation of the effects of physiology, age and environment on otolith composition. *Journal of Experimental Marine Biology and Ecology* 132: 151-178.
- Kalish, J.M. 1991. Determinants of otolith chemistry: seasonal variation in the composition of blood plasma, endolymph and otoliths of bearded rock cod, *Pseudophycis barbatus*. *Marine Ecology Progress Series* 74: 137-159.
- Kowalewska, M., and K. Korzeniewski. 1991. Trace metals from gastersteus aculeatus L. from the Gdansk Bay. *Polish Archive of Hydrobiology* 38: 475-484.
- Limburg, K.E. 1994. Ecological constraints on growth and migration of juvenile American shad (*Alosa sapidissima* Wilson) in the Hudson River Estuary, New York. Ph.D. Dissertation, Cornell University, Ithaca, NY. 352 p.

- Limburg, K.E. 1995. Otolith strontium traces environmental history of subyearling American shad *Alosa sapidissima*. Marine Ecology Progress Series 119: 25-35.
- Limburg, K.E., L. Westin, H. Wickström, and H. Svedäng. 1997. Revealing complex life histories of fishes through natural environmental storage devices: case studies of diadromous events as recorded by otoliths. Proposal submitted to SJFR.
- Löfvendahl, R., G. Åberg, and P.J. Hamilton. 1990. Strontium in rivers of the Baltic Basin. Aquatic Sciences 52/4: 315-329.
- Moyle, P.B., and J.J. Cech, Jr. 1988. Fishes: an introduction to ichthyology. 2nd Edition. Prentice Hall, Englewood Cliffs, New Jersey. 559 p.
- Pannella, G. 1971. Fish otoliths: daily growth layers and periodical patterns. Science 173: 1124-1127.
- Pethon, P. and U. Svedberg. 1996. Fiskar I färg. Nordstedts Förlag AB, Stockholm. 245 p.
- Radtke, R.L. 1984. Codfish otoliths: information storage structures. Flodevigen Rapportser. 1: 273-298.
- Sadovy, Y., and K.P. Severin. 1992. Trace elements in biogenic aragonite: correlation of body growth rate and strontium levels in the otoliths of the white grunt, *Haemulon plumieri* (Pisces: Haemulidae). Bulletin of Marine Science 50: 237-257.
- Secor, D.H., J.M. Dean, and S.E. Campana (editors). 1995a. Recent developments in fish otolith research. University of South Carolina Press, Columbia, South Carolina. 735 p.
- Secor, D.H., A. Henderson-Arzapalo, and P.M. Piccoli. 1995b. Can otolith microchemistry chart patterns of migration and habitat utilization in anadromous fishes? Journal of Experimental Marine Biology and Ecology 192:15-33.
- Stumm, W., and J.J. Morgan. 1996. Aquatic chemistry. Third edition. John Wiley and Sons, New York. 1022 p.
- Svantesson, S.-I. 1978. Översiktlig naturinventering: geologi. Länsstyrelsen i Gotlands Län. Visby.
- Townsend, D.W., R.L. Radtke, D.P. Malone, and J.P. Wallinga. 1995. Use of otolith strontium:calcium ratios for hindcasting larval cod *Gadus morhua* distributions relative to water masses on Georges Bank. Marine Ecology Progress Series 119: 37-44.
- Winterhalter, B., T. Flodén, H. Ignatius, S. Axberg, and L. Niemistö. 1981. Geology of the Baltic Sea, pp. 1-121 In The Baltic Sea (A. Voipio, editor). Elsevier Scientific Pub.Co., Amsterdam.

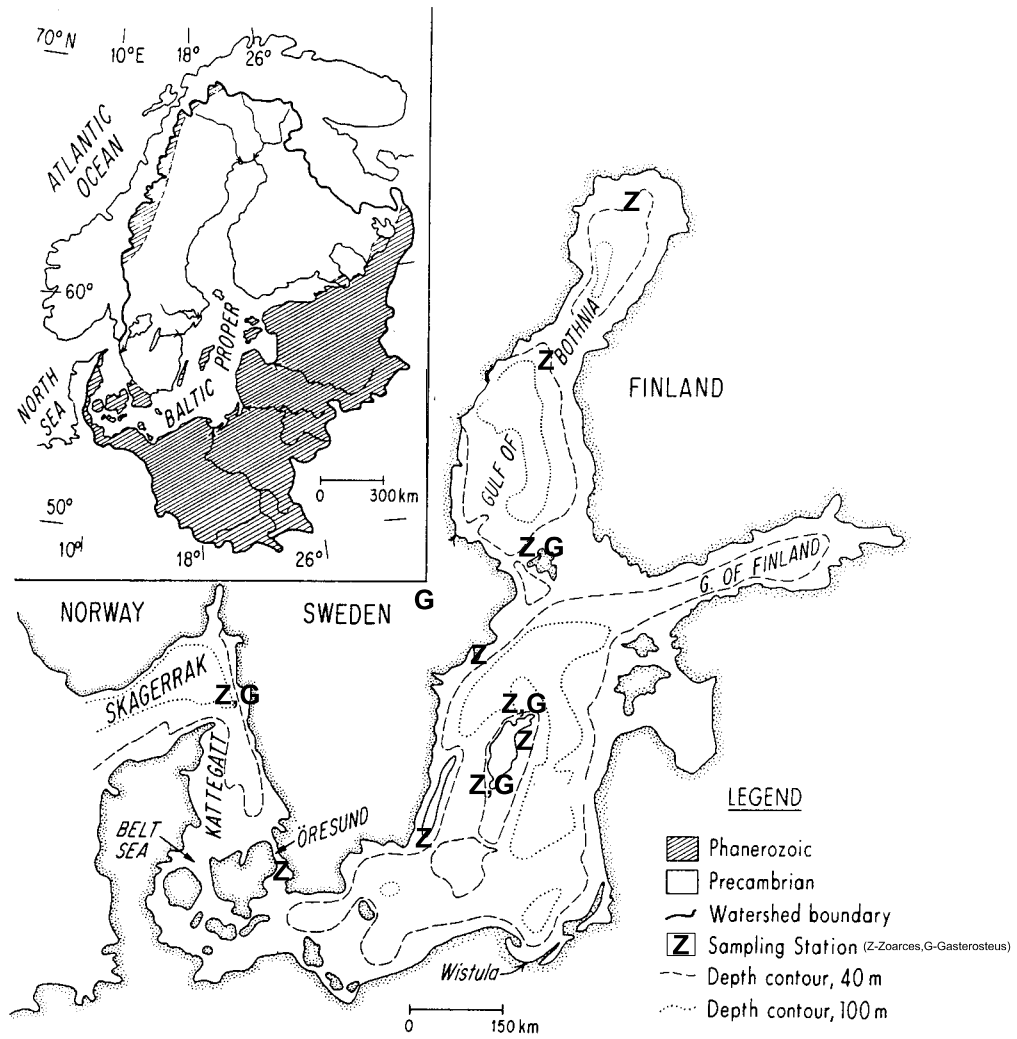


Fig. 1. Map of Baltic drainage basin, showing approximate locations of proposed sampling sites for *Zoarces* (Z) and *Gasterosteus* (G). Water will be collected at the same sites. Inset map shows major basin bedrock types.

FUNDING

- project applied for

Calculated costs (excluding overhead)

Applied for

	1998	1999	2000
Salaries and fellowship			
New postgraduate fellowship (doktorandtjänst)			
Junior researcher (forskarassistent)			
Lectorship grant (lektorsbidrag) %			
Adjunct professorship (adj professor) %			
Postgraduate exchange fellowship (öppet pd-stipendium)			
Guest researcher grant (gästforskarbidrag)			
Sum salaries and fellowships	0	0	0

Materials, technical personnel, travel costs, etc. (specify)

Fish collection (fees to contracted fishermen)	30 000	0	0
Laboratory assistant (Year 1: 4 mo, Year 2: 2 mo)	82 780	43 460	0
Travel for sampling	5 000	0	0
Water chemistry analyses (11 sites, 3 samplings, and 2 water fractions (filtered + unfiltered))	39 600	0	0
Otolith chemistry analyses (10 sites for <i>Zoarces</i> , 5 sites for <i>Gasterosteus</i> , 20 fish per site, plus 10 otolith samples for training. 160 samples will be run in Year 1, the rest (150) will be run in Year 2.)	152 000	142 500	0
Laboratory supplies (chemicals for otolith preparation, containers, etc.)	2 000	0	0
Travel to present results at international conference	0	13 500	0
Publication costs	0	5 000	0
Misc. running costs (postage, copying, phones, etc)	10 000	10 000	0
(Laboratory and office space rental charged by Stockholm University (8% of total grant) not included in total sum here, but shown on this line for completeness)	(29 217)	(19 497)	(0)
Sum materials etc.	321 380	214 460	0

Equipment (specify)

Sum equipment	0	0	0
Totals	321 380	214 460	0

RESEARCH RESOURCES

- overall summary

All funding available to the applicant
 (Amounts in SEK)

Source of grant	Project title	Granted 1997	Granted 1998	Applied for 1998
This NFR project				
Other NFR projects				
Other research councils	Revealing Complex Life Histories of Fishes Through Natural Information Storage Devices: Case Studies of Diadromous Events as Recorded by Otoliths (SJFR)			(estimated to run 3 years, total cost around 400 000 kr)
Sector authorities				
EU programme				
Other financial sources				

Balance brought forward

Balance (NFR grants) from previous year	from 95/96	estimated from 1997
---	------------	---------------------

List of personnel in the research group	Current post	Period of appointment	Source of funding	Period of funding
Main applicant Karin E. Limburg	Junior Researcher	1997-2000	SU	1997-2000
Other personnel (names) Lars Westin	Senior Lecturer	Until further notice	SU	Until further notice
Håkan Wickström	Senior Research Associate	Until further notice	Fiskeriverket	Until further notice
Henrik Svedäng	Senior Research Associate	Until further notice	Fiskeriverket	Until further notice