

中国水产科学研究院长江水产研究所
农业部淡水鱼类种质资源与生物技术重点开放实验室

论文汇编

(1996—1999.6)

Proceedings(1996—1999.6)

Key Laboratory of Freshwater Fish Germplasm Resources
& Biotechnology, Ministry of Agriculture Yangtze River
Institute of Fisheries, Chinese Academy of Fishery Sciences

一九九九年六月

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农业部淡水鱼类种质资源与生物技术 重点开放实验室简介

1. 实验室概况

淡水鱼类种质资源与生物技术实验室是 1985 年 7 月经国家计委审批并投资 300 万元人民币建立的国家重点实验室,其依托单位为中国水产科学研究院长江水产研究所。1989 年 6 月实验室建成并通过了国家验收,1990 年 2 月正式向国内外开放。1996 年 11 月更名为农业部淡水鱼类种质资源与生物技术重点开放实验室。

实验室设立了由所内外 12 名专家(其中所外专家 8 人)组成的学术委员会。吴清江研究员任学术委员会主任,余来宁研究员和张荣权副研究员任学术委员会副主任。学术委员会的主要任务是对实验室的研究方向、开放课题的设置和研究工作进展进行审核和评议,协调和组织国内外学术交流活动及监督经费使用等。

实验室主任由长江水产研究所常务副所长余来宁担任,陈松林研究员、张四明副研究员和危起伟副研究员任实验室副主任。实验室下设鱼类种质资源、鱼类基因工程、鱼类细胞工程、鱼类分子内分泌学和鱼类生态五个学科组。目前,实验室拥有固定人员 25 人,其中正、副研究员 11 人,助理研究员 7 人,其他人员 7 人。本室与华中农业大学水产学院共同拥有水产养殖博士点,同时与上海水产大学联合招收硕士研究生。本室可同时接纳 10 名客座研究人员及 10 名研究生。同时,农业部淡水养殖种质监测中心也依托本实验室。

2. 主要研究方向和内容

实验室的主要研究方向是:进行淡水鱼类生物学、种群组成和遗传结构及变化机理与规律,细胞和分子遗传学基础和应用基础研究;进行种质资源保存及利用,养殖及鱼病防治等应用基础和生物技术研究。

鱼类种质资源研究:主要进行淡水鱼类种质鉴定,鱼类原、良种种质标准参数建立及在群体、个体、细胞和分子水平上保存保护淡水鱼类种质资源的应用基础和应用技术研究,应用分子生物学技术进行鱼类种群遗传结构,遗传标记及种内与种间系统进化的研究。

鱼类基因工程研究:主要进行鱼类有用目的基因(生长激素、转铁蛋白等)的分离与克隆,转基因鱼构建和转基因细胞系构建等研究;进行主要养殖鱼类病原微生物(如嗜水气单胞菌)毒素基因表达与调控,核酸探针制备及疾病检测以及应用分子生物学技术进行鱼病防治研究。

鱼类细胞工程研究:主要进行鱼类多倍体诱导和单性化育种技术研究,进行鱼类细胞核移植培育抗病草鱼的研究,进行鱼类细胞培养、单克隆抗体制备及抗病细胞疫苗规模化生产的研究

究。

鱼类分子内分泌学研究:主要进行鱼类垂体及下丘脑激素分离纯化、生化性质的研究,进行鱼类垂体激素单克隆抗体制备及酶联免疫吸附测定技术的研究;进行鱼类生长、发育和繁殖过程中体内激素(如生长激素)变化规律及其调控技术的研究。

鱼类生态学研究:主要包括长江鲟鱼类保护对策研究,中华鲟资源变动和发展趋势研究,应用超声波遥测追踪技术研究中华鲟的洄游规律、产卵行为和产卵生态条件;中国鲢鱼保护生物学研究;进行水库移植银鱼基础生态学研究等。

3. 科学研究情况

近几年来,实验室先后共承担了国家自然科学基金、国际科学基金、国家“948 项目”,国家“九五”科技攻关及农业部重点项目等近 30 个项目的研究。在已完成的研究项目中,有些取得重大成果。例如“超低温保存淡水鱼类精液技术及冻精库设计与建设”的研究课题对鲤科养殖鱼类精子的低温生物学、抗冻剂、冷冻保存及解冻授精方法进行了系统研究,提出了一套行之有效,可应用于生产的鲤科鱼类精子冷冻保存和冻精授精技术,获得了农业部科技进步贰等奖;“草鱼出血病细胞培养灭活疫苗的研究”攻关专题研制出了纯度高、免疫效果好、稳定和安全的培养细胞,建立了工厂化生产工艺,在草鱼出血病防治中具有重要意义和应用价值,该子专题并入“草鱼出血病防治技术”专题,先后获得农业部科技进步一等奖和国家科技进步一等奖。“草鱼生长激素分离、单抗制备及酶标免疫测定技术建立和应用的研究”在国际上首次建立了鲤科鱼类生长激素单抗细胞株和 ELISA 测定技术,获农业部 1996 年科技进步贰等奖。国家“八·五”攻关项目“天鹅洲通江故道四大家鱼种质资源天然生态库研究”,获国家 1998 年科技进步叁等奖。近年来,采用先进的技术在国家一级保护鱼类中华鲟遗传资源的保护、开发以及群体遗传和鲟鱼类的分子进化研究方面取得了很大成就,其结果将会在《中国科学》、《自然科学进展》、《科学通报》等国家最权威刊物发表。建室以来,实验室固定人员在国内外学术会议、国内外学术刊物上共发表论文 200 余篇。

目前承担的研究课题主要有:(1)淡水养殖品种种质鉴定技术研究;(2)应用分子生物学技术进行主要养殖鱼类不同群体遗传结构与遗传多样性研究。(3)以遗传标记和生物工程(为雌核发育)为手段进行白鲢选育研究。(4)中华鲟分子群体遗传学和相关种类的分子进化研究。(5)鱼类性别决定因子研究;(6)鲫转铁蛋白基因和嗜水气单胞菌毒素基因克隆与应用研究;(7)“暴发性”鱼病防治技术的研究;(8)中华鲟产卵场地和产卵条件研究、超声波遥测技术对中华鲟繁殖生态的研究(中美合作)。(9)中华鲟资源数量变动和发展趋势的研究和中华鲟资源监测研究等系列课题。(10)中国鲢鱼驯化和人工繁殖技术的研究。

4. 实验室主要仪器设备

本实验室具有从事鱼类种质资源保存、细胞工程、基因工程及分子内分泌学研究所需的主要仪器设备:如 DNA 合成仪、多肽序列仪、高效液相色谱仪,超高速离心机、图象分析仪、液体闪烁计数器、基因转移仪、双波长薄层扫描仪、紫外可见分光光度计、透射电镜、电泳仪、微量电子天平、万能显微镜、倒置显微镜、PCR 仪、酶标仪、低温与超低温冻箱、水生动物超声波遥测追踪仪、探鱼仪、电子流速仪、GPS 接收仪,以及考察专用快艇等。

5. 对外开放、交流与合作

实验室始终坚持“开放、流动、联合”的运行机制,制订优惠政策,吸引科技人员来实验室合作研究,扩大实验室的开放度。在经费十分困难的情况下,积极筹措经费,设立开放课题基金。迄今,共组织评审和资助了三期开放课题。第一期开放课题基金资助了4个研究单位的7个研究课题,其中包括中科院发育所、南京农业大学及浙江省水产研究所的申请项目,第一期开放课题基本按计划完成任务,已于1993年底进行了验收和鉴定。第二期开放课题基金共资助了包括中科院水生所、中科院发育所及中国水产科学研究院淡水渔业研究中心和黑龙江水产研究所等单位的12项研究课题,97年结束。第三期开放课题共资助了武汉大学、华中农业大学、西南农业大学,上海水产大学等单位共12个研究课题,目前正在研究之中。

实验室目前与美国、加拿大、德国、法国及日本等国的7个实验室建立了合作交流关系。已有加拿大多伦多大学、美国 Tennessee 技术大学、美国野生生物与鱼类保护局,德国乌尔兹堡大学及日本广岛大学等国家的10余名专家教授来实验室访问、讲学与交流,正同美国国家生物考察局进行中华鲟合作研究,与德国乌尔兹堡大学进行鱼类性别决定因子的研究。同时,本室也派科技人员到美国、法国、加拿大、德国、日本、印度、泰国、巴基斯坦、新加坡、孟加拉国等进行合作研究,考察或出席国际学术讨论会。此外,本室曾经多次主持或参与主持全国性的学术讨论会,如1994年9月在沙市主持召开了“全国鱼类种质资源与生物技术学术讨论会”。由本实验室和中国科学院发育生物学研究所共同主办的第10届国际同工酶及基因家族国际学术大会1999年10月份将在北京召开。

本实验室热忱欢迎有志于鱼类种质资源与生物技术研究的国内外科技人员来室工作、合作研究或学术交流。对获得硕士、博士学位的科研人员,归国留学生及进修人员优先接纳,并提供生活及工作条件。

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A Brief Introduction of the Key Laboratory of Freshwater Fish Germplasm Resources & Biotechnology, Ministry of Agriculture

1. General conditions

The Laboratory of Freshwater Fish Germplasm Resources & Biotechnology based at Yangtze River Institute of Fisheries of CAFS was one of the state key laboratories that had been constructed with the approval of the state planning commission in July, 1985 and its investment of 1 million US dollars. In 1989, the laboratory got through the check and acceptance of the state departments concerned. Since February, 1990, it has been formally opened toward home and abroad. In November 1996 the name of laboratory was changed into Key Laboratory of Freshwater Fish Germplasm Resources & Biotechnology, Ministry of Agriculture.

The laboratory has established an academic committee, consisting of 12 experts within and outside the institute (including 8 outside experts), with Professor Wu Qingjiang as a chairman. Its main responsibilities are to examine the research direction of the laboratory and the emplacement of open subjects, to coordinate and organize academic exchange activities at home and abroad, and to supervise fund expenditures.

Professor Yu Laining, deputy director of the Yangtze River Institute of Fisheries, concurrently holds the post of director of the laboratory, under whom there are three deputy directors including Professor Chen Songling, associate professor Zhang Siming and associate professor Wei Qiwei. The laboratory is divided into five sections, namely, fish germplasm resources group, fish gene engineering group, fish cell engineering group, fish molecular endocrinology group and fish ecology group. The fixed personnel in the laboratory totals 25, of which 11 are senior scientists, and 7 assistant research fellows. The laboratory can jointly admit PhD and Ms graduate students with Huazhong Agricultural University and Ms student with Shanghai Fisheries University. The laboratory can simultaneously accommodate 10 guest researchers and 10 graduate students. The Monitoring Center of Ministry of Agriculture of Freshwater Fish Germplasm Resources is based at the laboratory.

2. Research directions and fields

The laboratory conducts researches on freshwater fish biology, population compositions and genetic structures, population dynamics and laws and on cytogenetics and molecular genetics as well. In addition, the laboratory is engaged in the researches on preservation and exploitation of germplasm resources, aquaculture, fish disease control, etc.

Research on fish germplasm resources: Mainly engaged in the identification of freshwater fish germplasm, the development of standard parameters in germplasm of the original fish species and their

improved varieties, the study on the preservation and protection of freshwater fish germplasm resources at population, individual, cell and molecule levels, the study on fish population genetic structures and micro- and macroevolution by the molecular approaches.

Research on fish gene engineering: Mainly engaged in the isolation and cloning of useful object genes, the construction of transgenic fishes and cell lines, the study on the expression and regulation of toxin gene from pathogenic microorganisms in major cultivated fishes, the preparation of nucleic acid probes and disease detection with them and the study on fish disease control by techniques in molecular biology.

Research on fish cell engineering: Mainly engaged in the study on the techniques for the induction of fish polyploids and for monosex breeding, the study on the breeding of disease resistant grass carp by nuclear transplantation and the study on fish cell culture, monoclonal antibody preparation and scale - production of disease - resistant cell vaccine.

Research on fish molecular endocrinology: Mainly engaged in the isolation and purification of fish pituitary and hypothalamic hormones and the study on their biochemical properties, the study on the techniques for the preparation of fish pituitary hormone monoclonal antibodies and for enzyme - linked immunosorbent assay, and the study on fish growth and reproduction endocrinology.

Research on fish ecology: The activities are involved in the studies on conservation strategies of sturgeons and paddlefish in Yangtze River, the population dynamics of Chinese sturgeon *Acipenser sinensis* and its trend in the near future, the migration, spawning behavior and habitat requirements for the sturgeon using ultra - sonic telemetry, conservation biology of Reeves shad, as well as the aspects of ecology of icefishes (salangids) introduced in reservoir.

3. Advances in scientific research

In recent years, the laboratory has undertaken near 30 projects supported by the national natural science foundation, international science foundation, national "948" project, national science & technology key projects and the major projects of ministry of agriculture, etc. Of the projects completed, some have made great achievements. For instance, the subject of 《Cryopreservation of freshwater fish sperms and construction of sperm bank》has made a systematic study on the cryobiology, antifreeze and cryopreservation of cultivated cyprinid fish sperms and on their thawing and insemination methods, put forward a set of effective techniques of cryopreservation, freezing and insemination of cyprinid fish sperms, and thus won the second prize of ministry of agriculture science & technology progress. The key subproject of 《Cell culture of killed vaccine for grass carp haemorrhage virus》has developed a stable, safe and cell - cultured killed vaccine of high purity and satisfactory immunization results, set up a process of factory - scale production and has great importance and applied value in the control of grass carp haemorrhage disease. After incorporation into the project of 《Grass carp haemorrhage disease control techniques》, this project won the national first prize of science & technology progress and the first prize of ministry of agriculture science and technology progress. The eighth - year project《The study on natural ecological pool of four major carps germplasm resources at Swan oxbow》won the national third prize of science & technology progress in 1998. In recent years, great success has been achieved in studies on conservation, utilization and population genetics of Chinese sturgeon, the first rank protected species in China, and on molecular

evolution of the order Acipenseriformes by utilizing new technologies. The data will be published in national top journals such as 《Sciences in China》, 《Progress in Natural Sciences》 and 《Chinese Sciences Bulletin》 etc. Since the establishment, the fixed personnel of the laboratory have published over 200 papers at symposia or in journals at home and abroad.

The main projects undertaken presently are as follows:

(1) Study on identification of the germplasm resources of the four major Chinese carps. (2) Study on population genetic structure and genetic marker of main farmed fishes by molecular biology techniques. (3) Selection of silver carp by using the molecular approaches and bioengineering (gynogenesis etc.). (4) Molecular population genetics of Chinese sturgeon and molecular evolution of related species. (5) Study on the factor of sex determination in fish. (6) Cloning of transferrin gene from crucian carp (*Carassius auratus*) and toxin gene from *Aeromonas hydrophila*. (7) Study on out breaking fish disease control techniques. (8) Studies on the spawning grounds and habitat requirements of Chinese sturgeon, studies on spawning ecology of Chinese sturgeon with ultra-sonic telemetry (US - China cooperation). (9) Studies on the population dynamics of Chinese sturgeon, monitoring Chinese sturgeon stock. (10) The domestication and artificial breeding of Reeves shad.

4. Main instruments and facilities

The laboratory has some instruments and facilities that are necessary for the preservation of fish germplasm resources and for the scientific research on cell and molecular biology in fishes and fish ecology as well, for instance, DNA synthesizer, polypeptide sequencer, high performance liquid chromatography system, ultracentrifuge, image analyzer, liquid scintillation counter, gene transfer system, dual-wave length thin layer scanner, ultraviolet spectrophotometer, transmission electron microscope, electrophoresis systems, electron microbalances, universal microscopes, inverted microscopes, DNA amplification system, microplate reader, hydro-ultrasonic receiver, fish detector, electron hydrodynamometer, GPS satellite Navigator and special purpose motor boat.

5. Opening, exchange and cooperation

The laboratory has always persevered in the operational mechanism of being "Open, Mobile and United". It has already made policies on favorable terms to attract scientists and technicians to the laboratory for cooperation research. In the case of financial constrain, the laboratory actively looked for money for the establishment of "open subject fund". It has hitherto organized and aided financially three terms of open subjects. "The first-term open subject fund" supported 7 subjects of 4 institutes. "The second-term open subject fund" subsidized 12 subjects applied by researchers from 5 different institutes. "The third-term open subject fund" funded 12 projects which are going on.

The laboratory has set up a relationship of cooperation and exchange with 7 laboratories in USA, Canada, Germany, France and Japan. Over 10 experts and professors from Toronto University, Tennessee Polytechnical University, United States Fish and Wildlife Service, University of Wurzburg and Hiroshima University have visited the laboratory for discussion and academic exchange. Simultaneously the laboratory sent scientists to USA, France, Canada, Germany, Japan, India, Thailand, Pakistan, Singapore and Bangladesh, etc. for cooperation research, investigation or symposium. Besides, the laboratory has presided over or participated many national symposia. Such as the National Symposium on Fish

Germplasm Resources & Biotechnology held in Shashi in September, 1994. The 10th International Conference on Isozyme and Gene Families, which will be held in Beijing in October, 1999, will be cosponsored by the laboratory and Institute of Developmental Biology, Chinese Academy of Sciences.

All the internal and external scientists devoted to the research on fish germplasm resources and biotechnology are warmly welcome to the laboratory for work, cooperation research or academic exchange. The laboratory will give priority to the researchers who have obtained master's degree or doctorate, returned students and refresher personnel for acceptance and provide them with living and working conditions.

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ery Sciences.**

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目 录

养殖鲢鱼性腺发育的研究	王汉平(1)
珠江口池塘幼鲢养成亲鱼的生理生态研究	王汉平(8)
池养鲢鱼全人工繁殖的机理	王汉平(13)
人工诱导圈养鲢鱼性腺发育、成熟、产卵和受精	王汉平(21)
鲢鱼的驯养与生物学研究Ⅲ.1 龄鲢鱼的驯养及其生长规律	王汉平(30)
养殖鲢鱼性腺发育的年周期变化	王汉平(37)
埋植和注射激素诱导驯养鲢鱼卵巢发育、成熟和排卵	王汉平(43)
中华鲟的渔业、养殖和繁殖	王汉平(50)
池塘条件下幼鲟驯化养成其生长特征与条件、温度的关系	王汉平(55)
中国鲢鱼资源的现状与保护	王汉平(62)
淡水鱼类种质资源人工生态库水质分析	方耀林(66)
十种常见淡水鱼类的 RAPD 鉴定	邓 怀(70)
雌核发育白鲢 RAPD 指纹和蛋白质电泳的研究	邓 怀(74)
草鱼生长激素单抗免疫亲和柱的制备及初步应用	邓文涛(80)
游动气单胞菌分离株对胞外溶血素和蛋白酶的生产	龙 华(84)
嗜水气单胞菌蛋白酶在鱼离体血清铁的利用中的作用	龙 华(88)
基因工程在水产上的应用前景	龙 华(93)
长江中华鲟超声波遥测技术	危起伟(100)
长江鲟鲑类的保护对策	危起伟(106)
我国鲟鱼和白鲟的生物学、渔业及保护	危起伟(116)
乳酸脱氢酶与酯酶同工酶同板染色法	余来宁(134)
用电融合结合继代移核方法构建草鱼抗病体细菌工程鱼	余来宁(138)
我国鱼类遗传育种研究的现状和展望	余来宁(143)
克隆技术及其在水产上的应用	余来宁(152)
分子生物学技术及其在渔业科学中的应用	张四明(155)
湖北淤泥湖团头鲂 mtDNA 限制性片段长度多态性的研究	张四明(167)
中华鲟天然群体蛋白质水平遗传多样性贫乏的初步证据	张四明(172)
中华鲟随机扩增多态性 DNA 及遗传多样性研究	张四明(179)
中华鲟 mtDNA 个体间的长度变异与个体内的长度异质性	张四明(186)
鲟鱼类基因组大小、倍性特点及鲟形目细胞进化的探索	张四明(194)
长江水系白鲢和草鱼遗传结构和遗传多样性的随机扩增多态性研究	张四明(201)
七种鲟形目鱼类亲缘关系的随机扩增多态性 DNA 研究	张四明(208)
十二种鲟形目鱼类 mtDNA ND4L - ND4 基因的序列变异及其分子系统学	张四明(214)
天鹅洲鱼类种质资源库环境质量评价	李 谷(222)
非离子型洗涤剂对浮游生物影响的研究	李 谷(230)

鱼用疫苗的现状及其发展趋势	杨先乐 (234)
中华鳖稚鳖对几种药物敏感性的研究	杨先乐 (244)
中华鳖出血性肠道坏死症初报	杨先乐 (251)
我国鳖病防治工作刍议	杨先乐 (253)
鳖用药物	杨先乐 (255)
长江大鳍鲩的人工繁殖	杨德国 (267)
胭脂鱼亲鱼的池塘培育技术	杨德国 (272)
秦岭水河太白段珍稀水生动物分布现状及保护对策	杨德国 (277)
里海流域鲟渔业现状和保护	杨德国 (283)
大鳍鲩亲鱼池塘驯养和培育技术	杨德国 (285)
大口鲶染色体组型和 DNA 含量的研究	邹桂伟 (289)
草鱼生长激素单克隆抗体的生成和鉴定	陈松林 (293)
作为水产动物遗传改良模型的青胚干细胞	陈松林 (299)
草鱼垂体催乳素的分离、纯化与鉴定的研究	陈松林 (305)
草鱼催乳素抗血清的制备与鉴定	陈松林 (308)
草鱼的生长及其血清生长激素水平的季节和日变化规律的研究	陈松林 (311)
鲤科鱼类生长激素夹心式 ELISA 测定技术建立和应用的研究	陈松林 (316)
草鱼生长激素非竞争性酶联免疫吸附测定法的建立和鉴定	陈松林 (323)
鲫鱼败血症病原菌的分离及毒素产生	汤伏生 (331)
长时间灌流式器官培养方法	陈细华 (335)
鱼类两性嵌合体及其在种质保存中的应用前景	陈细华 (338)
LHRH - A ₂ 及无机离子促进草鱼脑垂体离体分泌 GH 的初步研究	陈细华 (340)
长时间离体灌流草鱼脑垂体器官分泌生长激素技术的研究	陈细华 (343)
鱼类基因分子生物学及转基因鱼研究进展	昌永华 (348)
玻璃化液对鲢鱼胚胎成活的影响	章龙珍 (364)
卤虫胚胎和无节幼体超低温冷冻保存的研究	章龙珍 (369)
粗唇的驯养与人工繁殖技术研究	章龙珍 (374)
玻璃化液对泥鳅胚胎的渗透及毒性作用	章龙珍 (377)
温室内中华鳖性腺发育的研究	章龙珍 (380)
健康草鱼肠道气单胞菌的季节变化及胞外酶调查	曾 勇 (386)
草鱼出血病细胞培养灭活疫苗旋转瓶法大规模生产工艺	曾令兵 (390)
草鱼出血病病毒 854 株的理化、生物学特性及基因组结构	曾令兵 (395)
鱼用新药“康达富”对鳗鲡弧菌和福建爱德华氏菌的杀灭作用	曾令兵 (399)
鱼用新药“康达富”对中华鳖“疔疮”、“腐皮”并发病病原菌的杀灭作用	曾令兵 (403)
水产抗菌药物抗菌素作用的测定方法	曾令兵 (407)
淡水养殖鱼类病害研究的现状与发展方向	曾令兵 (410)
中华鲟精液的生物学特性和精浆的氨基酸成分	鲁大椿 (414)
鱼类精液冷冻保存技术操作规程	鲁大椿 (418)
热休克诱导鲤四倍体的研究	潘光碧 (421)

CONTENTS

Study on the gonadal development in cultured <i>Tenualosa reevesii</i>	Wang Han-Ping(1)
Physiological-ecological studies on domestication of <i>Tenualosa reevesii</i> juvenile to broodfish	Wang Han-Ping(8)
The mechanism and regulation of artificial propagation of pond-domesticated reeves shad	Wang Han-Ping(13)
Induction for development and maturation in gonads, spawning of captive Reeves shad and its egg fertilization	Wang Han-Ping(21)
Domestication of reeves' shad and its biology domestication of 1-year-old shad and its grow law	Wang Han-Ping(30)
Studies on the Year Cycle of the Gonadal Development on <i>Tenualosa reevesii</i>	Wang Han-Ping(37)
Induced Ovarian Development, Maturation and Ovulation of Domestic Reeves Shad by Hormone Implantation and Injection	Wang Han-Ping(43)
Fisheries, Aquaculture and Breeding Efforts of Chinese Shad	Wang Han-Ping(50)
On Pond Domestication of Reeves shad Juvenile to Adult and their Growth Characteristics in Relation to Condition and Temperature	Wang Han-Ping(55)
Status and Conservation of Reeves Shad Resources in China	Wang Han-Ping(62)
Water quality analysis of artificial ecological storage of freshwater fish germplasm resources	Fang Yao-Ling(66)
RAPD identification of ten common freshwater fish species	Deng Huai(70)
Genetic Analyses of Gynogenetic Silver Carp by Means of RAPD and protein Electrophoresis	Deng Huai(74)
Purification of Recombinant Carp GH Using Immunoabsorbent Column of McAb Against Grass Carp GH	Deng Wen-Tao etc(80)
Production of the Extracellular Hemolysin and Protease by an Isolated Strain of Motile <i>Aeromonas</i> spp	Long Hua(84)
The Role of <i>Aeromonas hydrophila</i> Protease in the Utilization of Fish Serum Iron in vitro	Long Hua(88)
Applied Perspective of Fish Gene Engineering	Long Hua(93)
Technique of Ultrasonic Telemetry for Chinese Sturgeon, <i>Acipenser sinensis</i> , in Yangtze River	Wei Qi-Wei(100)
Strategy on conservation of sturgeons in the Yangtze River	Wei Qi-Wei(106)
Biology, fisheries and Conservations of Sturgeons and Paddlefish in China	Wei Qi-Wei(116)
A Method for Staining Lactate Dehydrogenase and Esterase isozymes on the Slab GEL	Yu Lai-Ling(134)
Cell-engineering Grass carp Produced by The Combination of Electric Fusion and Nuclear Transplantation	Yu Lai-Ling(138)
Present Status and prospects of fish genetics and breeding in china	Yu Lai-Ling(143)
Cloning techniques and their application to fisheries	Yu Lai-Ling(152)
Molecular biology techniques and their Applications to fisheries sciences	Zhang Si-Ming(155)

mt DNA RFLP analysis of blunt snout bream (<i>Megalobrama amblycephala</i>) from lake Yuni poulation	Zhang Si-Ming(167)
The preliminary evidence for low genetic diversity in Chinese sturgeon(<i>Acipenser sinensis</i>) revealed by protein electrophoresis	Zhang Si-Ming(172)
Random amplified polymorphic DNA(RAPD)and genetic diversity of CHinese Sturgeon(<i>Acipenser sinensis</i>)	Zhang Si-Ming(179)
Mitochondrial DNA Length Variation and Heteroplasmy in Chinese Sturgeon(<i>Acipenser sinensis</i>)	Zhang Si-Ming(186)
Genome Size, Ploidy Characters of Several Species of Sturgeons and Paddlefishes With Comment on cellular Evolution of Acipenseriformes	Zhang Si-Ming(194)
Population structure and genetic diversity of silver carp and grass carp from populations of Yangtze River systems revealed by RAPD	Zhang Si-Ming(201)
Systematic Relationships Among 7 Species of Acipenseriformes Inferred from Random Amplified Polymorphic DNA	Zhong Si-Ming(208)
Variation in mtDNA ND4L-ND4 Gene Sequences of 12 Species of Acipenseriformes and Their Molecular Systematics	Zhang Si-Ming(214)
Evaluation of Environmental Quality of Freshwater Fish Germplasm Resources Pool in Tianezhou	Li Gu(222)
Study on the Effects of Nonionic Detergent on Plankton	Li Gu(230)
The Existing Situation and Tendency in Development of Fish Vaccine	Yang Xian-Le(234)
Susceptibility of CHinese soft-shelled Turtle to Medicines Commonly Used	Yang Xian-Le(244)
A Preliminary Report on Haemorrhagic Gut Necrosis of Chinese Soft-shelled Turtle(<i>Trionyx sinensis</i>)	Yang Xian-Le(251)
A Tentative Suggestion for Control of Soft-shelled Turtle Diseases in China	Yang Xian-Le(253)
Drugs for Soft-shelled Turtles	Yang Xian-Le(255)
Parent Fish Cultivation in Ponds and Artificial Propagation of <i>Mystus Macropterus</i>	Yang De-Guo(267)
The Technique of Parent Fish Cultivation of Chinese Sucker in Pond	Yang De-Guo(272)
The Distributing Actuality and Protect Countermeasure of Rare Aquatic Animal in Xushui River of Qinling Mountains	Yang De-Guo(277)
The Precent Status and Conservation of Sturgeons(<i>Acipenseridae</i>) in the Caspian Basin	Yang De-Guo(283)
Training and Culturing Techniques of Parent Fish of the Bagridae Catfish <i>Mystus Macropterus</i> in Pond	Yang De-Guo(285)
Studies on Karyotype and Cellular DNA Content of <i>Silurus meridionalis</i>	Zhou Gui-Wei(289)
Generation and Characterization of Monoconal Antibodies Against Grass Carp(<i>Ctenopharyngodon idellus</i>) Growth Hormone	Chen Song-Ling(293)
Medakafish Embryonic Stem Cells as a Model for Genetic improvement of aquaculture livestock	Chen Song-Ling(299)
Isolation、Purification and Identification of Prolactin from Grass Carp(<i>Ctenopharyngodon idellus</i>)	Chen Song-Ling(305)
Preparation and Identification of Antibody Against Grass carp Prolactin	Chen Song-Ling(308)

Seasonal and Daily Variations in Somatic Growth Rates and Serum Levels of Growth Hormone in The <i>Ctenopharyngodon idellus</i>	Chen Song-Ling(311)
The Development of a Sandwich ELISA for Growth Hormone of Carps	Chen Song-Ling(316)
Development and Validation of a Noncompetitive Enzyme-Linked Immunosorbent Assay For The Growth Hormone of Grass Carp(<i>Ctenopharyngodon idellus</i>)	Chen Song-Ling(323)
Isolation and Toxin Production of The Pathogens of THE Hemorrhagic Goldfishes	Tang Fu-Sheng(331)
A Method of Long-Term Organ Culture With Perfusion System	Chen XI-Hua(335)
The Amphigentic Mosaics of Fish and Their Application to The COnservation of Fish Germplasm Resources	Chen XI-Hua(338)
LHRH-A ₂ and Inorganic Ions Stimulate Growth Hormone Secretion in Vitro in The Pituitary of Grass Carp	Chen XI-Hua(340)
Studies On The Technique of perfusing Organ Culture of Grass Carp Pituitaries for Growth Hormone Secretion	Chen XI-Hua(343)
Researches In Molecular Biolgy of Fish Genes And Transgenic Fish	Chang Yong-Hua(348)
Effects of Vitrification of The Survival of Silver carp Embryo	Zhang Long-Zhen(364)
Cryopreservation of Embryo And Nuaplil of <i>Aptemia</i> SPP	Zhang Long-Zhen(369)
Domestication and Artificial Propagation of <i>Leiocassis crassilabris</i>	Zhang Long-Zhen(374)
Osmosis and Toxic Effect of Glassified Liquid on Loach(<i>Misgurnus anguillicaudatus</i>)	Zhang Long-Zhen(377)
Gonadal Development of Chinese Turtle, <i>Trionyx sinensis</i> , Cultured in Hothouse	Zhang Long-Zhen(380)
Investigation on Seasonal Variations of <i>Aeromonas</i> in Healthy Grass carp Intestines and Their Exoenzymes	Zheng Yong(386)
Batch Processing Technology of Cell Cultured Killed Vaccine Against the Hemorrhage of Grass carp ...	Zhang Lin-Bing(390)
Physical-Chemical and Biological Characterisitics and Cenome Structure of Grass carp Hemorrhage Virus854	Zhang Lin-Bing(395)
In Vitro Bactericidal Action of A New Fish Drug "KANGDAFU" Against <i>Vibrio</i> and <i>Edwardsiella</i> <i>fujianensis</i> of The EEL, <i>Anguilla Japonica</i>	Zhang Lin-Bing(399)
Bactericidal Action of A New Fish Drug "KANGDAFU" To The Pathogen of Complicaiton of Farunculosis and Putrid Skin Disease in Softshelled Turtle	Zhang Lin-Bing(403)
Methods for Measuring the Co-Action of Anti-Bacteria medicines in Aquaculture	Zhang Lin-Bing(407)
The Present Situation and Developmental Trends of The Study on Freshwater Fish Diseases	Zhang Lin-Bing(410)
Biological characteristics of Chinese sturgeon semen and plasma amino acid compositions of the seminal	Lu Da-Chun(414)
Regulations for technical operation in cryopreservation of fish sperms	Lu Da-Chun(418)
Tetraploidy Induction of Ying Hybrid carp by Heat Shock	Pan Guang-Bi(421)

养殖鲢鱼性腺发育的研究

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摘要 经激素处理和生态调控的养殖鲢鱼, 能完成性腺发育的全过程, 其可分为 6 个时期, 卵细胞发育可相应分为 6 个时相。与其它鱼类不同, 细胞中液泡最早出现在胞质的内缘而不是外缘。大、小核仁数随卵母细胞的发育而变化。成熟卵巢成熟系数为 8.54%~12.64%。成熟期卵径为 628.5~835.3 μm 、精子头径为 0.74~1.55 μm 。达性成熟的鲢鱼, 冬季卵巢为 II 期、精巢为 II~III 期。精、卵巢发育呈现出明显的不同步现象。前者 5 月底开始进入成熟期, 后者 7 月初进入成熟期。初级卵母细胞由 II 时相发育到 IV 时相基本上是同步的。第 IV 期卵巢卵径的频率仅出现 1 个高峰。养殖鲢鱼属 1 年 1 次产卵类型。

关键词 鲢鱼 驯养 性腺发育 成熟 产卵类型

鲢鱼 (*Tenualosa reevesii*) 是我国最名贵的洄游性鱼类之一, 其肉质鲜美, 富含脂肪, 经济价值极高。但近 20 年来, 由于人类的生产活动和环境污染, 鲢鱼资源急剧衰退, 目前, 长江、西江和钱塘江鲢鱼资源已濒临灭绝 (Wang, 1996)。因此, 有必要积极开展和完善鲢鱼的全人工繁殖研究。

性腺发育是鱼类繁殖的基础。对天然鲢鱼生殖洄游过程中阶段性性腺发育, 国内外已有过一些研究。陆桂等 (1964) 曾记述过钱塘江鲢鱼 IV 期和 V 期性腺的外形特征** 邵炳绪等 (1978) 曾描述过长江鲢鱼在溯河洄游过程中性腺发育规律; 张克俭等 (1990) 曾报道了鲢鱼在中国东南近海生殖洄游期间性腺发育情况; Mylonas 等 (1995) 曾研究美洲鲈 (*Alosa sapidissima*) 的性腺发育特征。但至今对鲢鱼性腺发育的全过程缺乏系统资料, 尤其是对鲢鱼性腺发育规律的研究尚属空白。为此, 我们在进行鲢鱼的驯化养殖和全人工繁殖试验 (王汉平等, 1992a, b, 1995, 1997) 的同时, 开展了这方面的研究。

1 材料和方法

从珠江采集鲢鱼幼鱼, 在池塘中驯化养成亲体。采用外源激素和生态调控方法诱导性腺发育成熟和排卵。在定期催熟的过程中, 解剖取整体或用挖卵器取部分性腺材料, 共获样品

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** 陆桂, 钟展烈, 赵长春 1964 钱塘江鲢鱼的自然繁殖和人工繁殖. 上海水产学院论文集. 1~28

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68个。每个时期样品取自5~10尾亲鱼。采用 Bouin 氏液固定,制成4~8 μm 厚的石蜡切片,用 Delafield 苏木精-伊红染色,部分材料作了显微摄影。卵母细胞时相的划分,基本上采用 Meüet (1939) 的原则。采用西德产 IBAS-2000 图象分析仪测定卵径和核径及其面积,求出各时相卵母细胞所占面积比例,分别计算各期早晚时相的面积比例。采用 Packard Bell 586 计算机分析各期卵径组成。成熟系数定义为 $100 \times \text{性腺重} / \text{净体重}$ 。

2 结果

2.1 性腺发育的特征和组织学期

2.1.1 卵巢: 鲢鱼卵巢可分为6个时期,其生殖细胞可相应分为6个时相。

I 期,即原始生殖细胞期。卵巢细薄膜状,银白色,透明,位于腹腔壁背面前后两侧,不见血管,肉眼不能辨别雌雄。

在 I 期卵巢中,主要为由生殖上皮细胞分生而来的第 1 时相卵原细胞。此时卵巢腔已形成,卵巢壁主要由结缔组织和生殖上皮构成,产卵板尚未形成,卵原细胞排列杂乱无章,形态不规则,有圆形、梨形或椭圆形,直径为 7.55~23.61 μm 。位于细胞中央的核较大,其直径 4.68~9.76 μm ,核内有核仁 1~3 个,分布于核中央(图版 I: 1)。

II 期,即增殖期至早期生长期。卵巢为扁囊状,宽 3.0~9.0mm,长 115.0~140.0mm,长宽比为 15.5~38.3。有微血管分布,呈淡红色或肉红色。卵巢固定后,可见片状的蓄卵板,肉眼可辨认卵巢,但只能在显微镜下见到卵粒,成熟系数为 0.21%~1.04%。

此期卵巢内主要为第 2 时相卵母细胞。早期卵母细胞紧密排列在产卵板上,多数为多角形,少数为卵圆形。核圆形或卵圆形,核仁 7~18 个,多靠近核膜分布,卵径 19.21~45.85 μm ,核径 11.58~26.65 μm ,此时相滤泡细胞尚不明显,卵母细胞间存在少量卵原细胞,胞质中出现卵黄核(图版 I: 2, 3)。中期卵母细胞有规则地排列在产卵板上,有圆形、卵圆形或不规则。核膜外缘胞质中出现大小不等的液泡。胞核为椭圆形,核仁 10~21 个,仍分布于核边缘(图版 I: 4)。卵径为 32.81~65.69 μm ,核径为 17.68~30.76 μm ,此进细胞膜外的单层滤泡已比较明显。晚期卵细胞排列松散,体积增大,呈卵圆形,卵黄核少见,胞质内液泡数增加,并出现脂肪滴(图版 I: 5)。卵径为 65.58~139.22 μm ,核径为 24.39~68.59 μm ,核仁径为 3.10~4.30 μm ,卵膜外围的单层滤泡明显变厚。

III 期,即生长早期。中部稍大的长囊状,长 140~180mm,宽 11~15mm,浅黄色,血管明显且分支多,透过卵巢膜可见到细的卵粒,成熟系数为 0.85%~2.10%。

此期卵巢内主要为第 3 时相卵母细胞。早期呈卵圆形,核膜外缘的胞质中出现一层液泡,数量为 4~41 个(图版 I: 6),直径为 2.65~5.62 μm 。卵黄核尚存在,接近细胞膜,细胞外有两层滤泡细胞。胞膜与滤泡层厚度 3.17~4.93 μm ,卵径 111.10~165.10 μm ,核径 41.65~65.90 μm 。中期胞体呈圆形或椭圆形,卵膜附近开始出现卵黄粒,直径为 4.78~14.25 μm ,靠近核膜的胞质中形成 2~3 层液泡(图版 I: 7),直径 1.60~10.57 μm 。细胞核近圆球形,数目 4~7 个,直径为 2.32~4.19 μm 。滤泡细胞双层,中间长梭状的核清楚可见,放射带出现。卵径为 127.8~249.3 μm ,核径为 47.4~106.9 μm 。晚期卵黄粒增多,并向细胞中央延伸,分布于液泡之间的细胞中,细胞核内的核仁数目 4~8 个,仍分布在核膜边缘(图版 I: 8)。卵径 308.2~442.1 μm ,核径 79.74~134.4 μm 。

IV 期,即生长晚期。卵巢体积更加增大,近圆筒状,长 163.0~195.0mm,宽 23.0~

38.0mm, 呈浅黄色, 血管粗大且分支伸展面广, 卵粒饱满, 早期卵粒为褐黄色, 不易分离, 成熟系数为 3.50%~6.20%。晚期卵粒为黄色, 易分离, 成熟系数为 8.54%~12.64%。

Ⅳ期卵巢内主要以 4 时相卵母细胞为主。其早期呈圆球形, 卵黄颗粒迅速增加, 但靠近细胞核的卵黄颗粒稀少。卵黄颗粒圆球形, 大小不等, 直径为 6.15~13.59 μm 。细胞质中除卵黄颗粒外, 还有液泡, 脂肪滴等结构。细胞核位于细胞的中央, 为不规则圆形, 核仁数目减少, 核膜逐渐变成波浪状。滤泡细胞层增厚至 6.80~9.84 μm , 此时卵细胞径为 366.80~552.50 μm , 核径为 45.93~131.60 μm (图版Ⅱ: 9, 10)。中期卵黄颗粒充满卵细胞质, 其大小差异悬殊, 变幅为 3.36~13.38 μm , 平均为 8.61。液泡明显缩小, 被挤压到胞质外缘。细胞核仍位于细胞的中央, 核膜仍为波浪状, 滤泡细胞层厚度为 6.42~10.06 μm , 附着膜厚度为 2.02~2.42 μm , 放射带厚度为 0.63~1.27 μm , 卵膜总厚度为 4.07~5.93 μm , 卵径为 477.10~613.10 μm , 核径为 60.51~92.85 μm (图版Ⅱ: 11)。晚期卵黄颗粒充满, 呈板块状充满卵母细胞, 卵黄颗粒直径为 6.10~18.74 μm 。核膜仍呈波浪状, 随着卵黄物质的充满, 卵细胞逐步达到生理成熟, 卵核逐步向动物极移动, 整个细胞出现极性 (图版Ⅱ: 12)。此时卵径为 503.0~810.5 μm 。

V 期, 即成熟期。只能经过人工催产后得到。卵巢极为膨大, 充满整个腹腔。卵巢松软, 呈橙黄色或桔黄色, 卵细胞游离, 光泽透明。V 期卵巢主要以 5 时相卵母细胞为主, 其细胞核移至动物极, 核膜逐渐消失, 核质与胞质融合, 卵细胞从外周滤泡层细胞中分离出现, 落入卵巢腔中, 卵母细胞中充满松散排列的卵黄颗粒。产出的卵无论受精与否均能吸水膨胀, 此时相的卵径为 593.50~815.3 μm 。

Ⅳ期, 即排空期。卵巢呈囊状, 萎缩, 充血, 呈暗红色。经人工催产, 卵巢内的大部分成熟卵细胞排出体外, 残留一些滤泡和部分未产出的 4、5 时相卵细胞 (图版Ⅱ: 13)。这些剩余卵母细胞将被卵巢自身吸收并退化, 退化吸收后, 卵巢回到第Ⅱ期。

2.1.2 精巢: 由精原细胞到精子形成和衰老, 精巢也可分为 6 个时期, 用肉眼难以对早期精巢进行准确分期。第Ⅰ期为细线状, 宽度在 2mm 以下, 第Ⅱ期精巢为细带状, 长 50~80mm, 宽 2~4mm, 呈紫红色。成熟系数为 0.065%~0.30%。Ⅲ期精巢为带状, 长 60~110mm, 宽 55~98mm, 呈紫红色, 有少量血管分布, 成熟系数为 0.18%~0.45%。Ⅳ期精巢为乳白略带红色, 表面血管减少, 长 98~130mm, 宽 110~140mm, 用力挤压腹部有少量精液流出, 成熟系数为 0.50%~1.59%。V 期精巢形态大小变化不大, 白色, 无明显血管分布, 轻压腹部, 有粘稠的乳白色精液流出, 成熟系数为 0.93%~1.61%。Ⅳ期精巢体积明显缩小, 淡红色, 可见明显的微血管分布。

按养殖鲢鱼精细胞不同发育阶段的特征可分精原细胞, 初级精母细胞, 次级精母细胞和精子细胞 (图版Ⅱ: 14)。精子细胞经过系列变态成为精子, 成熟的精子分布均匀, 嗜碱性强, 染深蓝色 (图版Ⅱ: 15)。精子头部呈圆颗粒状, 直径为 0.74~1.55 μm , 平均为 1.01 μm , 尾部不十分明显。

2.2 不同时相卵巢细胞的组成

2.2.1 切片上的面积和数量组成: 在第Ⅱ期卵巢中, 第 2 时相卵母细胞占绝大多数, 其数量均值占 75.3%, 面积均值占 87.5%, 余下为第 1 时相卵原细胞。第Ⅲ早期至晚期卵巢中, 第 1、2 时相卵母细胞数量比例下降为 50.55%~28.1%, 面积下降为 27.7%~1.7%; 而第 3 时相卵母细胞上升为主导地位, 其早期至晚期数量均值为 48.2%~61.6%, 面积均值为