Bonneville Power Administration
Fish and Wildlife Program FY99 Proposal

Section 1. General administrative information

Nutritional Status Of Columbia River White Sturgeon

Bonneville project number, if an ongoing project 9150

Business name of agency, institution or organization requesting funding
Department of Food Science and Technology, and Marine/Freshwater Biomedical Sciences Center Oregon State University

Business acronym (if appropriate) OSU/MFB

Proposal contact person or principal investigator:

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Subcontractors.

Organization	Mailing Address	City, ST Zip	Contact Name

NPPC Program Measure Number(s) which this project addresses.
10.4 Sturgeon Mitigation

NMFS Biological Opinion Number(s) which this project addresses.
Though this project does not propose to directly study the Endangered Kootenai River Population of White Sturgeon, the findings of this project may benefit restoration efforts for the Kootenai Sturgeon.

Other planning document references.

Subbasin.
Lower Columbia, Lower Mid-Columbia Mainstem

Short description.
Investigate the possible role of nutritional factors in reproductive dysfunction and early life stage mortality problems limiting recruitment to white sturgeon populations in the Columbia River above Bonneville Dam.

Section 2. Key words

Mark	Programmatic Categories	Mark	Activities	Mark	Project Types
*	Anadromous fish		Construction		Watershed
X	Resident fish		O & M		Biodiversity/genetics
	Wildlife		Production	*	Population dynamics
	Oceans/estuaries	X	Research		Ecosystems
	Climate	*	Monitoring/eval.		Flow/survival
	Other		Resource mgmt	X	Fish disease
			Planning/admin.		Supplementation
			Enforcement		Wildlife habitat en-hancement/restoration
			Acquisitions		

Other keywords.
Micronutrient, antioxidant, vitamin E, iodine, selenium, thiamine, thyroid function, endocrine disruption, halogenated aromatic hydrocarbon, polychlorinated biphenyl, early life stage mortality

Section 3. Relationships to other Bonneville projects

Project #	Project title/description	Nature of relationship
8605000	White Sturgeon Productivity Status and Habitat Requirements	Source of young of year sturgeon

Section 4. Objectives, tasks and schedules

Objectives and tasks

Obj		Task	

1,2,3	Objective	a,b,c	Task
1	Determine levels of micronutrients and vitamins in serum and liver of adult white sturgeon from the Columbia R. above and below Bonneville Dam.	a	Perform ICP-MS analyses for iodine, selenium.
		b	Perform HPLC analyses for vitamin E.
		c	Use thiochrome method to assess thiamine.
2	Determine levels of certain micronutrients and vitamins in early life stages and juvenile sturgeon above and below the Bonneville Dam.	a	Perform ICP-MS analyses for iodine, selenium.
		b	Perform HPLC analyses for vitamin E.
		c	Use thiochrome method to assess thiamine.
3	Evaluate histology of thyroid of adult and juvenile white sturgeon from the Columbia R. above and below Bonneville Dam.	a	Obtain samples of thyroid tissue, fix in formalin, prepare and examine histologic sections.
4	Correlate histologic lesions of thyroid with micronutrient and vitamin status.	a	Perform statistical analysis of thyroid histologic and nutritional data to determine correlations.
5	Determine minimal dietary iodine levels, with and without sufficient dietary vitamin E and selenium, to ensure normal thyroid function in juvenile white sturgeon.	a	Raise young of the year white sturgeon in the laboratory while feeding diets containing various levels of iodine, selenium and vitamin E.
		b	Assay serum thyroxin (T4) and triiodothyronine (T3) at bimonthly interals during feeding of diets containing various levels of nutrients.
		c	At midpoint and end of feeding trials, prepare thyroid tissue from sturgeon for histologic examination.
		d	Statistically evaluate nutritional influences on thyroid hormone

			data and thyroid histologic data.
6	Determine the influence of iodine and/or vitamin E/selenium deficiency on sensitivity of juvenile white sturgeon to disruption of thyroid function by halogenated aromatic hydrocarbons.	a	Raise young of the year white sturgeon in the lab while feeding various levels of iodine and vitamin E/selenium in combination with Arochlor 1254 or a coplanar PCB.
		b	Assay serum T4 and T3 at bimonthly intervals during dietary studies.
		c	At midpoint and end of feeding trials, prepare thyroid tissue from sturgeon for histologic examination.

Objective schedules and costs

Objective #	Start Date mm/yyyy	End Date mm/yyyy	Cost %
1	10/1998	9/1999	10.00%
2	10/1998	9/1999	10.00%
3	10/1998	9/1999	7.00%
4	10/1998	9/1999	2.00%
5	10/1999	9/2001	35.00%
6	10/1999	9/2001	36.00%
			TOTAL 100.00%

Schedule constraints.
Difficulty obtaining sufficient numbers of early life stages, young of the year or yearling juvenile sturgeon at all sites for nutritional analysis during the initial year may cause delays of some of those analyses until subsequent years.

Completion date.
2001

Section 5. Budget

FY99 budget by line item

Item	Note	FY99
Personnel	Technician, 25% effort, PI, 25% effort	$21,907
Fringe benefits		$8,701
Supplies, materials, non-	For analyses of Se, I, thiamine, vitamin E;	$42,450

expendable property	preparation of histology slides	
Operations & maintenance		
Capital acquisitions or improvements (e.g. land, buildings, major equip.)		
PIT tags	# of tags:	
Travel	For sample collection—10 days X $100/d	$1,000
Indirect costs	42.5%	$31,815
Subcontracts		
Other	Publication costs, 1 manuscript	$ 800
TOTAL		$106,673

Outyear costs

Outyear costs	**FY2000**	**FY01**	**FY02**	**FY03**
Total budget	$112,960	$114,648		
O&M as % of total				

Section 6. Abstract

This project aims to clarify effects of hydropower projects on nutritional status of white sturgeon in the Columbia River. We plan to investigate levels of micronutrients, antioxidants and vitamins including iodine, selenium, vitamin E and thiamin in adult and juvenile sturgeon in the Columbia River below Bonneville Dam and to compare the nutritional status of these fish to sturgeon in impounded upstream portions of the river. Histological examination of thyroid tissue from fish collected at 5 sites will be used to correlate endocrine status with nutritional status. Dietary investigations in the laboratory using juvenile sturgeon at 2 months post-hatch will assess iodine and vitamin E/selenium requirements for optimal thyroid function and will investigate the influence of dietary deficiency of these nutrients on endocrine disruption by xenobiotics. In these laboratory studies, thyroid endocrine status will be evaluated using both histological parameters and serum thyroid hormone (T3 and T4) levels. In the first year of this study, nutritional status of fish from the Columbia River will be analyzed. In the second and third years of the study, the influence of nutritional parameters on PCB-induced thyroid dysfunction will be evaluated. These studies will yield important insights into the subtle dose-response interactions of diet and xenobiotics in determining endocrine status. Thus these proposed studies will aid in future programs to protect and augment populations of this valuable commercial species in the this river basin. These studies may also suggest novel approaches for increasing recruitment and facilitating restoration of the endangered Kootenai River white sturgeon.

Section 7. Project description

a. Technical and/or scientific background.

Historically a highly productive population of white sturgeon inhabited the Columbia River, with free access of these anadromous fish to major spawning habitats in upper reaches of the river and its tributaries, except where natural barriers prevailed. Such a natural barrier occurred at Bonnington Falls on the Kootenai River, isolating the Kootenai River white sturgeon from fish downstream, following the Wisconsin glaciation approximately 10,000 years ago. Over the past hundred years, populations of various sturgeon species worldwide have been adversely impacted by man's activities. In the late 1800's Columbia River white sturgeon populations plummeted due to overharvesting. Additional adverse effects due to habitat alteration then occurred, beginning in 1938, as a series of dams built for hydropower limited the mobility of sturgeon in the Columbia River ecosystem, and dramatically altered the structure and hydrology of riverine habitats in the system (Craig and Hacker, 1940; Birstein, 1993; Parsley and Beckman, 1994; Graham, 1981; Scott and Crossman, 1979)

Currently, portions of the Columbia River downstream of Bonneville Dam are the most productive waters for the white sturgeon throughout its range (Devore et al, 1995). In contrast, reproduction and recruitment of white sturgeon in impounded segments of river upstream of Bonneville Dam are severely limited (Beamesderfer et al, 1995; Beamesderfer and Nigro, 1993). Due to the relatively slow growth and delayed maturity of white sturgeon, these populations are slow to recover from overharvesting or environmental insults (Doroshov et al, 1997; Scott and Crossman, 1979). With the recent profound decline in several Pacific salmon populations in the Pacific Northwest, the thriving white sturgeon population in the lower Columbia River is gaining in economic importance to sport and commercial fisheries (Devore et al, 1995).

In order to address the complex task of rehabilitating sturgeon populations in the Columbia River upstream of Bonneville Dam, scientists, engineers and regulatory agencies must develop a clearer understanding of the many factors which may contribute to the poor reproduction and recruitment in these river reaches. Alterations in streamflow characteristics as well as degradation of spawning and nursery habitat have occurred throughout most of the Columbia River drainage due to damming , as well as development and agriculture in the watershed (Beamesderfer and Nigro, 1993). Clearly such environmental perturbations contribute to suboptimal productivity of sturgeon populations upstream of Bonneville Dam. However, additional factors may also play a role in limitation of the reproductive potential and recruitment success of these white sturgeon populations.

Nutritional factors have not yet been investigated as possible agents influencing productivity of impounded white sturgeon populations. Also little research has focused on possible subtle effects of environmental contaminants on sturgeon reproduction and the survival of early life stages. Possible interactions between nutritional factors and environmental contaminants in influencing health and productivity of sturgeon populations have not been addressed. Traditional anadromous sturgeon populations inhabiting the Columbia River watershed moved freely between open ocean, estuarine, and upstream freshwater spawning and nursery habitats. Thus white sturgeon evolved relying upon the rich and diverse nutritional environment of oceanic and estuarine waters.

Most inland regions of the Pacific Northwest, including the majority of the watershed of the Columbia River, are profoundly deficient in certain dietary micronutrients essential for optimal health and reproduction of vertebrate species. Domestic animal species consuming unsupplemented diets grown in these regions suffer from nutritional deficiencies of iodine and selenium (Osweiler, 1985). Profound lack of iodine and associated severe hypothyroidism impairs reproduction in adult vertebrates. Early in life, hypoiodinism causes more extreme effects, with marked dwarfism, severe neurologic impairment and neuromuscular weakness or paralysis (Hetzel, 1989). Selenium deficiency in fish and other vertebrates can result in mild, subtle problems such as immunologic impairment in the case of mild deficiency, or in severe deficiency, can result in death due to cardiovascular, hepatic or neurologic lesions (National Research Council, 1993; Fairbrother et al, 1996). Severity of goiter and the degree of impairment of human and animal reproduction and development are most pronounced in those parts of the world in which both iodine and selenium are low and diets are unsupplemented (Contempre et al, 1995).

Man's introduction of exotic fish species has perturbed aquatic food chains in certain cases and has resulted in unexpected adverse effects on the nutritional status of important game fish populations. An early life stage mortality syndrome occurs in landlocked Atlantic salmon in New York's Finger Lakes, characterized by stage-specific lesions and death affecting 100% of offspring in certain lakes. Investigations into possible causes of the syndrome ruled out toxicants and infectious agents and confirmed that the syndrome occurred due to a thiamine deficiency in offspring of female fish which consume a diet composed principally of alewives. The thiaminase content of alewives causes a subclinical thiamine deficiency in adult salmon. Because of the higher requirement for thiamine in the rapidly growing early life stages, pathologic lesions and mortality occur (Fisher et al, 1996; Fisher et al, 1995). A wide variety of freshwater and saltwater finfish, molluscs and crustaceans are known to contain differing levels of thiaminase (National Research Council, 1983), however, the distribution of thiaminase in sturgeon dietary components in the Columbia River watershed has not been investigated..

In the past 5 years, increasing concern by scientists, governmental regulatory agencies and the public has focused upon possible adverse effects on endocrine systems of animals following exposure to xenobiotic agents in the environment. With a dizzying array of species studied, target organs or tissues, assay systems and research groups, much controversy has arisen regarding the validity and significance of reported findings (Ashby et al. 1997; Colborn et al. 1993; Colborn and Clement 1992; Goodbred et al, 1997). Much of the research on environmentally-induced endocrine disruption has evaluated toxicants for estrogenic activity and associated reproductive dysfunction. Fewer investigators have considered xenobiotic effects on thyroid function, including secondary effects on other endocrine systems. Because the thyroid is a central integrative control point in many endocrine and physiologic systems, its role in toxicant-related endocrine alteration is important to consider. In any case, all endocrine systems are highly interrelated and reciprocally influence other neuroendocrine systems as well as essentially all physiologic mechanisms in the body of vertebrates. In both mammals and fish, perturbation of the thyroid can impair reproduction (Chapin et al. 1996; Cyr and Eales 1996). During initial development of standardized protocols for assessment of contaminant-related endocrine disruption, a holistic, intact vertebrate animal system is needed to define the complex interrelationships of the various segments of the neuroendocrine system as a whole. Only whole animal systems will allow accurate clarification of the many confounding variables such as diet which may influence xenobiotic-induced endocrine perturbation (LeBlanc et al. 1997). Subsequently, more reductionistic in vitro systems will be essential to dissect cellular and molecular mechanisms of toxicant action in particular segments of the neuroendocrine system.

For 20 years scientists studying halogenated aromatic hydrocarbons (HAH) including polyhalogenated biphenyls, dibenzodioxins and dibenzofurans have recognized morphologic and functional alterations in the thyroid of vertebrate species exposed to this family of xenobiotics (Mann 1997; Soontornchat et al. 1994). Depending on the extent of intoxication, thyroid impairment can be subtle and detectable only histologically or with thyroid hormone assays, or frank goiter may be evident. In addition, increased TSH secretion, resulting from lowered circulating T3 and T4 in HAH-treated rodents, may act as a tumor promoter, increasing the incidence of spontaneous and carcinogen-induced thyroid neoplasia. HAH disrupt thyroid function at least in part by increasing peripheral degradation of thyroid hormones via induction of hepatic microsomal enzymes (Capen 1997). HAH also increase the levels of oxidant damage in treated animals, and this oxidant-induced tissue damage may contribute to thyroid dysfunction (Hassoun et al. 1994; Palace et al, 1996). Since thyroid hyperplasia, due to a variety of nutritional or toxicant causes, increases production of hydrogen peroxide in thyroid (Capen 1997), concurrent iodine deficiency, selenium deficiency and treatment with HAH may act synergistically in disrupting thyroid dysfunction. Elevated levels of PCBs and halogenated dioxins and dibenzofurans are present in fish in the Columbia River watershed (Parsons et al, 1991; Washington State Department of Health/Oregon Health Division, 1996; Tetratech, 1996). Public and regulatory concern centers on unanswered questions regarding subtle chronic effects of these agents on the health, endocrine status and reproduction of fish and wildlife in this region. Possible health effects in people following consumption of fish and wildlife from this watershed are also of concern.

A variety of Great Lakes fish species, particularly salmonids, have exhibited endemic goiter (Leatherland, 1992). The Great Lakes geologic region is known to be low in both selenium and iodine, requiring nutritional supplementation of domestic animals consuming diets grown in these regions (Osweiler, 1985). However, the possible relative role of nutritional factors in goiter and reproductive endocrine disruption in Great Lakes watersheds has not yet been clarified. Leatherland and his colleagues have extensively investigated endocrine parameters and effects of Great Lakes contaminants, particularly organochlorines and HAH, on endocrine systems and reproduction in salmonids (Leatherland, 1992; Leatherland and Sonstegard, 1987). These investigations did not examine possible interactions between micronutrient status of fish and contaminant effects.

Fish pathologist Dr. Jerry Hendricks in the Department of Food Science and Technology at Oregon State University has observed a high prevalence of grossly or microscopically visible thyroid follicular hyperplasia in certain toxicant experiments conducted in rainbow trout (Jerry Hendricks, personal communication). In these experiments, control fish which received no xenobiotic treatment had grossly normal thyroids. Rainbow trout fed 100 ppm Aroclor 1254 in the diet for 1 year had essentially 100% prevalence of microscopic thyroid follicular hyperplasia if they were fed diets marginal in iodine, but not if fed diets containing optimal iodine levels. Dose-response relationships in this interaction between PCBs and dietary iodine have not yet been explored, so that the lowest dietary level of particular PCB mixtures or specific congeners which might cause thyroid dysfunction in the face of iodine deficiency of varying degrees remains uncertain. Since iodine requirements of various species of fish are not yet well defined (National Research Council, 1993), sturgeon may require different levels of dietary iodine for optimal physiologic function than do those species in which nutritional requirements have been more completely defined.

This project will advance efforts in mitigation of adverse effects of hydropower projects on white sturgeon populations above the Bonneville Dam. This work fits within the objective of the 1994 Fish and Wildlife Program to protect and increase populations of white sturgeon in the Columbia River Basin. Consideration of nutritional factors in white sturgeon populations might mitigate losses in place and in kind. Dr. Spitsbergen has collaborated over the past year with Dr. Marty Fitzpatrick of the Department of Fisheries and Wildlife at Orgeon State University and with Dr. Gene Foster of the Oregon Department of Environmental Quality to evaluate the reproductive and endocrine status of white sturgeon collected at various sites above and below Bonneville Dam. Dr. Spitsbergen has examined gonadal and liver samples from these fish for histologic lesions. Also, while on the faculty at Cornell University, Dr Spitsbergen collaborated with Dr. Kofi Fynn-Aikins of the Tunison Laboratory of Aquatic Science (then a U.S. Fish and Wildlife Service Lab, now part of USGS) to evaluate possible pathologic effects of high carbohydrate diets on Atlantic sturgeon . In these studies, light microscopic and ultrastructural features of liver, as well as serum biochemical factors indicative of liver function were investigated. Dr. Spitsbergen and colleagues from the University of Wisconsin at Madison, including Dr. Richard Peterson, studied early life stage toxicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in lake trout and found this species highly sensitive to cardiovascular toxicity of TCDD following egg exposure to TCDD at toxicant levels present in more contaminated regions of the Great Lakes. Dr. Spitsbergen and former Ph.D. candidate at Cornell, Dr. Jeff Fisher, investigated etiologic factors involved in the early life stage mortality syndrome of landlocked Atlantic salmon from New York's Finger Lakes, demonstrating that a thiamin deficiency resulting from a diet high in thiaminase-containing alewives was the principal cause of this mortality syndrome.

b. Proposal objectives.

Objective 1. To determine the nutritional status of Columbia River white sturgeon below Bonneville dam and upstream to waters including the McNary Reservoir.

a. To test the hypothesis that white sturgeon impounded above the Bonneville Dam are deficient in dietary iodine and selenium in comparison to sturgeon which have access to marine and estuarine habitats below Bonneville Dam. Vitamin E levels are evaluated as this antioxidant strongly influences the requirement for the antioxidant micronutrient

selenium (Poston et al, 1976).
c. To test the hypothesis that white sturgeon impounded above the Bonneville Dam are deficient in dietary thiamine due to alterations in dietary components resulting from habitat changes associated with damming of the upper Columbia River.

Objective 2. To evaluate thyroid histology as an indication of the integrity of the endocrine system of Columbia River whiter sturgeon below the Bonneville Dam and above the Bonneville Dam including the McNary Reservoir.

a. To test the hypothesis that low dietary iodine levels above the Bonneville Dam result in thyroid dysfunction in white sturgeon indicated by thyroid follicular hyperplasia, whereas sufficient dietary iodine available to sturgeon inhabiting waters below the Bonneville Dam results in normal thyroid function and histology.

Assumption: Effects of stress on thyroid hormone parameters, will contraindicate evaluation of thyroid status by measurement of thyroid hormones in sturgeon held for commercial slaughter. Only in rare cases where sturgeon could be caught and blood sampled immediately could one safely assess serum thyroid hormones as an indication of thyroid status of field-collected fish.

Objective 3. To determine the minimal dietary iodine level to ensure optimal thyroid function in white sturgeon and to determine the influence of dietary selenium and vitamin E levels on this iodine requirement.

a. To test the hypothesis that dietary iodine levels below 1 ppm will result in thyroid impairment in white sturgeon as indicated by reduced serum levels of thyroxine (T4) and triiodothyronine (T3) and by histologic thyroid follicular hyperplasia.

b. To test the hypothesis that marginal or low dietary levels of both vitamin E and selenium in combination with dietary iodine below 1 ppm will result in more severe thyroid impairment than a deficiency of iodine alone, as indicated by more severe reduction in serum levels of thyroxine (T4) and triiodothyronine (T3) and by more severe histologic thyroid follicular hyperplasia.

Objective 4. To determine the influence of marginal or low dietary iodine alone, or in combination with low dietary vitamin E and selenium on the sensitivity of juvenile white sturgeon to disruption of thyroid function by xenobiotics, such as a mixture of polychlorinated biphenyls or a pure coplanar PCB.

a. To test the hypothesis that low or marginal dietary iodine levels will increase the sensitivity of white sturgeon to disruption of thyroid function following dietary exposure to Aroclor 1254 or the coplanar polychlorinated biphenyl 3,3',4,4',5,5'-hexachlorobiphenyl (HB), as indicated by lower serum thyroid hormone levels and increased severity of histologic thyroid follicular hyperplasia in fish with combined nutritional deficiency and toxicant exposure.

b. To test the hypothesis that more severe thyroid dysfunction will occur in white sturgeon with combined dietary deficiency of iodine, selenium and vitamin E, in comparison to iodine deficiency alone, when exposed to dietary Aroclor 1254 or HB.

c. Rationale and significance to Regional Programs.

Sturgeon restoration programs funded by the Bonneville Power Administration have involved extensive collaboration among state and federal agencies as well as academia, particularly following the Pacific Northwest Electric Power Planning and Conservation Act of 1980 which emphasized mitigation of hydropower impacts on fish and wildlife in the Columbia River system. To date, these initiatives have assessed population levels, growth, reproductive status and recruitment of white sturgeon throughout the Columbia basin. The influences of streamflow and habitat structure on reproductive activity and survival or abnormalities of young have been studied. Feeding ecology has been investigated in terms of dietary components and selection of prey (Beamesderfer et al, 1995; Beamesderfer and Nigro, 1993; Devore et al, 1995). Techniques for hatchery rearing of sturgeon have been developed and strategies for preserving genetic diversity have been considered (Dettlaff et al, 1993; Cui et al, 1997; Kincaid, 1993). Particular concern has focused on the Kootenai population of white sturgeon following their designation as an endangered species (Federal Register, 1996). Currently, a basin-wide collaborative project (8605000) is administered by the Oregon Department of Fish and Wildlife titled “White Sturgeon Productivity Status and Habitat Requirements”. Additional aquaculture and ecosystem improvement projects focus on particular subbasins or waterways such as the Kootenai River.

Investigations of nutritional status and possible interactions of nutritional status with xenbiotic effects on endocrine function, reproduction and early life stage survival would be a valuable addition to these ongoing studies and would not duplicate current work. During the past year, I have begun collaborating with Dr. Gene Foster of the Oregon Department of Environmental Quality and with Dr. Marty Fitzpatrick of the Department of Fisheries and Wildlife at Oregon State University in their work with the Oregon Department of Fish and Wildlife to assess endocrine and reproductive parameters in white sturgeon from unimpounded and impounded portions of the Columbia River. I have contacted Mike Parsley of the USGS in Washington State to arrange possible collaboration in collection of early life stages and juvenile white sturgeon from the Columbia River in the fall of 1998. Mr. Parsley stressed the need for more detailed assessment of both caloric and nutrient components available in the diets of sturgeon in various Columbia River habitats. The findings of these studies will be reported at fish health meetings and in peer-reviewed scientific journal articles. Hopefully findings from these studies will lead to management changes increasing recruitment in impounded sturgeon populations in the Columbia River basin.

d. Project history

e. Methods.

Assessment of Nutritional Status and Thyroid Histology of Columbia River White Sturgeon—Objectives 1 and 2

Fish will be sampled from 5 sites in the Columbia River, downstream of the Bonneville Dam and from each of the reservoirs above the Bonneville, Dalles, John Day and McNary Dams. Adult white sturgeon will be obtained from commercial fisheries and juvenile sturgeon and eggs or larvae will be obtained by collaboration with federal or state agencies performing field collections. Blood will be collected by caudal vein puncture from adult sturgeon. Following clot retraction, blood will be centrifuged and serum will be transferred to vials. Liver samples or whole eggs and larvae will be placed into sealed vials. Samples will be placed on dry ice until transferred to a -20 C freezer for storage. Liver from 10 adult fish from each of the 5 sites will be analyzed for iodine, selenium, vitamin E and thiamin levels, as well as for a panel of macronutrients, micronutrients and metals. At each of the 5 collection sites, liver will be obtained from each of 5 young of the year or year-old juvenile fish. At each of these 5 sites, 5 pools of 200 eggs or larvae will be obtained. Selenium and iodine will be quantified in serum or tissue by inductively coupled plasma mass spectrometry (ICP-MS) (Abou-Shakra et al, 1997). Inductively coupled argon plasma emission spectrometry (ICP)(Poston and Ketola, 1989) will be used to determine levels of minerals or metals including K, P, Ca, Mg, Mn, Fe, Cu, Zn, Mo, Al, Co, Cd, Cr, Ni, Pb, S, V and As in tissue. Vitamin E levels in liver will be determined by high performance liquid chromatography (HPLC) (Cowey et al, 1981). Thiamin will be determined by the thiochrome method (AOAC International, 1990).

Thyroid tissue will be dissected from the pharyngeal region of adult and juvenile sturgeon and will be fixed in 10% neutral buffered formalin. If larvae are obtained live, whole individuals will be anesthetized in tricaine methanesulfonate (MS222) and placed whole into neutral buffered formalin. Tissue from adults or juveniles will be embedded in paraffin, sectioned at 4 micrometer intervals and stained with hematoxylin and eosin. Tissue from larvae will be embedded in glycol methacrylate, sectioned at 4 micrometer intervals and stained with hematoxylin and eosin.

Dietary Studies Evaluating Iodine and Vitamin E/Selenium Requirement for Optimal Thyroid Function in White Sturgeon and Evaluation of the Influence of These Nutrients on Xenobiotic-Induced Thyroid Dysfunction—Objectives 3 and 4

General Experimental Design

The following table outlines the factors studied in dietary experiments. Basal, low and very low levels of vitamin E, selenium (sodium selenite as source) and iodine (potassium iodide as source) are 150 ppm, 20 ppm and 2 ppm, 0.2 ppm, 0.05 ppm and 0.02 ppm, and 1 ppm, 0.3 ppm and 0.1 ppm, respectively. In year 2, Aroclor will be examined together with the 3 nutritional parameters. In year 3, HB will be examined in dietary experiments. In each of years 2 and 3, 45 treatment combinations will be studied in a 3 X 3 X 5 factorial design, with dietary vitamin E/Se level, iodine level and dietary PCB level serving as the 3 factors. Note that vitamin E and Se levels will be varied together, not independently.

Vitamin E	Selenium	Iodine	Aroclor 1254	HB[1]
150 ppm	0.2 ppm	1 ppm	50 ppm	10 ppm
20 ppm	0.05 ppm	0.3 ppm	25 ppm	3 ppm
2 ppm	0.02 ppm	0.1 ppm	5 ppm	1 ppm
			1 ppm	0.1 ppm
			0 ppm	0 ppm

[1] HB=3,3',4,4',5,5'-hexachlorobiphenyl

Diet Formulation and Preparation

Basal Oregon Test Diet will be formulated and prepared as previously described (Lee et al, 1991). PCBs will be mixed with the fish oil component overnight prior to diet preparation. Dietary residues of I, Se and vitamin E will be confirmed using the same methodology applied to fish tissues. Dietary PCB residues will be confirmed by the Mass Spectrometry Center of the Environmental Health Sciences Center at OSU using GC/MS.

Fish Husbandry and Sampling

Fish will be obtained from the Abernathy Salmon Culture Center, U.S. Fish and Wildlife Service, Longview, WA or from the aquaculture program at the University of California at Davis at approximately 45 days post-hatch. Following a 2-week acclimation to the laboratory conditions, treatment groups of 100 fish will be randomly assigned to each of the 45 treatment combinations and will be maintained in flowing well water at 18-20 C. Fish will be fed experimental diets at a rate of 2% of body weight per day. At bimonthly intervals up to 8 months following the onset of dietary treatments, 20 fish will be sampled from each treatment group. To ensure consistent thyroid hormone values relative to diurnal fluctuations, fish will be sampled at a consistent time of day. Fish will be anesthetized in MS222 and blood will be obtained from the caudal vein. Following clot retraction, blood will be centrifuged, serum withdrawn and stored at -20 C until analyzed. Thyroid tissue will be fixed and processed for histologic examination as previously described in the above section. T3 and T4 assays will be conducted using radioimmunoassay (Eales and Brown, 1993;Youson et al, 1994). PCB residues will be assessed in tissues collected from fish at the termination of the dietary experiments. Two pools of tissue, each prepared from 5 fish, from each of the PCB treatment levels will be assessed using GC/MS. At termination of the experiments, serum and liver vitamin E, Se, thiamin and other micronutrients/metals will be assessed as in field studies in at least 10 fish on basal diet, 10 fish on diets with low nutrient content, and 10 on diets with the maximum dose of PCBs in combination with the various nutrient levels in diets.

Statistical Data Analysis

Data will be analyzed using the General Linearized Modeling (GENMOD) procedure using SAS 6.11 statistical software (SAS, 1996). Appropriate parametric or nonparametric multiple comparisons of treatment group means will be used as needed. Lesion prevalence data will be analyzed using a Chi-square test, Fisher Exact test or Mantel-Haenszel test (Matthews and Farewell 1996).

Links to Other Work

Sample collection from adult sturgeon will be conducted in conjunction with reproductive and endocrine studies to be pursued by Dr. Gene Foster of Oregon Department of Environmental Quality and Dr. Marty Fitzpatrick of the Department of Fisheries and Wildlife at Oregon State University, so that body length/weight, gonadal-somatic index, gonadal histology and sex steroid parameter assessment can be correlated with nutritional factors and thyroid histology to be assessed in this proposed study.

f. Facilities and equipment.

Oregon State University has coordinated a National Institutes of Health/National Institutes of Environmental Health Sciences-Funded Environmental Health Sciences Center since 1967, and has also housed a Marine/Freshwater Biomedical Sciences Center for the past 11 years. Oregon State University is the only institution in the United State to house both an Environmental Health Sciences Center and a Marine/Freshwater Center. These centers provide core support in mass spectrometry, analytical chemistry, flow cytometry, image analysis and cell culture. The Food Toxicology and Nutrition Laboratory in the Department of Food Science and Technology is the world's largest experimental rainbow trout research facility, allowing us to conduct large-scale carcinogenisis studies to study the low end of carcinogen dose-response curves. The fish hatchery and histopathology complex is a 15,000-sq. ft. facility housing broodstock and supporting all stages of salmonid aquaculture. The facility houses 511 fiberglass fish tanks, ranging in size from 2 to 10 ft in diameter. A separate 1,400 sq.ft. portion of the facility provides a southern hemisphere lighting regimen to allow two a fall and a spring spawning season. A 544-sq.ft. warmwater fish room is available and has been used for rearing medaka, zebrafish and tilapia. Chilling and heating capacity can provide water at a variety of constant temperatures for research purposes. OSU has developed a semi-purified diet into which a variety of toxicants and nutritional supplements can be incorporated. Dr. Spitsbergen's office at the Food Toxicology and Nutrition Laboratory (FTNL) is equipped with a Pentium-based IMB PC, with CDROM drive and runs Windows 95 software with SAS, Minitab and Statgraphics statistical software. A microprobe apparatus for immunohistochemical staining of microscope slides is available at the FTNL. Several center investigators have fully equipped laboratories for molecular biology research regarding proteins, RNA or DNA.

Collaboration with Dr. Marty Fitzpatrick in the Department of Fisheries and Wildlife at Oregon State Univeristy will facilitate conducting the radioimmunoassays for thyroid hormones. Dr. Fitzpatrick works closely with the Oregon Cooperative Fishery Research Unit at OSU and is fully equipped and has extensive experience conducting radioimmunoassays for a variety of hormones of fish. Dr. Fitzpatrick also has raised white sturgeon at OSU had can provide advice on sturgeon husbandry.

g. References.

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Section 8. Relationships to other projects

The proposed work complements and will involve collaboration with NWPPC FY 98 Project Number 8605000 titled "White Sturgeon Productivity Status and Habitat Requirements" which will evaluate and work to restore sturgeon populations throughout the Columbia Basin. Sample collection from adult sturgeon for the proposed study will be conducted in conjunction with reproductive and endocrine studies to be pursued by Dr. Gene Foster of Oregon Department of Environmental Quality and Dr. Marty Fitzpatrick of the Department of Fisheries and Wildlife at Oregon State University, so that body length/weight, gonadal-somatic index, gonadal histology and sex steroid parameter assessment can be correlated with nutritional factors and thyroid histology to be assessed in this proposed study. Collection of early life stages and juvenile sturgeon will be conducted in cooperation with the USGS through Washington State contact Mike Parsley.

Section 9. Key personnel

Jan Spitsbergen, DVM, PhD. - Dr. Spitsbergen is the PI coordinating the project and will spend 25% of her effort on the project during the funding years. She is a board-certified veterinary pathologist who has studied toxicant effects on fish since 1982. She has conducted or supervised fish toxicology research in immunotoxicology, reproductive/developmental toxicology, virology/cell culture, nutritional pathology, field tumor epidemiology and experimental carcinogenesis.

EDUCATION:

Michigan State University, B.S. in Fisheries and Wildlife, March 1976. Major field of study fisheries and limnology, minor biochemistry.

Michigan State University College of Veterinary Medicine, D.V.M., June, 1980.

Cornell University, Ph.D. in immunology and pathology, January, 1986. Minor field of study toxicology.

RESEARCH AND PROFESSIONAL EXPERIENCE:

1995-present Research Associate, Department of Food Science and Technology, Oregon State University, Corvallis, OR.

1988-1995 Assistant Professor, Department of Avian and Aquatic Animal Medicine, Cornell University, Ithaca, NY.

1986-1988 Research Associate, School of Pharmacy, University of Wisconsin, Madison

HONORS, AWARDS, CERTIFICATIONS AND PROFESSIONAL ACTIVITY

Phi Zeta, June, 1979; Catherine Patton Award in Veterinary Physiology, June, 1978; Diplomate, American College of Veterinary Pathologists, 1987; Most Significant Paper in Journal of Aquatic Animal Health in 1995 (7:269-283); Member of American Veterinary Medical Association, Fish Health Section of American Fisheries Society, Society of Toxicologic Pathologists

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Section 10. Information/technology transfer

Findings from proposed field and laboratory studies will be presented at scientific conferences such as those held by the Fish Health Section of the American Fisheries Society, the Society of Environmental Toxicology and Chemistry or the Society of Toxicologic Pathologists. Manuscripts reporting research findings will be published in peer-reviewed scientific journals. The Environmental Health Sciences and Marine/Freshwater Biomedical Sciences Centers at OSU cooperate in Outreach programs explaining the significance of new research findings to professionals, students and the lay public.