中华人民共和国农业部
海洋水产增养殖生态学重点开放实验室

Y LABORATORY OF MARICULTURE ECOLOGY MINISTRY OF AGRICULTURE THE PEOPLE'S REPUBLIC OF CHINA

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(1997-1998)

大連水産学院

大连水产学院

农业部海洋水产增养殖生态学重点开放实验室

年　　报

（1997—1998）

主　编：王子臣　张国范

编　辑：王翠娥　周一兵
宋　坚　丁　君
朱　莹

王子臣　教 授

序　言

为发挥和发展大连水产学院海水增养殖生态学理论与技术的优势，促进我国海洋经济，特别是我国北方海水养殖业的发展，农业部在大连水产学院建立了海洋水产增养殖生态学重点开放实验室，并于1998年6月正式举行揭匾仪式暨召开第一次学术委员会会议。

实验室总体目标是建成具有较高学术水平、较强研究能力；具有北方海洋水产养殖特色的，能承担国家和行业重大海洋水产应用基础研究攻关项目，国内一流、国际著名的海洋水产研究机构。本实验室现设有三个研究方向：1、海水增养殖生物学和生态学；2、养殖遗传学与生物技术；3、水产医学。其研究内容包括新的种质资源的开发，营养与饲料，渔业环境和资源管理，养殖生物抗病机理和疾病防治等。

本实验室可承担各级各类海水增养殖学科的研究课题，以应用基础理论研究为主，向基础理论研究和应用技术研究拓延，培养高层次专门人才，为发展我国现代化可持续海水增养殖渔业提供理论和技术支撑。

实验室经过两年的建设，已经取得丰硕成果。本实验室承担了包括国家海洋“863”在内的27项研究课题，其中4项已获省市级奖励，6项已结题或鉴定，发表论文共32篇。实验室的各种软硬件设施趋于完善，现拥有实验研究场地700余平方米，其中有生态室、活体培育室、生物技术操作室等18个专用或公用实验室。拥有流式细胞仪、电子显微镜等一大批先进的仪器设备，可基本满足海水增养殖生态学、遗传育种、生物技术及水产医学研究的需要。本实验室对国内外开放，并设有开放课题，欢迎国内外学者广泛利用实验室的基础条件，共促我国海洋水产事业的发展。

本年报编辑了1997-1998年我室研究人员在各种学术刊物上发表的论文，其中有三篇被SCI索引，还选登了加强实验室建设的有关资料。由于初次从事这项编纂工作，缺乏经验，不妥之处敬请读者指正。

最后，向关心、支持和帮助我院重点实验室组建和发展的各级领导，各位专家及兄弟院校同仁，表示衷心的感谢和诚挚的敬意！

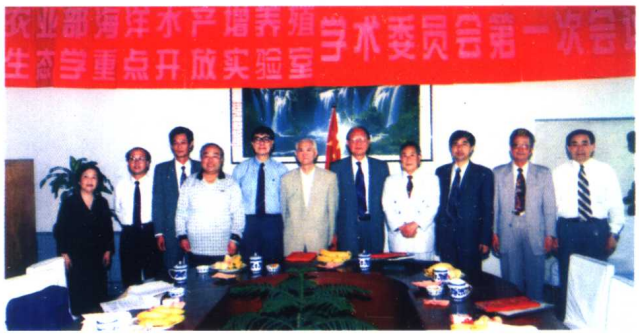
农业部海洋水产增养殖生态学
重点开放实验室学术委员会主任　王子臣

1999年5月5月

大连水产学院李鸿鸣院长
在实验室揭匾仪式上的讲话

中国水产科学院赵法箴院士
在实验室揭匾仪式上的讲话

美国新泽西州立大学郭希明
博士被聘为我院兼职教授



实验室学术委员会第一次会
议合影

我校“863”课题组成员在进
行研讨

太平洋牡蛎三倍体规模生产
技术研究鉴定会

张国范博士在澳大利亚昆士
兰大学进行学术交流

美国新泽西州立大学郭希明
博士来我室作学术报告

大连水产学院院长李鸿鸣在农业部海洋水产增养殖生态学重点开放实验室揭匾仪式上的讲话

各位领导、各位专家、各位来宾、同志们：

今天，我们汇集在滨城大连，隆重举行农业部海洋水产增养殖生态学重点开放实验室的揭匾仪式。首先请允许我代表大连水产学院广大师生向光临今天揭匾仪式的各级领导、专家和来宾，表示最热烈的欢迎。向多年来关心、支持和帮助我校建设和发展，特别是协助组建重点实验室的各级领导、各位专家及兄弟院校同仁，表示衷心的感谢和诚挚的敬意！

我校是农业部直属的北方地区唯一的一所以水产学科为主，兼顾工程与管理等学科的多科性高等水产院校，现有六系两所 24 个专业，办学规模逐步扩大，教学质量不断提高，我校水产学科已被列为农业部、辽宁省重点学科。在搞好教学同时，学校还致力于科学研究，多年来获重大科技成果奖 45 项。其中国家级 4 项，省部级 28 项。近年来，又承担了国家海洋“863”重大课题。1996 年末，经农业部批准我校建立了“农业部海洋水产增养殖生态学重点开放实验室”，这标志着我校的科研工作又上一个新台阶。

组建这个部级重点开放实验室不仅对我国海洋水产增养殖业的发展起着促进作用，而且对我校整个学术水平的提高也将起着促进作用。同时，为我校迎接国家教育部的教学合格评价也增添了色彩。在组建重点实验室的过程中，院党政领导班子专门听取了有关人员的汇报，并召开了几次会议，专门研究落实重点实验室的建设工作。尽管我校经费十分紧张，并在学校底子薄、条件差，各方面都要用钱的情况下，院领导班子仍决定单列一部分经费，用于重点实验室的基本建设。并由一名院领导主抓这项工作，由科研处和养殖系专门配备领导负责筹划这项工作，总务后勤负责基本建设工作，设备科的同志负责仪器设备的建设工作。并且调整配齐了重点实验室的领导班子，成立了重点实验室的学术委员会，由院领导亲自审查了重点实验室制定的主要研究方向和各项规章制度。经过多方努力，目前已经建成一个拥有实验室场地 700 余平方米，配有生态室、活体培育室、生物技术操作室、电镜室等 18 个专用或公用实验室，形成能正常进行研究工作并初具规模的重点实验室。

21 世纪是世界开发海洋的世纪，海洋的开发就成为各国竞争的热点，尤其是 21 世纪人们将向海洋要“粮食”，索取蛋白质，历来作为海洋各产

业中的支柱产业海洋渔业就显得尤为重要，而我国是海洋水产大国，也是海水增养殖业的大国，到那时，海水增养殖业的地位，也就显而易见了，目前，我国水产品年总产量在 3000 万吨左右，占世界总产量的 1/3，居世界各国首位，而在这 3000 万吨左右的水产品产量中，有半数以上来自海洋，近些年来，我国近海自然渔业资源严重衰竭，近海捕捞业已向两个方向发生转移，一是远洋，一是海水增养殖业，近两年的海产品中有 1/3 以上来自海水增养殖业，而且这个比例也正在逐年增长。因此，发展海水增养殖业势在必行。在海洋渔业大力发展的形势下，在海水增养殖业受到病害困扰的情况下，加强对海水增养殖的研究就显得更有必要，这个海洋水产增养殖生态学重点开放实验室肩上重担也就更加沉重。

根据当前形势的要求和我们的技术、设备力量，重点实验室确定了三个主要研究方向：1. 海水增养殖生物学和生态学；2.养殖遗传学与生物技术；3.水产医学。确立这三个研究方向的目的是，即要从事海水增养殖业的基础科学研究，又要从事海水增养殖业的应用科学研究。“九五”期间，重点实验室的科研任务，有包括国家海洋“863”在内的 26 个研究项目，值得庆幸的是，重点实验室虽然仅组建一年多，但已取得了可喜的成果，有 5 个项目已结题或鉴定，还有 4 个项目准备今年鉴定，3 个项目已获得省政府和大连市政府科技进步奖，发表了有价值的学术论文 30 余篇，出版专著 1 部。我们的研究成果或阶段研究成果，有的已在大连市和辽宁省得到应用，有的已经扩展至山东的烟台、威海等市，产生了明显的经济效益，对海水养殖业的发展起到了推动作用。

各位领导、各位专家，农业部海洋水产增养殖生态学重点开放实验室今日揭匾，我们认为：这既是一份荣誉也是一份责任，我们要充分利用这块高科技的基地，在实践中不断发展，在发展中不断完善，既然是开放实验室，就要努力搞好对外开放，真诚而热切地欢迎各位专家同仁志士来我校进行科学研究，校内校外，同心协力，为祖国的水产事业，为迎接 21 世纪这海洋的世纪，联合攻关，协同作战。

最后，让我再次向在百忙中抽出时间参加我们今天揭匾仪式的各级领导、各位专家和来宾表示诚挚地谢意！

1998 年 6 月 12 日

实 验 室 简 介

大连水产学院是一所农业部所属的，以水产学科为特色兼顾工程与管理等学科的多科性水产高等院校。自 1952 年建校以来，大连水产学院十分重视水产养殖学科的发展，使该学科成为农业部和辽宁省的重点学科。

为了优化和发展大连水产学院水产养殖学科在海水增养殖理论和技术方面的优势，促进我国海洋经济的发展，农业部于 1996 年在大连水产学院建立部级海洋水产增养殖生态学重点开放实验室。

实验室的研究目标是：为实现我国可持续发展的海水增养殖业而服务；研究方向为：1. 海水增养殖生物学、生态学；2. 养殖遗传学与生物技术；3. 水产医学。其研究内容包括：增养殖生态学；新品种的开发和繁育；营养与饲料；渔业环境和资源管理；病害生态防治等。

本实验室对国内外开放，承担各级各类海水增养殖学科的研究课题。以应用基础理论研究为主，向基础理论研究和应用技术研究拓延，培养高层次专门人才，为发展我国现代化可持续海水增养殖渔业提供理论和技术支撑。

一批有较高学术造诣的海水增养殖专家被聘为实验室的研究人员，这些专家在海水增养殖的理论和技术研究方面取得了很多重要成果，曾获全国科学大会奖和国家科技进步奖等多项奖励。鲍和牡蛎多倍体育种技术的研究课题已被列为国家“八六三”计划的重点项目。

本实验室拥有实验研究场地 700 余平方米，其中有生态室等 18 个专用或公用实验室。拥有流式细胞仪、电子显微镜、万能研究显微镜、气相和液相色谱仪、液闪仪、紫外和原子吸收分光光度计、超低温冰箱、高速冷冻离心机、PCR 仪等大型先进的仪器设备，可基本满足各种海水增养殖研究的需要。

实验室的发展目标是：争取建成具有较高学术水平、较强研究能力；能承担国家重大海洋水产应用基础研究攻关项目，国内一流、国际著名的海洋水产研究机构。

实验室主任：张国范教授
地址：大连市黑石礁街 52 号
邮编：116023
电话：0411-4663572
传真：0411-4660163
电子邮件：dlmcl@pub.dl.lnpta.net.cn

实验室学术委员会名单

序号	姓 名	性别	出生年月	职 称	学委会职 务	专 业	工 作 单 位
1	王子臣	男	1932.04	教 授	主 任	贝类增养殖	大连水产学院
2	丁德文	男	1941.02	研究员 院士	副主任	环境生态	国家海洋局海洋 环境监测中心
3	赵法箴	男	1934.10	研究员 院士	副主任	海水养殖	中国水产科学研究院黄 海水产研究所
4	张福绥	男	1927.12	研究员	委 员	海水增养殖	中国科学院海洋研究所
5	相建海	男	1948.05	教 授	委 员	生物技术	中国科学院海洋研究所
6	何志辉	男	1927.04	教 授	委 员	水生生物	大连水产学院
7	刘焕亮	男	1933.08	教 授	委 员	鱼类增养殖	大连水产学院
8	陈介康	男	1937.05	研究员	委 员	海洋生物	辽宁省海洋水产研究所
9	王如才	男	1933.08	教 授	委 员	贝类养殖	青岛海洋大学
10	蔡完其	女	1939.12	教 授	委 员	养殖病害	上海水产大学
11	张国范	男	1954.04	教 授	委 员	遗传育种	大连水产学院

实验室固定研究人员名单

序号	姓 名	性 别	出生年月	职 称	专 业
1	王子臣	男	1932.04	教 授	贝类养殖
2	雷衍之	男	1938.03	教 授	环境化学
3	李永函	男	1937.12	教 授	生物饵料
4	张国范	男	1954.04	教 授	遗传育种
5	王吉桥	男	1950.09	教 授	养殖生态
6	周一兵	男	1957.08	副教授	海洋生态
7	张泽宇	男	1950.11	副教授	藻类栽培
8	高悦勉	男	1954.08	副教授	贝类养殖
9	李 霞	女	1961.05	副教授	组织胚胎
10	李 华	女	1958.02	副教授	养殖病害
11	姜志强	男	1960.09	副教授	鱼类养殖
12	赵兴文	男	1958.11	副教授	鱼类养殖
13	常亚青	男	1967.03	副教授	贝类养殖
14	张 硕	男	1962.11	讲 师	海水养殖

实验室管理人员名单

序号	姓 名	姓别	出生年月	职 称	职 务	专 业	工作性质
1	张国范	男	1954.04	教 授	主 任	遗传育种	研 究
2	王吉桥	男	1950.09	教 授	副主任	养殖生态	研 究
3	周一兵	男	1957.08	副教授	副主任	海洋生态	研 究
4	宋 坚	男	1971.06	助 研		海水养殖	研究管理
5	丁 君	女	1973.11	助 研		生态遗传	研究管理
6	朱 莹	女	1962.11		秘 书	文 秘	行政管理

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Journal of Shellfish Research, Vol. 17, No. 3, 783–788, 1998.

TRIPLOID INDUCTION IN PACIFIC ABALONE *HALIOTIS DISCUS HANNAI* INO BY 6-DIMETHYLAMINOPURINE AND THE PERFORMANCE OF TRIPLOID JUVENILES

GUOFAN ZHANG,[1] ZICHEN WANG,[1] YAQING CHANG,[1] JIAN SONG,[1] JUN DING,[1] YONGPING WANG,[1] AND RENBO WANG[2]

[1]*Key Laboratory of Mariculture Ecology*
Dalian Fisheries University
Dalian, China 116023
[2]*Dalian Pacific Sea Food Ltd.*
Dalian, China 116041

ABSTRACT Pressure shock, thermal shock, and cytochalasin B treatment have been the main treatments used in inducing triploid gastropod. In this study 6-dimethylaminopurine (6-DMAP) was attempted to induce triploid in Pacific abalone, *Haliotis discus hannai* Ino, by inhibiting formations of either polar body 1 (pb1) or polar body 2 (pb2). Although the highest triploid (73.5%) was obtained by blocking pb2 for 15 min with a 6-DMAP concentration as high as 300 μM (the recommended dose for triploid induction in bivalve), no veligers could survive. At the ranges of 75–150 μM 6-DMAP, blocking pb2 for 20 min resulted in higher abnormalities than blocking pb2 for 15 min. The relative survival rate was higher and the abnormality was lower in the pb2 group than those in the pb1 group. No significant difference in triploid induction ($p > 0.05$) was found both between 15- and 20-min treatments and between treatments targeting pb1 and pb2 at 75–150 μM 6-DMAP. At 6-DMAP concentrations of 75, 100, 125, and 150 μM, in groups that blocked the pb 2 formation, the triploid yields (number of triploid larvae/fertilized eggs) were 30.0, 46.0, 47.0, and 54.0% and the relative survivorships at the trochophore stage were 96.8, 95.3, 94.8, and 90.5% for 15-min treatments, respectively. The triploid yields were 30.1, 49.0, 51.2, and 56.0% and the relative survivorships at the trochophore stage were 95.8, 90.2, 88.5, and 82.6% for 20-min treatments, respectively; in groups that blocked pb1 for 15 min, the triploid yields were 30.4, 43.0, 46.6, and 51.0% and the relative survivorships at the trochophore stage were 70.6, 68.4, 68.0, and 61.8%, respectively. The shell dimensions of triploids and controls were measured at 4 mo postfertilization. In the large-size group (1.2–1.3 cm), differences between 3n and 2n groups in both dimensions and weight were significant ($p < 0.01$), whereas in the small-size group (0.7–0.8 cm), no significant differences were found in dimensions ($p > 0.05$), but they were found in total weight ($p < 0.01$). Overall, the optimal treatment criteria for triploid production in Pacific abalone with 6-DMAP appear to be 125–150 μM for 15–20 min at 500–600/mL zygote density and 23.0°C.

KEY WORDS: abalone, triploid, 6-dimethylaminopurine (6-DMAP)

INTRODUCTION

Pacific abalone, *Haliotis discus hannai* Ino, is one of the most valuable seafoods in the Asian market and has been farmed in northern China for many years. Abalone are now the major marine products in this area. However, in recent years, abalone farming has dropped tremendously in terms of scale and productivity. Deterioration of environments and disease contamination were found to be the main reasons. For example, 80–90% of land-based abalone hatcheries in Dalian, China, were contaminated by disease in 1996, which caused up to 85–90% mortalities.

The large-scale abalone mortality happening in Dalian might also result from, in addition to the above-mentioned reasons, decreases in heterozygosity, so animals became more sensitive to environmental changes and more easily attacked by the pathogens. Triploid animals, especially polar body 1 (pb1) triploids should, theoretically, have higher heterozygosity than their diploid siblings; therefore, triploid chromosome manipulation techniques would provide a novel approach in protection against disease. Chromosome engineering such as polyploidy induction has also been considered one of the genetic tools that could improve shellfish production. Chemical (cytochalasin B) and physical (thermal and pressure shocks) treatments were applied shortly after fertilization for triploid induction in shellfish (Stanley 1981, Stanley 1984, Allen 1987). However, the methods usually result in low survival rates and high levels of aneuploid.

Thermal shock, cytochalasin B, and pressure shock were applied, respectively, by Arai et al. (1986), Wang et al. (1990), Rong et al. (1990), Sun et al. (1993), and Stepto and Cook (1998) to induce triploid in *Haliotis discus hannai* Ino, *Haliotis diversicolor*, and *Haliotis midae*. Some of those treatments could result in high percentages of triploids, but because of the very high mortalities in treated animals, none of those methods could be commercially used in triploid abalone hatcheries. Recent work of Desrosiers et al. (1993) and Gerard et al. (1994) showed that treatment with 6-dimethylaminopurine (6-DMAP) was far more effective than other methods in generating viable triploid oysters (*Crassostrea gigas*) and mussels (*Mytilus edulis*). The effectiveness of this chemical in triploid induction in abalone, *H. discus hannai* Ino, is reported in this article.

MATERIALS AND METHODS

Broodstock

Pacific abalone, *H. discus hannai* Ino, about 6.5–8.0 cm in shell length, were collected in the vicinity of coastal Dalian. The animals were conditioned for 80–100 days at 20°C, during which they were fed *Laminaria japonica*, and the effective accumulation temperature reached 1000–1200°D. The gonad of a well, mature individual is gray-blue in color in females and milk-white in males, with a distinct incise between gonad and alimentary glands.

Gamete Collection and Insemination

In each experiment, gametes from at least two females and several males were collected by drying the broodstock in darkness

with 75–80% moisture first, and then spawning them in ultraviolet- (400–600 mw/h per L) treated seawater and raising the water temperature to 3–4°C above the conditioning temperature (20°C). High-quality oocytes are round and even blue in color, with a well-distributed yolk and clear membrane. The average egg diameter is 220 μm; the average yolk diameter is 180 μm. Successful fertilization could be obtained by using fresh sperm, which could stand erectly on the egg surface when checked under the microscope. Sometimes, a preexperiment fertilization was used to determine the vigor of sperm. Eggs and sperm were pooled within 30 min after being released at 23°C.

6-DMAP Solution Preparation

The commercial product of 6-DMAP is white powder. The stock solution used in this study was prepared in 1/100 (wt/vol) sterilized filtered seawater 1–2 h before the commencement of experiments and was stored at 4°C in a refrigerator.

Determination of Commencement Times and Treatment Duration

Although the first polar body could be found as early as 8 min postfertilization at 22.8–23.0°C, 45–50% zygotes released their pb1 within 22 min. Therefore, 6–7 and 20–22 min postfertilization were, respectively, chosen as the timings of the commencements of chemical treatments for inducing pb1 or pb2 triploids. On the basis of the observations and the previously published data, 15–20 min exposure durations were determined.

The time when sperm was added to the suspension of eggs was considered as the start of the experiment. Eggs and sperm were mixed gently. The fertilized eggs were equally divided into beakers according to replicates needed in each experiment.

Triploid Induction

In the first experiment, eggs were subjected to 6-DMAP at concentrations of 150 mm, 300 μM, and 450 μM. Chemicals were added either 6–7 min postfertilization to inhibit pb1 formation or 20–22 min postfertilization to retain pb2. Water volume was individually adjusted before 6-DMAP solution was added; the beakers were then stirred gently. The first experiment revealed that Pacific abalone appeared to be more sensitive to 6-DMAP than bivalves, so 6-DMAP concentrations were reduced to 75, 100, 125, and 150 μM in the later experiments. A 15- or 20-min treatment was applied in all experiments; eggs were washed with clean seawater and hatched under the same conditions as controls. During treatments, the zygote density remained at 200–600/mL, and the temperature was 22.6–23.0°C.

Rearing of Larvae and Juveniles

After treatment, each replicate was stocked at a density of 30–50/mL for embryos, 5–8/mL for trochophore larvae, and 4–5/mL for veliger larvae. At about 65 h postfertilization at 20°C, the eyed larvae were settled on the transparent waved polyethylene plates (42 × 33 cm), which were preplanted with benthic diatoms and turned to brown-yellow in color. The settlement plate density was kept consistently at 80/m^2, and 4.0–4.8 ($\times 10^4$) healthy eyed larvae were used for settlement in each replicate.

Juvenile abalone were kept on the transparent plates for 2 mo, until they reached a size of 4–5 mm in shell length; they were then transferred to the black waved plates for growout until the animals were about 1.5–2.0 cm in shell length. Juveniles from treated and control groups were maintained under the same conditions.

Ploidy Verification

Twelve to 15 h postfertilization, trochophore larvae swimming in water were selected and placed in a 0.01% colchicine solution for 0.5–1.0 h and then subjected to a hypotonic treatment for 1 h with 0.075M KCl and fixed in four changes of Carnoy solution (3:1, methanol:acetic acid) at 15-min intervals. The samples were stored at 4°C overnight in a refrigerator. Cell suspension was made by replacing Carnoy suspension was made by replacing Carnoy solution with 50% acetic acid and shaking heavily. The suspension was then dropped onto a warmed (48–52°C) microscope slide, air dried, and stained in 4% Giemsa solution (pH 6.8) for 30–60 min. According to Arai et al. (1986), diploid chromosome number of *H. discus hannai* Ino is 36. The following ploidy classification was applied in this study: metaphase spreads with about 36 chromosomes were classified as diploid; those with 52–54 chromosomes were classified as triploid (Fig. 1). A total of 80–100 metaphase spreads were counted in each replicate.

Formulations

Fertilization rates and survivorship at trochophore and veliger stages were calculated. Deformed trochophores or trochophores with irregularly divided cells were counted as abnormal.

Fertilization rate (FR) = fertilized eggs/total eggs (100%).

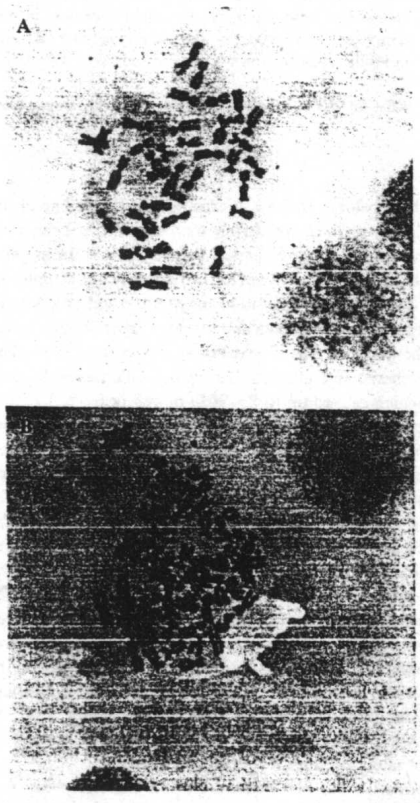

Figure 1. (A) A metaphase of commercial (2N = 36). (B) A metaphase of chromosome (3N = 54).

TABLE 1.

The induction of triploid in *H. discus hannai* Ino by 6-DMAP in meiosis II lasting 15 min (fertilized rate is 52.1%, treated density is 140-250/mL, and hatchery density of zygote is 30–55/mL).

6-DMAP (μM)	Trochophore Larvae					E. Veliger		P. Veliger
	SR	RSR	AR	RAR	TY	SR	RSR	RSR
150	49.2 ± 1.5	74.4	52.5 ± 5.9	106.9	55.9	22.5 ± 2.1	66.9	69.6
300	42.1 ± 0.4	63.7	93.2 ± 1.3	189.8	73.5	—	—	—
450	2.0 ± 2.8	3.0	100.0 ± 0.0	203.7	—	—	—	—
Control	66.1 ± 3.5	100.0	49.1 ± 3.5	100.0	0.0	33.6 ± 5.1	100.0	100.0

Survival rate (SR) = trochophore or veliger numbers/fertilized eggs (100%).

Relative survival rate (RSR) = survival rate of each replicate/survival rate of control (100%).

Veliger survivorship = veliger number/trochophore number (100%).

Abnormal trochophore rate (AR) = abnormal trochophore larvae/(normal trochophore + abnormal trochophore) (100%).

Relative abnormal rate (RAR) = abnormal rate of treated group/abnormal rate of control (100%).

Triploid yield (TY) = $N_{3n}/N_{3n} + N2_n$ (100%).

N_{3n} is the number of metaphase chromosomes of triploids, and N_{2n} is the number of chromosomes of diploids.

RESULTS

Effect of 6-DMAP Concentration on Triploid Induction by Blocking Meiosis II Division for 15 min

At concentrations of 150, 300, and 450 μM, the normal veliger larvae were found in the 150 μM group and their relative survival rate was 66.9%. In the other two groups, only 2.9% of fertilized eggs reached the trochophore stage in the 300 μM group and no normal trochophore larvae were found in the 450 μM group. The triploid yields were 55.9 and 73.5% in the 150 and 300 μM 6-DMAP groups, respectively (Table 1).

With increases of concentrations form 75 to 150 μM, the triploid yields increased from 30.0 to 55.1%, whereas the relative survival rates of veligers remained more than 77.2% (Table 2). The increases of triploid yields were 16.0% from 75 to 100 μM treatments, only 1.0% from 100 to 125 μM treatments, and 7.0% from 125 to 150 μM treatments.

Effect of 6-DMAP Concentration on Triploid Induction by Blocking Meiosis II Division for 20 min

At a given 6-DMAP concentration, treatments lasting for 20 min resulted in higher triploid yields but lower survival rates than did treatments lasted for 15 min. The number of abnormal larvae in the 20-min group was 10.0% higher than that in the 15-min group (Table 3). As in the 15-min groups, triploid yields also depended on concentrations of 6-DMAP. From 75 to 100 μM, the increment of triploid yield was 18.9%, which was the biggest increase between groups treated with chemicals at the other concentrations. Comparison of triploid yields and relative survival rates of postveligers revealed significant differences ($p < 0.01$) between treatments with different 6-DMAP concentrations, but no significant differences (*Argopecten purpuratus* > 0.05) between treatments with the same 6-DMAP concentration for different durations (15 or 20 min). The relative survival rate decreased 4.8% from the 15- to the 20-min treatments, whereas relative abnormality increased 37.0%. The relative survivorships were almost the same at early veliger stage in both 15- and 20-min treatments at the given concentration, but 5.1% higher in 15-min treatments than that in 20-min treatments at the postveliger stage.

Effect of 6-DMAP Concentration on Triploid Induction by Blocking Meiosis I Division for 15 min

Except at 75 μM 6-DMAP, at other given concentrations, the triploid percentages resulted from blocking meiosis I division was lower than that from blocking meiosis II division. The relative survival rate of the pb1 triploid group was 26.9% lower and the abnormal rate was 8.18 times higher than those of pb2 triploid group. The relative survival rates at early and postveliger stages were only 2.5 and 27.2% of those from meiosis II treatments (Table 4).

Comparison of the Effects of Different Egg Densities on Triploid Yields

No positive correlation was found between triploid percentages produced and different egg densities (150–500/mL) at the given chemical concentrations. Differences in survivorship, relative sur-

TABLE 2.

The induction of triploid in *H. discus hannai* Ino by 6-DMAP in meiosis II lasting 15 min (fertilized rate is 71.2%).

6-DMAP (μM)	Trochophore Larvae					E. Veliger		P. Veliger
	SR	RSR	AR	RAR	TY	SR	RSR	RSR
75	65.5 ± 4.8	96.9	6.6 ± 6.5	103.1	30.0	76.8 ± 10.3	89.3	96.8
100	64.1 ± 4.6	94.3	7.4 ± 7.8	115.6	46.0	74.1 ± 8.4	86.1	95.3
125	64.1 ± 5.2	94.8	9.3 ± 5.6	145.3	47.0	73.3 ± 8.3	85.2	94.8
150	61.2 ± 6.4	90.5	7.1 ± 6.3	110.9	54.0	66.4 ± 7.3	77.2	90.5
Control	67.6 ± 4.7	100.0	6.4 ± 5.1	100.0	0.0	86.0 ± 3.4	100.0	100.0

TABLE 3.

The induction of triploid in *H. discus hannai* Ino by 6-DMAP in meiosis II lasting 20 min

6-DMAP (μM)	Trochophore larvae					E. veliger		P. veliger
	SR	RSR	AR	RAR	TY	SR	RSR	RSR
75	79.4 ± 4.1	95.9	14.2 ± 7.9	124.6	30.1	82.7 ± 10.3	89.4	95.8
100	74.7 ± 4.8	90.2	18.1 ± 6.1	158.8	49.0	81.4 ± 3.7	88.0	90.2
125	73.3 ± 7.4	88.5	19.4 ± 5.1	170.2	51.2	77.5 ± 6.0	83.3	88.5
150	68.4 ± 7.2	82.6	19.3 ± 5.0	169.3	56.0	75.2 ± 11.2	81.3	82.6
Control	82.8 ± 5.2	100.0	11.4 ± 5.5	100.0	0.0	92.5 ± 7.3	100.0	100.0

vivorship, and abnormality seemed dependent on the fertilization rates only (Table 5).

Comparison of the Developmental Speeds Between Treated and Control Groups

The development of treated embryos was slower than that of control embryos (Table 6).

Effects of 6-DMAP Treatments on Growth of Juvenile Abalone

In the first after treatments, the growth rate (54.1 μm/day) in shell length of treated group was still slower than that in control (59.1 μm/day) and showed a significant difference ($p < 0.01$). The growth rate in shell width of the treated group was similar to that of the shell length (Table 7).

Four months postfertilization, the growth rates in both treated and control groups were calculated again. In large-size groups (1.2–1.3 cm), triploid and diploid abalone showed a significant difference ($p < 0.01$) in all dimensions and weights. In small-size groups (0.7–0.8 cm), however, no significant difference ($p < 0.01$) was found between triploid and diploid groups in their dimensions, but differences were found in total weights (Table 8). Comparisons between Tables 6, 7, and 8 revealed that the growth rates in triploid groups were slower than those in controls at the early stage, whereas at the later stage, the mean shell dimension(s) of triploid groups were significant longer than those of diploid siblings in large-size groups.

DISCUSSION

Cytochalasin B is one of the most important chemicals in oyster polyploidy induction and has been extensively used in shellfish chromosome manipulations. Although cytochalasin B could induce as high as 100% triploids in some experiments (Allen et al. 1982, Downing and Allen 1987, Wang et al. 1990, Nell et al. 1996, Stepto et al. 1998), its high toxic nature and expense impede its use in commercial hatcheries. High mortality of larvae (about 85–90%) after treatment is another consideration. Therefore, new approaches that should be inexpensive, nontoxic, and consistent with high percentage of triploid yields are needed, especially new chemicals and comprehensive techniques. *C. gigas* may produce more than 40–50 million eggs by strip spawning a 2-y-old oyster. Therefore, 10% survivorship after cytochalasin B treatment, the common cases in most experiments, could be tolerated in hatcheries. Pacific abalone, on the other hand, could not be strip spawned. A 7–8 cm female abalone could produce only 0.8–1.2 million eggs in a spawning season, even after 70–80 days conditioning in the hatchery. Without any treatments, the survival rate of abalone from zygotes to juveniles about 1.0 cm in shell length is usually less than 10.0%. If eggs are treated with cytochalasin B, the survivorship will drop to 1–5% (or less) at as early as veliger stages (Wang et al. 1990). Therefore, techniques of including triploid abalone by the use of cytochalasin B could hardly be applied in commercial scales. The results from this study suggested that the 6-DMAP technique could overcome the above-mentioned disadvantages in triploid induction and thus meet the large-scale requirement in abalone industries.

pb1 Triploids should, theoretically, have higher heterozygosity than their pb2 triploid and diploid siblings; therefore, they should be very useful in aquaculture (Stanley et al. 1984). In this study, however, no high performances were found in pb1 triploids. In addition, at optimal 6-DMAP concentrations (125–150 mM), the relative abnormalities of pb1 triploids increased to 1,317–1,625%, which were much higher than those of pb2 triploids. Therefore, as in oysters, pb2 triploid techniques would be the potential methods that could be used in large-scale triploid productions in abalone.

As in a previous report on bivalves (Desrosiers et al. 1993), increasing periods of incubation with 6-DMAP also improved the

TABLE 4.

The induction of triploid in *H. discus hannai* Ino by 6-DMAP in meiosis I lasting 15 min

6-DMAP (μM)	Trochophore larvae					E. veliger		P. veliger
	SR	RSR	AR	RAR	TY	SR	RSR	RSR
75	56.6 ± 16.6	70.6	11.0 ± 0.2	282.1	30.4	85.8 ± 6.6	92.7	70.6
100	54.8 ± 16.8	68.4	25.8 ± 14.2	661.5	43.0	78.9 ± 1.8	85.2	68.4
125	54.5 ± 12.5	68.0	51.4 ± 19.8	1317.9	46.6	77.8 ± 10.9	84.0	68.0
150	49.5 ± 13.7	61.8	63.4 ± 15.9	1625.6	51.0	72.4 ± 8.4	75.7	61.8
Control	80.1 ± 8.7	100.0	3.9 ± 4.4	100.0	0.0	92.6 ± 2.6	100.0	100.0

TABLE 5.

The induction of triploid in *H. discus hannai* Ino by 6-DMAP in meiosis II lasting 15 min in different treated densities.

6-DMAP (μM)	DT* (egg/mL)	FR	Trochophore Larvae			E. Veliger	P. Veliger
			SR	AR	TY	SR	RSR
150	150	52.1	48.5	48.3	45.2	65.1	53.9
150	250	52.1	50.4	56.7	66.7	71.3	69.2
150	500	71.2	51.2	7.1	54.0	77.2	90.5

* DT, density of treatment.

production of triploid eggs in Pacific abalone. However, when the eggs were exposed to 6-DMAP for periods of time covering all of the meiosis II and overlapping the first mitotic division in the control, the proportion showing abnormal chromosome behavior increased and survival decreased sharply. These results suggest that a high percentage of triploids and good yield of normal larvae may be obtained after exposure to 6-DMAP for a period of incubation that is shorter than the duration of meiosis II.

The purine analog 6-DMAP was initially used as a cleavage inhibitor in sea urchin eggs (Rebhun et al. 1973). Recent studies on 6-DMAP have demonstrated that the drug exerts its actions on protein phosphorylation, microtubule organization, metaphase spindle formation, and cortical filament organization in different animals (Dufresne et al. 1991, Rime et al. 1989, Szollosi et al. 1991). These cellular effects can explain how the triploidy state can be induced by blocking chromosome movement and extrusion of polar bodies by 6-DMAP.

Comparison between methods used in previous studies and methods used in this study clearly reveals that treatment with 6-DMAP is the most simple, inexpensive, and efficient technique to induce triploidy in abalone. 6-DMAP treatment does not require expensive or specialized equipment. 6-DMAP is water soluble and can be easily washed out in seawater after the treatment. 6-DMAP is safer than cytochalasin B, which is known to be a carcinogenic product. Treatment with 6-DMAP in abalone was as effective as in bivalves (Desrosiers et al. 1993, Gerard et al. 1994). Therefore, 6-DMAP should be an efficient and safe alternative method to induce triploidy with a high potential in commercial aquaculture in abalone.

The best yield of 6-DMAP to produce triploid abalone was 73.5%, which is slightly lower than that to produce triploid bivalves (Desrosiers et al. 1993, Gerard et al. 1994). The main reason for lower triploid yields in this research could result from the eggs, which were not well synchronized, because the efficiency to induce triploid depends on the synchrony of meiosis in the eggs (Downing and Allen 1987), the time of initiation, and the duration of treatments (Desrosiers et al. 1993).

In Pacific abalone, the optimal 6-DMAP concentrations for triploid induction are much lower than those recommended for bivalves. For example, the relative abnormalities at trochophore stages were as high as 93.2% at 300 mM 6-DMAP and 100.0% at 450 mM, although the doses were within the optimal ranges suggested for oyster ploidy manipulations. At a concentration of 150 mM the performance of abalone larvae improved tremendously, the survival and relative survival at trochophore stage increased to 49.2 and 74.4%, respectively, and the relative postveliger survival remained at 69.6%. The abalone industries in China have already shown much interest in the methods.

The high survivorship of juveniles expressed in treated groups remained to at least 4-mo-old abalone. At 125–150 mM 6-DMAP, the survival rate from shell sizes of 3–4 mm in length to 10–12 mm was about 1–5% in controls, whereas it was 30.0–35.0% in treated groups.

Unlike oysters, in which the growth rate of triploid and diploid larvae is generally similar (Downing and Allen 1987), the growth rates of abalone larvae and early juveniles in treated groups were lower than those in controls. At 20.0°C, 47.2% of the embryos in control groups reached archenteron 8.25 h postfertilization, whereas only 43.5% in treated groups turned up at this stage at the same time. The slight difference might result from the prohibition of development in treated groups. However, this situation changed 4 mo later; triploid groups showed faster increases in both shell dimensions and total weight. The bigger the size of the groups, the faster the triploids grew.

Much of the utility of triploid shellfish to aquaculturists or to fisheries' managers is a consequence of their sterility or semisterility, which precludes the possibility of unwanted reproduction

TABLE 6.

The developmental comparison between the treated and control groups of *H. discus hannai* Ino (22 ± 0.5).

D	T (h)	Group	Developing Rate (%)
2 Cells	1.05	Treated	45.4
		Control	64.3
4 Cells	1.35	Treated	40.3
		Control	49.0
Archenteron	8.15	Treated	43.5
		Control	47.2
Trochophore	12.30	Treated	43.0
		Control	47.9

Note: D, developmental stage; T, time of postfertilization.

TABLE 7.

The comparison of early growth between the treated for triploid and control groups of *H. discus hannai* Ino that settled on May 13 (n = 20).

Group	Item	2/6	5/6	13/6
Treatment	SL (μm)	785.4 ± 53.9	1.035.4 ± 92.0	1.358.0 ± 108.1
	SW (μm)	710.8 ± 64.3	956.9 ± 81.1	—
Control	SL (μm)	902.9 ± 64.5	1.154.6 ± 117.2	1.552.7 ± 173.0
	SW (μm)	824.0 ± 71.5	1.056.4 ± 125.7	—

Note: SL, shell length; SW, shell width.

TABLE 8.

The comparison of growth in triploid treated and control groups of *H. discus hannai* Ino rearing for about 4 mo (n = 30 for each group).

Group		Shell Length (cm)	Shell Width (cm)	Shell Thickness (cm)	Total Weight (g)
3N	Group 1	1.37 ± 0.12	0.95 ± 0.08	0.23 ± 0.04	0.38 ± 0.14
	Group 2	0.82 ± 0.22	0.58 ± 0.13	0.15 ± 0.04	0.09 ± 0.06
2N	Group 1	1.27 ± 0.12	0.89 ± 0.09	0.21 ± 0.03	0.29 ± 0.10
	Group 2	0.78 ± 0.15	0.55 ± 0.11	0.14 ± 0.04	0.08 ± 0.03
(3N-2N)/2N	Group 1	7.87%	6.74%	9.52%	34.48%
	Group 2	5.13%	5.45%	7.14%	12.50%

because they produce, theoretically, aneuploid gametes. The mature individuals show fast growth and high quality of meat in the reproductive season. However, juvenile triploid abalone produced in this study began to show better performance in growth than did diploid controls at about 1 cm in shell length. The advantages of triploid groups could result from the relatively high heterozygosity of the triploid itself or from the selection effects of a chemical on individuals or both. Zygotes sensitive to chemical treatments stopped development or became abnormal, whereas the remaining were triploids and/or selected zygotes. Animals developed from those zygotes might, therefore, have greater vigor and thus the ability to withstand environmental variability at juvenile stage.

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