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TRIPLOIDÍA EN ALMEJA CATARINA (*Argopecten ventricosus*, Sowerby 1842): INDUCCIÓN, CRECIMIENTO, GAMETOGÉNESIS Y COMPOSICIÓN BIOQUÍMICA

T E S I S

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Uso, Manejo y Preservación de los Recursos Naturales
(Orientación Acuacultura)

p r e s e n t a

César Arturo Ruiz Verdugo

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ACTA DE REVISION DE TESIS

En la Ciudad de La Paz, B. C. S., siendo las 12:00 horas del día 5 del Mes de Diciembre del 2000, se reunieron los miembros de la Comisión Revisora de Tesis designada por la Dirección de Estudios de Posgrado del Centro de Investigaciones Biológicas del Noroeste, S. C., para revisar la Tesis de Grado titulada:

" TRIPLOIDÍA EN ALMEJA CATARINA (*Argopecten ventricosus*, Sowerby 1842): INDUCCIÓN, CRECIMIENTO, GAMETOGÉNESIS Y COMPOSICIÓN BIOQUÍMICA "

Presentada por el alumno:

César Arturo Ruiz Verdugo

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Después de intercambiar opiniones los miembros de la Comisión manifestaron su **APROBACION DE LA TESIS**, en virtud de que satisface los requisitos señalados por las disposiciones reglamentarias vigentes.

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Con todo mi ser

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RESUMEN

La inducción a la triploidía ha sido uno de los métodos más empleados para el mejoramiento genético en moluscos bivalvos. Teóricamente, la presencia de un tercer cromosoma homólogo afecta a la sinapsis, inhibiendo el proceso meiótico durante la formación de gametos. Esto da como resultado que los organismos triploides presenten una gónada pequeña o muy reducida, pero con un mayor tamaño o biomasa que los diploides, debido a que canalizan la energía al crecimiento y no a procesos de maduración. La almeja catarina *Argopecten ventricosus* es la única especie nativa de bivalvo que se cultiva en Baja California Sur. La temprana edad en que la almeja catarina alcanza su primera madurez sexual es una de las limitaciones biológicas para obtener óptimas producciones con esta especie, ya que esta ocurre a una talla pequeña (2 cm), afectando la tasa de crecimiento posterior. El objetivo de esta investigación fue por lo tanto el de evaluar los efectos de la triploidía sobre el crecimiento, madurez sexual, y composición bioquímica en almeja catarina.

Para la inducción a la triploidía se usaron dos concentraciones del antibiótico Citocalasina B (0.1 mg/l y 0.5mg/l), encontrando que en el tratamiento de 0.5 mg/l fue el mejor para la inducción a la triploidía en función de la estimación del éxito obtenido y supervivencia. El crecimiento se evaluó en condiciones de cultivo en campo observando que las diferencias en crecimiento fueron mas marcadas después de que los diploides alcanzaron su madurez sexual. La mayor diferencia se encontró en el peso del músculo, el cual fue 180% mayor en triploides que en diploides. Una característica interesante fue que los triploides desarrollaron una gónada anormal, la cual fue fácilmente reconocible por medio de observación visual, que permitió separar en forma individual a los triploides de los diploides para análisis comparativo. La gametogenesis y la condición de hermafroditismo, evaluadas por cortes histológicos, fueron afectadas por la triploidía, de tal forma que se describen nuevos estadios de gametogénesis para las almejas catarinas triploides. La porción femenina de triploides desarrolló pocos ovocitos, mientras que la masculina fue severamente afectada en los estadios primarios, permaneciendo en el estadio de espermatogonias. En organismos de mayor edad los acinos masculinos fueron reemplazados por acinos femeninos, produciendo organismos exclusivamente hembra, suprimiendo la condición de hermafroditismo hasta en un 96% de los organismos. Los ovocitos de los triploides fueron más grandes que los de los diploides en estadios avanzados de madurez, posiblemente como consecuencia del mayor

tamaño nuclear. En cuanto a la composición bioquímica entre diploides y triploides, las diferencias fueron principalmente en el contenido de lípidos y triglicéridos en la gónada durante los periodos de madurez de los diploides, siendo ese contenido mayor en diploides, indicando que en los organismos triploides no se almacena lípidos en gónada debido a su reducida capacidad de formar gametos. El contenido de carbohidratos en músculo por otro lado fue superior en triploides solo después del desove de los diploides. Se evaluó también el efecto del tratamiento (Citocalasina B) a nivel de familias, encontrándose diferencias entre familias en la producción de triploides, familias con 0% de triploides y otras con 100% de triploides. Los resultados de crecimiento en campo, a nivel de familia, permitieron corroborar las grandes diferencias entre diploides y triploides, siendo el músculo aductor hasta un 167% mayor en triploides.

Finalmente, este es el primer trabajo sobre triploidía en México, y con los resultados de este trabajo se plantea una alternativa real para el mejoramiento de la producción de la almeja catarina a través de la aplicación de esta biotecnología de ploidía.

ABSTRACT

Triploidy induction has been one of the methods most successful for the genetic improvement of bivalve mollusks. Theoretically, the presence of a third homologous chromosome affects the synapses, inhibiting the meiotic process during gamete formation. This results in that triploid organisms have a small or very reduced gonad, but a larger size or biomass that diploids, because they channel the available energy to growth and not to maturation processes. The catarina scallop, *Argopecten ventricosus* is the only native bivalve species cultured in Baja California Sur. The early age at which the catarina scallop reaches its first sexual maturity is one of the biological limitations to obtain better productions with this species, because it reaches that size when only 2cm, decreasing thereafter its growth rate. The objective of this research was to evaluate the effects of triploidy on growth, sexual maturity, and biochemical composition of catarina scallop.

For the induction to triploidy, two concentrations of the antibiotic Cytochalasin B were used (0.1 mg/l and 0.5mg/l), finding that the treatment with 0.5 mg/l was the best in function of the estimated success and survival. Growth was evaluated under growout conditions, observing that the differences in growth between diploids and triploids were significant after diploids reached sexual maturity. The largest difference was on the weight of the adductor muscle, which was 180% heavier in triploids that in diploids. An interesting finding was that triploids developed an abnormal gonad, which was easily recognizable by means of visual observation, which allowed to separate triploids from diploids for further comparative analysis. Gametogenesis and the hermaphroditism condition, evaluated by means of histology, were affected by triploidy in such a way that new gametogenesis stages were described for triploid catarina scallop. The female portion triploid gonads developed few oocytes, while the male portion was severely affected from the primary developmental stages, remaining in the spermatogonies stages. In older organisms, the male acinus was replaced by female acinus, producing exclusively female organisms, and suppressing the hermaphrodite condition in 96% of the organisms. Triploid oocytes were bigger than diploids in advanced maturity stages, possibly as a consequence of a largest nuclear size. As for the biochemical composition between diploids and triploids, the differences were primarily in lipids acylglicerides in gonad during the time of maturity in the diploids. Diploids had larger concentrations of those biochemical components than triploids, indicating that in triploid

organisms lipids were not stored in gonad because its reduced capacity to form gametes. Carbohydrate contents in muscle were superior in triploids only after diploid scallops spawned. The last study was for the evaluation of the effect of treatment (Cytochalasin B) at the family level, finding differences among families in the success triploid to production. Families with 0% triploidy and others with 100% triploidy were observed regardless of all having treated with the same CB concentration. It was concluded that one of the main factors affecting the success in triploid production is the quality of the used eggs. The results of growout in these families allowed for the corroboration of large differences between diploids and triploids.

This was the first work on evaluation of triploidy in a Mexican mollusk species. With the results achieved with this research we propose an alternative for the genetic improvement on catarina scallop production through the application of this biotechnology.

INTRODUCCION

Las investigaciones encaminadas al mejoramiento genético en acuacultura en moluscos con valor comercial han llevado a la producción de organismos triploides como una de las mejores alternativas para incrementar la producción. La característica más importante que confiere la triploidía a un organismo es la esterilidad parcial o total. Un organismo triploide es estéril debido a una falla en la sinapsis de los juegos de cromosomas homólogos en el transcurso de la meiosis I, ya que se involucra a un tercer juego de cromosomas, lo que dificulta la sinapsis. La esterilidad se puede detectar por una falta total de producción de gametos, pero también por la producción de gametos no funcionales o funcionales pero en números muy reducidos. Esto resulta en que los organismos triploides se caractericen como animales con una gónada pequeña o muy reducida, pero con un mayor tamaño o biomasa que los diploides, debido a que canalizan toda la energía al crecimiento y no a procesos de maduración (Allen, 1988; Allen et al., 1989; Beaumont y Fairbrother, 1991).

Los estudios sobre la inducción a la triploidía en moluscos bivalvos, indican que las técnicas de manipulación cromosómicas como método de mejoramiento genético, han alcanzado ya un nivel de madurez (Allen y Downing, 1986; Allen et al., 1989; Beaumont y Fairbrother, 1991; Guo y Allen, 1994a). De esta forma la producción de bivalvos triploides se ha logrado con éxito en diferentes especies de importancia comercial, por ejemplo, en ostiones (Stanley *et al.* 1981; Allen et al ., 1989; Nell et al., 1994; Eudeline, 2000), mejillones (Yamamoto y Sugawara, 1988), almejas (Allen et al., 1982), ostras perleras (Jiang et al., 1991) y pectínidos (Tabarini, 1984; Komaru y Wada, 1989) (Tabla 1).

Fundamento

Los procesos citológicos que ocurren durante la meiosis sustentan el principio de la manipulación cromosómica. La meiosis es un tipo de división celular presente en las células germinales de los organismos que se reproducen sexualmente. Es un proceso de reducción cromosómica por el que los cromosomas se reducen a un numero haploide. La meiosis consta de dos divisiones sucesivas (meiosis I y II) intercaladas por una muy corta interfase. En la meiosis I (primera etapa reduccionaria) se reduce el número de cromosomas replicados a la mitad, esto es, cada uno de los cromosomas homólogos ya replicados migra a polos opuestos para su primera división reduccional. En la meiosis II (etapa ecuacional) los cromosomas replicados se separan, y migran a polos opuestos, lográndose así la 2da división

reduccional. Los procesos esenciales de la meiosis son: el apareamiento o sinapsis de los cromosomas homólogos; la formación de quiasmas, que representan la recombinación genética; y la segregación de los cromosomas homólogos. Los estadios de la meiosis son profase I, metafase I, anafase I, telofase I, interfase I, profase II, metafase II, anafase II y telofase II. El inicio del proceso de meiosis esta precedido por una interfase durante la cual se duplica el material genético. La primera división de la meiosis tiene una larga profase I, en donde la envoltura nuclear y el nucleolo se desorganizan, los centríolos migran a los polos opuestos duplicándose y se ordena el huso acromático. Durante la profase I se lleva a cabo la sinapsis y ocurre la recombinación entre las cromátidas homologas. Se divide en 5 etapas: Leptoteno, Cigoteno, Paquiteno, Diploteno y Diacinesis. En la prometafase I la condensación de los cromosomas alcanza su máximo. La envoltura nuclear se rompe y los microtúbulos del huso se unen a los centrómeros, los cromosomas migran al plano ecuatorial de la célula, y da inicio la metafase I, donde los cromosomas homólogos se alinean en el plano ecuatorial. Los 2 cromosomas homólogos se unen por medio del centrómero a la misma fibra del uso acromático. Durante la anafase I los 2 cromosomas homólogos se repelen y migran a polos opuestos. Cada cromosoma está formado por 2 cromatidas, la telofase I comienza cuando los cromosomas llegan a los polos, se desorganiza el huso acromático y los ásteres; se reorganizan la envoltura nuclear y los nucleolos. El resultado de la primera división meiótica es la formación de los nucleos hijos, que en los animales se denominan espermacitos primarios (macho) y ovocito primario mas el primer cuerpo polar (en la hembra). La citocinesis se produce simultáneamente con la telofase, y da como resultado 2 células hijas con un número haploide de cromosomas. En la interfase I, entre las dos divisiones meióticas, no hay duplicación de los cromosomas replicados. En la meiosis II, los procesos de esta división son semejantes a los de una mitosis en una célula haploide. Durante la profase II, se condensan los cromosomas, se desintegran los nucleolos; los centríolos migran a los polos y se duplican, se forma el huso acromático y se desorganiza la envoltura nuclear. En la prometafase II, los cromosomas condensados migran a la placa ecuatorial de la célula. Posteriormente en la metafase II, los cromosomas se disponen en el plano ecuatorial, los centrómeros se separan y las dos cromátidas hijas se dirigen a los polos opuestos. Durante la anafase II se separan las 2 cromátidas de cada cromosoma. Cada una migra a un polo diferente. En la telofase II, los grupos cromosómicos llegan a los polos, el huso acromático se desorganiza; se reorganizan la envoltura nuclear y el nucleolo, se dispersan los

cromosomas y se transforman en cromatina. En la citocinesis, se separan los citoplasmas de las células hijas. El resultado de la meiosis es la formación de cuatro células haploides, que en los machos corresponde a los espermatozoides y en hembras al óvulo con dos cuerpos polares. (De Robertis y De Robertis, 1981; Alberts et al., 1998). Es conveniente señalar que durante la meiosis de las células germinales femeninas, la división del citoplasma se da en manera desigual, de tal forma que una de las células contiene casi la totalidad del citoplasma y los cuerpos polares contienen prácticamente solo los juegos de cromosomas. Al final de la meiosis en hembras se produce un ovocito con dos cuerpos polares, el segundo cuerpo polar se observa adherido al ovocito e intermedio al primer cuerpo polar (Beaumont y Fairbrother, 1991).

Producción de triploides

Se pueden inducir cambios en la ploidía de los bivalvos artificialmente, mediante la manipulación del proceso de meiosis del ovocito, ya que en los bivalvos los ovocitos son desovados antes de la metafase de la meiosis I, que corresponden al estadio de ovocito primario. El ovocito reinicia su desarrollo hasta el momento en que es fertilizado por el espermatozoide. La triploidía ha sido inducida por diferentes tipos de tratamientos (Allen, 1987; Beaumont y Fairbrother, 1991), los cuales se pueden aplicar durante ambas fases de la meiosis, bloqueando la formación del primero o segundo cuerpo polar. Estas técnicas permiten la división de los cromosomas (cariocinesis) mientras que previenen la división del citoplasma (citocinesis) con fines de obtener un huevo diploide, el cual al unirse al pronúcleo haploide normal del esperma da lugar a la formación de un cigoto triploide (Allen et al., 1989).

En la actualidad existe una variedad de técnicas que están disponibles para la producción de organismos triploides. Dentro de los factores importantes que intervienen en la inducción a la triploidía los más importantes han sido descritos como: el tiempo de aplicación; la duración del tratamiento; la temperatura del agua y finalmente la dosis del inductor. Los dos primeros factores están en función del proceso meiótico de los ovocitos recién fertilizados; la aplicación del tratamiento debe ser en el momento en el que se observa en los ovocitos la aparición del primer cuerpo polar, y el tiempo de duración del mismo debe ser igual al tiempo en que el ovocito libera el segundo cuerpo polar. Por otra parte, la

temperatura es uno de los factores más importantes que determinan la duración del desarrollo de los ovocitos (Allen et al., 1989), ya que este es dependiente de la temperatura. A diferentes temperaturas la extrusión de los cuerpos polares ocurre a distintos periodos de tiempos. Así mismo la temperatura interviene en la sincronía del desarrollo de los ovocitos, lo cual es fundamental para lograr un mayor porcentaje de triploides. Este es otro de los aspectos importantes en la producción de triplolde s, ya que si la mayoría de los ovocitos se encuentran en una misma etapa del desarrollo, y en el momento exacto de desarrollo de ovocitos en el que el tratamiento es efectivo, mayor será el éxito. Recientemente ha sido demostrado que la mejor manera de obtener ovocitos con mayor sincronía es trabajando con familias en lugar de producir triploides en masa (diferentes hembras), debido a que se tiene una mejor sincronía cuando los ovocitos provienen de una sola hembra (Eudeline et al., 2000).

Por otra parte es importante considerar a la condición del reproductor como otro factor para la producción de triploides, ya que de esto depende la calidad del huevo, la cual es importante para que se logre una mejor sincronía en el desarrollo de los ovocitos (Allen et al., 1989). Así mismo, una buena calidad del huevo medida en contenido de lípidos, ayuda a que estos puedan soportar los tratamientos químicos y el desarrollo embrionario (Utting y Doyou, 1992). Para lograr una buena calidad de huevo en bivalvos es importante optimizar los factores que intervienen en el proceso de acondicionamiento de los reproductores. En resumen, la existencia de metodologías óptimas para el acondicionamiento de reproductores, y en menor grado el estandarizar un protocolo preciso para la inducción a la triploidía, son los factores de mayor importancia en el éxito de esta Biotecnología.

En todas las técnicas de inducción a la triploidía se trata de seguir un protocolo en el cual se varían los diferentes factores ya mencionados. En la tabla 1 se resumen la mayoría de los trabajos sobre triploidía en moluscos bivalvos, así como el porcentaje de éxito obtenido. Los tratamientos pueden ser químicos como: citocalasina B (CB), calcio, cafeína y 6-dimetilaminopurina; o físicos como cambio de temperatura o aplicación de presión (Tabla 1). El químico más comúnmente utilizado en la inducción a la triploidía en moluscos bivalvos es la citocalasina B (CB), un antibiótico extraído del hongo *Helmintosporium dematioideum*. La citocalasina-B actúa inhibiendo la polimerización de la actina. Normalmente las fibras de

actina producen la constricción necesaria para la formación de los cuerpos polares y de las células de segmentación. En presencia de CB, el anillo contráctil no se forma y no hay división celular, inhibiendo así la reducción cromosómica durante la conclusión de la meiosis (Longo, 1972). Este inductor se ha aplicado con éxito para diferentes especies de peces, moluscos y crustáceos. Las distintas concentraciones de CB que han sido utilizadas para producir triploides varían en función de la especie a inducir, por ejemplo en *Argopecten irradians* se reportó la producción de triploides con concentraciones de CB de 0.1 mg/l (Tabarini 1984), mientras que en *Saccostrea commercialis* se han utilizado concentraciones de CB de 1.5 mg/l (Tabla 1). Otro químico, 6-Dimetilaminopurina (6-DMAP) se ha usado con éxito en ensayos de inducción de triploidía (Desrosier et al., 1993; Gérard et al., 1994). Los resultados obtenidos en cuanto a porcentaje de triploides son muy similares a los obtenidos con la citocalasina B. Los otros inductores químicos han sido probados solamente a nivel experimental ya que producen triploides en un porcentaje sumamente bajo (Scarpa et al., 1994). A pesar del éxito en la producción de triploides, la supervivencia en estadios larvales de larvas tratadas con respecto a los ovocitos inducidos es muy baja (Beaumont y Fairbrother, 1991). Recientemente se ha reportado un método que garantiza un 100% de triploides, que es mediante la producción de triploides biológicos al unir gametos de tetraploides (2n), con gametos de organismos diploides (n). Por medio de este método se obtiene una sobrevivencia significativamente más alta si se compara con las técnicas que utilizan químicos (Guo et al., 1996). Sin embargo, es importante señalar que para producir organismos tetraploides, ha sido necesario el contar con hembras triploides con alta fecundidad (Guo et al., 1996), al menos en ostión japonés.

Ventajas de la triploidía.

Es generalmente aceptado que los triploides no crecen más rápido que los diploides previo a la madurez sexual. Mientras que la maduración gonádica se lleva a cabo en los diploides, donde una gran parte de la energía es utilizada en la producción de gametos, esta energía en organismos triploides y estériles es desviada hacia la producción de tejidos somáticos como el músculo. De esta forma, los organismos triploides pueden alcanzar tallas más grandes en menor tiempo que un organismo diploide. Por ejemplo, Stanley et al. (1981) encontraron que los triploides y diploides de *Crassostrea virginica* promediaron la misma talla después de 8 meses de edad. Sin embargo a los 3 años de edad, los ostiones triploides

tenían un 40% de volumen y 12% de altura de la concha mayor que el de sus hermanos diploides. Las ventajas en crecimiento de bivalvos triploides han sido encontradas también en otras especies, siendo en los pectínidos el grupo dentro de los bivalvos en donde se han observados las mayores diferencias, particularmente en músculo aductor (Tabla 2). Como ya se mencionó, en casi todas las especies de bivalvos las diferencia en crecimiento entre los diploides y triploides normalmente se observan cuando la maduración es iniciada en los organismos diploides, así que la característica principal de los organismos triploides es la supresión de la gametogénesis produciendo una esterilidad generalmente parcial. Sin embargo esta supresión es diferencial entre y dentro de las especies. En triploides de *Mya arenaria* (Allen et al., 1986), *Chlamys nobilis* (Komaru y Wada, 1989) y *Mytilus galloprovincialis* (Kiyomoto et al., 1996), la esterilidad fue total, los triploides no produjeron ni espermatozoides ni ovocitos. En contraste con estos resultados, en *Crassostrea gigas, Pinctada fucata, Mullinia lateralis, y Saccostrea commercialis (*Komaru y Wada, 1989; Guo y Allen, 1994b; Cox et al., 1996*)* se han encontrado triploides con una gametogenesis reducida, con producción de algunos gametos. Aun más, en el caso de *C. gigas, y P. fucata* la fertilización de gametos triploides es posible (Guo y Allen, 1994c; Komaru y Wada, 1994). Los estudios sobre la fecundidad de triploides son muy importantes porque a partir de ovocitos de triploides (de hembras triploides con una fecundidad alta) es que se han producido los únicos organismos tetraploides. Por ejemplo, en ostión japonés se inhibió el primer cuerpo polar de ovocitos triploides fertilizados con espermas haploides, dando lugar a la producción de tetraploides. En el caso de los pectínidos la gametogenesis se ha descrito razonablemente bien para diploides hermafroditas, sin embargo la gametogenesis en pectínidos triploides sólo se ha estudiado en *Chlamys nobilis* (Komaru y Wada, 1989) un hermafrodita no funcional. Para *Argopecten irradians*, Tabarini (1984) solo reportó una reducción en el tamaño de la gónada de triploides con relación al tamaño de pectínidos maduros diploides, lo cual fue determinado con base en su morfología, pero la histología del proceso gametogénicos no fue investigada.

Durante la gametogenesis los cambios en la composición bioquímica de las partes del cuerpo de los moluscos bivalvos nos indican cual de los componentes bioquímicos contribuyen al metabolismo energético. En bivalvos marinos los ciclos estaciónales de almacenamiento y uso de energía son conocidos (Gabbot, 1975; Barber y Blake, 1981). En general, previo a la

gametogenesis cuando el alimento es abundante, la energía es almacenada en forma de lípidos, proteína, y glucógeno, y más tarde esta energía es utilizada durante la maduración, cuando la demanda metabólica es alta (Gabbot, 1975; Barber y Blake, 1981 y 1991; Sphigel et al., 1992).

En los pectínidos, las reservas de nutrientes se almacenan principalmente en la glándula digestiva y músculo aductor durante el período de crecimiento somático cuando el alimento es abundante. Los carbohidratos (principalmente el glucógeno en el músculo aductor) parecen ser la fuente energética más importante para el sostenimiento de la gametogenesis. (Barber y Blake, 1991; Martínez y Mettifogo, 1998). Estas reservas pueden ser catabolizadas para apoyar el metabolismo de mantenimiento a fin de aportar el material para la vitelogénesis. De echos, es significativo el efecto de la maduración sobre el peso e índice de condición del músculo aductor. Por ejemplo, Barber y Blake (1981 y 1983) reportan que el peso seco del músculo y el contenido de glucógeno disminuyen hasta un 75% durante la maduración de *Argopecten irradians irradians* . Así mismo, en *Argopecten irradians concentricus*; Epp et al. (1988) reportan que un 63-99% de la energía total utilizada por la gónada durante la gametogénesis proviene del músculo aductor, y es principalmente a expensas de la proteína del músculo. Por otra parte, la movilización de las reservas bioquímicas en relación con el ciclo reproductivo, parece depender del alimento disponible en el medio al menos en un pectínido. En *Chlamys varia* durante la maduración en primavera cuando el alimento es abundante, solamente las reservas de carbohidratos son utilizadas para el desarrollo de las gónadas, mientras que durante la maduración en otoño, cuando el alimento es menos abundante, todas las reservas (lípidos, proteínas y glucógeno) disminuyen con el desarrollo gonadal (Shafe, 1981).

Debido a la condición de triploidía, el desarrollo de la gónada es alterado como ya se describió anteriormente, lo cual se manifiesta por una reducción de los índices gonadales (Komaru y Wada, 1989) causados por una esterilidad parcial o total (Allen et al., 1986; Allen y Downing, 1990; Komaru y Wada, 1990; Guo y Allen, 1994a; Cox et al., 1996; Eversole et al., 1996; Kiyomoto et al., 1996), por lo cual se esperaría que en triploides la movilización de los componentes energéticos sea alterada. Existen algunos estudios que comparan las características fisiológicas y bioquímicas de diploides y triploides, aunque la mayoría de ellos se orientan hacia las diferencias presentes en el contenido de carbohidratos entre los

triploides y diploides. Por ejemplo, en *Crassostrea gigas,* durante la gametogénesis de diploides la concentración de glucógeno en el tejido completo de triploides es más alto (26.4% del peso seco) que en diploides (4.8% del peso seco) (Allen y Downing 1986). Sin embargo en los meses posteriores del periodo no reproductivo se observaron valores similares de concentración del glucógeno en ostiones triploides y diploides. Para la misma especie, Akashige (1990) encontró que la cantidad de glucógeno fue dos veces mas alta en triploides que en diploides. Para la almeja *Tapes philippinarum*, los triploides tuvieron una concentración de carbohidratos y un índice de condición mas altos (Utting et al., 1996). Con relación a pectínidos, Tabarini (1984) encontró que los índices gonadales de los triploides de *A. irradians* fueron más bajos que los diploides, sin embargo el contenido de glucógeno, el peso seco de la biomasa y músculo aductor fueron más altos en triploides que en diploides. Al parecer, la esterilidad parcial observada en *A. irradians* fue la responsable de la falta de movilización de reservas de energía del músculo aductor hacia la gónada (Tabarini, 1984).

Además de la hipótesis que plantea la redirección de la energía destinada para la maduración en organismos triploides hacia el crecimiento, existen otras dos hipótesis propuestas para tratar de explicar el mayor incremento en peso de los organismos triploides. La primera es la hipótesis propuesta por Stanley et al. (1981), quienes señalan que el incremento en mayor peso de los organismos triploides es debido a un incremento en la heterocigocidad. En apoyo a esta hipótesis, Stanley et al. (1984) y Hawkins et al. (1994) encontraron que los triploides de meiosis I fueron superiores a los triploides de meosis II y diploides. La segunda hipótesis fue propuesta por Guo y Allen (1994b), quienes proponen que el mayor tamaño de los moluscos triploides se puede explicar por un fenómeno llamado gigantismo poliploide. Debido a que las células triploides tienen un mayor volumen que las diploides, y a que los moluscos no presentan un mecanismo de compensación en el numero de células como ocurre en peces, esto resulta en un "gigantismo poliploide" (Guo y Allen, 1994b).

Almeja catarina.

La almeja catarina *(Argopecten ventricosus,* Sowerby II, 1842) es el único molusco bivalvo nativo que se cultiva a escala comercial en Baja California Sur, y su pesquerías es una de las más importantes del estado. Esfuerzos considerables desde hace 17 años se han llevado a cabo para lograr su cultivo comercial (Maeda-Martínez et al., 2000). La almeja catarina es una pectínido pequeño (talla máxima 60 mm), siendo esta una de las limitaciones biológicas

principales para su cultivo. Así mismo, en la almeja catarina se ha encontrado que la gametogenesis tiene un efecto significativo en la productividad, ya que la cosecha comercial ocurre a la edad de 1 año ó 6 cm de longitud, mientras que la edad de primera madurez sexual ocurre en un tiempo corto del ciclo de vida (Cruz et al., 2000). Sin embargo esto puede variar en función de las condiciones del medio. Por ejemplo, en Bahía Magdalena la población alcanza su madurez sexual a una talla 1.8 cm y 4 meses de edad, pero en Bahía Concepción, un ambiente caracterizado por temperaturas altas y baja productividad, la población alcanza su madurez sexual a 1 año de edad (Villalejo-Fuerte y Ochoa, 1993).

En este sentido es necesario buscar alternativas para mejorar la talla de la almeja catarina. Con estos argumentos el grupo de Genética Acuícola del CIBNOR inicia en 1993 investigaciones sobre el mejoramiento genético de esta especie, con resultados alentadores, Ibarra et al. (1995) evalúan el efecto de la autofecundación en el desarrollo larval y crecimiento; Cruz et al. (1998) trabajan con cruzas reciprocas de dos poblaciones diferentes de almeja catarina encontrando que el cruzamiento no es una alternativa adecuada para el mejoramiento productivo; Ibarra, (1999) y Ibarra et al. (1999) realizan un trabajo de selección sobre peso y ancho estimando la heredabilidad y las correlaciones genéticas en esta especie, obteniendo una mejora del 16.3% en la primera generación a través de la selección por peso (Ibarra et al., 1999). Para concluir con un marco integral de posibles métodos de mejoramiento genético para esta especie se planteo la hipótesis de que el efecto de la triploidía en *Argopecten ventricosus* podría ser un mayor incremento productivo que el observado con los otros métodos. Si los diploides maduran a una edad temprana, y los triploides resultan en esterilidad, se podrían obtener mejores tasas de crecimiento. Esto con base en que como se ha señalado anteriormente, los pectínidos es el grupo de bivalvos donde se han observado las mayores diferencias entre diploides y triploides (Tabla 2).

Por lo anterior, el presente trabajo pretende ser una aportación para el conocimiento del efecto de la triploidía sobre la producción de un pectínido hermafrodita funcional, a fin de plantear esta biotecnología como una alternativa para el mejoramiento genético de esta especie.

Tabla 1. Resumen de experimentos en inducción a la triploidía en moluscos bivalvos.

Referencia	Especie	Inductor	Concentración mg/L (CB), µM/l 6-DMAP	Inicio tratamiento (min)	Duración tratamiento (min)	Exito %	Sobrevivencia Larva-D (%)
Allen y Downing (1986)	*Crassostrea gigas*	CB	1	30-45	15	(30) 96.3	
Barber et al., 1992	*Crassostrea virginica*	CB	0.1, 0.5 y 1 0.25 y 0.5 0.1 y 0.25	25 y 20 13 15	10 y 22 15 10	(0.25,13,10-15) 96	84
Baron et al., 1989	*Chlamys varia*	CB	1	10,20,30	15	(1,20,15)78.5	87.5
Beaumont 1986	*Pecten maximus*	CB	0.1 0.5	20	30	23 36	
Beaumont y Kelly (1989)	*Mytilus edulis*	CB	0.1 0.5 1	5 (M I) 30 (M II)	15 20	(0.1,30,15) 38 (0.5,30,15)60 (1,30,15) 50	38.7 62.5 48.7
		Choque caliente	↑Δ5, 10 y 15°C	10	10		
Desrosiers et al., 1993	*Crassostrea gigas* *Mytilus edulis* *Placopecten magellanicus*	6-DMAP	300 (0 to 600) 600 400 (0 to 600)	15 30 70	20 y 15 20,40,60 5 to 60	(300,15,20) 90 ostión (400,70,15) 95 pectínido	
	Crassostrea gigas *Mytilus edulis* *Placopecten. magellanicus*	CD,CB	0.5-1, 0.5-1 0 to 10	20, 20 10	15 y 20	6DMAP (300,15,20) 90 CB(0.5) 94-100	
Downing y Allen (1987)	*Crassostrea gigas*	CB	1	0, 15, 30, 45, 60, 75, 90, 105,120	15	(1,30,15) 88	56
Gerard et al., 1994	*Crassostrea gigas*	6-DMAP	150, 300, 450 y 600	15	20		
		6-DMAP	300	5, 10, 15, 20, 25, 30, 35 y 40	20	(300,15,20) 68 (300,39,20) 65	29 16
		6-DMAP	300 y 400	15	10, 15, 20 y 25	(300,15,15) 51 (300,15,10) 52 (400,15,10) 72	71 52 58

Continuación tabla 1. Resumen de experimentos en inducción a la triploidía en moluscos bivalvos.

Referencia	Especie	Inductor	Concentración mg/L (CB), µM/l 6-DMAP	Inicio tratamiento (min)	Duración tratamiento (min)	Exito %	Sobrevivencia Larva-D (%)
Hawkins et al., 1994	*Ostrea edulis*	CB	1	40 y 90	20	N.A	
Jiang et al., 1993	*Pinctada martensii*	CB	0.75 y 1	2 y 17	15 y 17		
Nell et al., 1994	*Saccostrea commercialis*	CB	0.5	23	20	81 85 (semilla)	
Nell et al., 1996	*Saccostrea commercialis*	CB	0.75 1.5	30	15	83	43
Scarpa et al., 1994	*Mytilus galloprovinciallis*	CB Calor Calcio Cafeína	1 mg/l 30°C 0.1 M 15 mM	20	15	86 73 4.7 71	
Shen et al., 1993	*Pinctada fucata*	H. P.	200-250 kg/cm^2	5-7 (MI) 17-19 (MII)	10	76	
Stanley et al., 1981	*Crassostrea virginica*	CB	0.1, 1 y 5	50	20		
Tabarini (1984)	*Argopecten irradians*	CB	0.05 y 0.1	10	20	(0.10) 94 (0.05) 66	6 20
Utting y Child (1994)	*Tapes philippinarum*	CB	0.5	15 20	15 20	70 77	45
Wada et al., 1989	*Pinctada fucata*	CB	0.5	3, 6, 9, 12, 15, 18, 21, 24, 27 y 30	15	(0.5,20,15) 100	39 larva normal
		CB	0.1	5, 10, 15, 20, 25 y 30 5 y 20	15	(0.1,20,15) 80	
		Choque frio	↓20°C ↓Δ19°C	5 15	5 y 10 5, 10 y 15	(↓20°C,5,10) 52	78 larva normal

Tabla 2. Ventajas de los organismos triploides sobre triploides en moluscos bivalvos.

Especie	Tejido	Superioridad de los triploides sobre los diploides (%).	Referencia
Argopecten irradians	Biomasa Húmedo	36	Tabarini, 1984
	Músculo aductor	73	"
Chlamys nobilis	Biomasa Húmedo	32	Komaru y Wada, 1989
	Biomasa Seco	52	"
Chlamys farreri	Músculo Aductor	44-96	Yang, et al., 2000
	Biomasa Húmedo	81	"
Ostrea edulis	Biomasa Seco	61	Hawkins et al., 1994
Pinctada martensii	Biomasa Húmedo	27-58	Jiang et al., 1993
Crassostrea gigas	Biomasa Húmedo	80	Akashige y Fushimi, 1991
	Biomasa Húmedo	70	Landau y Guo, 1999
Mulinia lateralis	Biomasa Húmedo	72	Guo y Allen, 1994a
Saccostrea commercialis	Biomasa Húmedo	31	Hand et al., 1998

OBJETIVO GENERAL

El objetivo principal de este trabajo fue el de evaluar los efectos de la condición de triploidía en la almeja catarina (*Argopecten ventricosus,* Sowerby 1842).

Objetivos específicos:

Para examinar el efecto de la triploidía en la Almeja catarina fue necesario realizar diferentes experimentos, los objetivos particulares de cada uno de ellos son descritos en cada uno de los siguientes capítulos:

Capítulo 1. Desarrollo de la técnica y evaluación del crecimiento y gametogenesis en triploides de almeja catarina.

En este estudio, se pretende evaluar la técnica de producción de triploides en almeja catarina mediante el uso de citocalasina B y estudiar los efectos de la triploidía en el crecimiento, la gametogénesis, y madurez gonadal para este organismo hermafrodita funcional (*Argopecten ventricosus,* Sowerby 1842).

Capítulo 2. Evaluación de la composición bioquímica en diploides y triploides de almeja catarina.

En este estudio, se pretende elucidar algunas de las causas de las diferencias que se encontraron en crecimiento, comparando estas diferencias con los componentes bioquímicos entre diploides y triploides de almeja catarina.

Capítulo 3. Mejoramiento de la técnica para la producción de triploides a través de la inducción en familias. Evaluación de la fecundidad en triploides.

Uno de los objetivos de esta investigación fue establecer si la inducción a la triploidía a nivel de familias individuales produce el mismo rendimiento que cuando la inducción es con una mezcla de familias. Como un segundo objetivo, se pretende comparar la fecundidad y el tamaño de los ovocitos en organismos triploides de *A. ventricosus,* para establecer bases para una posterior producción de tetraploides.

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Capítulo 1.

Desarrollo de la técnica y evaluación del crecimiento y gametogénesis en triploides de almeja catarina.

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Triploid catarina scallop (*Argopecten ventricosus* Sowerby II, 1842): growth, gametogenesis, and suppression of functional hermaphroditism.

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Abstract

Triploidy was induced in catarina scallop, *Argopecten ventricosus*, using two concentrations of cytochalasin-B (CB) . Growth of triploid scallops exceeded that of diploids in all evaluated traits. The largest percent difference between the diploid control group and the treated groups was seen after diploid scallops reached the peak of sexual maturation and began spawning. The gonad of triploid scallops was easily recognizable by visual inspection because of a brownish discoloration and lack or few egg masses present. That characteristic allowed for the separation of 'putative triploids' (PTs) , which when compared with diploid scallops from within the same treatment treated diploids , had a significantly larger muscle weight than the differences seen between treated and control groups. Gametogenesis and the condition of hermaphroditism in this normally functional hermaphrodite were greatly affected by the triploid condition. In the female portion of the gonad, few oocytes developed. The male portion of the gonad was arrested early during development, and the male acini were replaced by female acini, producing all female gonads in increasing percentages during the culture period, suppressing the normal condition of hermaphroditism. Oocytes of diploids were larger than those of triploids early in the culture, probably because of delayed maturation in triploids. Later during the culture, oocytes of triploids were larger than those of diploids only when compared with treated diploids.

1. Introduction

The catarina scallop (*Argopecten ventricosus = circularis*, Sowerby II 1842) is commercially one of the most important bivalve resources in Baja California Sur, MX. This functional hermaphrodite has been studied for 15 years, and there have been considerable efforts to grow it commercially (Felix-Pico, 1991). Recently, studies to establish the best genetic improvement method for this species were undertaken (Cruz et al., 1998; Ibarra, 1999; Ibarra et al., 1999). These demonstrated that potential improvement by selective breeding was high. Additional research on age–size at first sexual maturity in catarina scallop has also shown that the maturation process can have a significant effect on productivity, because commercial harvest occurs at age 1 year (6-cm length) , and the age of first sexual maturation can be as early as 4 months 2-cm length (Cruz, Rodriguez-Jaramillo and Ibarra, unpublished results). For commercial-scale culture, it is necessary to look for ways to achieve maximum growth of market products, and the use of triploids is one alternative. The principal value of triploid organisms is their larger size, thought to be caused by their total or partial sterility such that more energy is available for growth (Allen and Downing, 1986) , or caused by an increased heterozygosity in triploids (Beaumont and Fairbrother, 1991) , especially in triploids produced by blocking meiosis I (Hawkins et al., 1994) , or because of cell gigantism caused by polyploidy, with lack of cell-number compensation (Guo and Allen, 1994a) . Research to improve the culture potential of fish and shellfish with commercial value has led to the viable production of many polyploid organisms. For bivalves, triploids have been successfully produced and evaluated for growth or gametogenesis for many commercially important species, e.g., oysters (Stanley et al., 1981, 1984; Allen and Downing, 1986, 1990; Allen et al., 1989; Yamamoto et al., 1988; Nell et al., 1994; Hand et al., 1998) , pearl oysters (Wada et al., 1989; Komaru and Wada, 1990; He et al., 1996), mussels (Yamamoto and Sugawara, 1988; Beaumont and Kelly, 1989), clams (Allen et al., 1982, 1986; Eversole et al., 1996), and scallops (Tabarini, 1984; Komaru and Wada, 1989; Zeng et al., 1995; Heasman et al., 1998) . Gonad maturation and sterility have been studied for several triploid species. In *Mya arenaria* (Allen et al., 1986) and *Chlamys nobilis* (Komaru and Wada, 1989), sterility was confirmed. However, total sterility has not been the rule among triploids; spermatozoa have been detected in triploid *Crassostrea gigas*, *Pinctada fucata*, *Mulinia lateralis*, and *Saccostrea commercialis* (Komaru and Wada, 1990; Guo and Allen, 1994a; Cox et al., 1996). Furthermore, for *C. gigas*, fertilization of

triploid gametes is possible (Guo and Allen, 1994b). Although gametogenesis has been reasonably well-described for diploid hermaphroditic scallops, in triploid scallops it has been previously studied only for the sequential hermaphrodite *C. nobilis* (Komaru and Wada, 1989). There are no histological descriptions of gonad morphology for triploids of any functional hermaphroditic bivalve species.

In this study, we report on the results of triploid research in catarina scallop. Two concentrations of cytochalasin-B CB were evaluated for triploid induction, and the effects of triploidy on growth, gametogenesis, and gonadal maturity were studied for the functional hermaphroditic catarina scallop, *A. ventricosus*.

2. Materials and methods

2.1. Maturation and spawning

Spawners for this study were taken from the lagoon of Rancho Bueno at Bahia Magdalena, B.C.S., MX. Scallops larger than 40 mm and with similar gonadal development were visually selected and transported to the CIBNOR Genetics Laboratory at La Paz, B.C.S. Conditioning for spawning consisted of adding a mixture of *Isochrysis galbana*, *Chaetoceros muelleri*, and *Thalassiosira pseudonana* at a total concentration of 6 x 4=10 cell r scallop r day, using a continuous drip feeding system (Racotta et al., 1998; Ramirez et al., 1999). Water temperature was kept constant at 19 °C and salinity at 38 ppt. After 15 days, when the breeding organisms reached sexual maturity, they were induced to spawn by injecting 0.2 ml of serotonin 0.5 mM intramuscularly.

2.2. Egg collection

The catarina scallop is a functional hermaphrodite that sheds sperm and eggs intermittently during the same spawning. However, when serotonin is used, it induces the release of sperm allowing for the partial separation of gametes. After discharge of sperm, the scallops were kept in a 1000-l tank until they began releasing eggs. They were then washed and each was placed in a 2-l container to release the remaining eggs. Eggs were washed using a 15-mm screen and allowed to stand until verification of lack of or reduced self-fertilization was microscopically made. All water used in the Genetics Laboratory is filtered to 1mm and UV-sterilized before use.

2.3. Triploid induction

Mass production of triploids was done by mixing the eggs of 10 females fertilized with a mixture of stripped sperm from five males. To synchronize development after fertilization, sperm was added at a high concentration approximately 20 sperm per egg . CB was evaluated for its effects in inducing triploidy at two concentrations 0.1 and 0.5 . mg/l , in both cases applied for 15 min after fertilization, beginning when 50% of the eggs had released the first polar body to inhibit extrusion of the second polar body PB II . Water temperature was kept at 21°C during the treatments. The CB was dissolved in the same amount of DMSO for stock solutions Allen et al., 1989 . Three replicates for each or the two CB treatments 0.1 and 0.5 mg/l and for the control were done. Treatments were compared for survival from egg to first D-larvae, and from D-larvae to Eyed-larvae. Ten spat from each replicate were shipped to the Haskin Shellfish Research Laboratory to determine percent triploidy by flow cytometry.

2.4. Larval culture

After fertilization, zygotes were held in 20-l containers until they reached the D-larvae stage, after which, they were transferred to a 50-l tank for larvae culture. The larvae were grown at densities between 10 and 12 per ml, and fed *I. galbana*, *Monochrysis lutheri*, and *C. muelleri* at varying concentrations depending on age; 30,000–50,000, 60,000–80,000, and 90,000–120,000 cells ml y 1 , for larvae at ages 3–6, 8–11, and 12–15 days, respectively. Water was exchanged every other day, keeping it between 198C and 218C.

2.5. Growout study

Spat 2–4 mm were transported to the Lagoon of Rancho Bueno, where they were kept for 382 days. Thirty individuals per replicate were sampled at 81, 118, 146, 205, 280, and 382 days of growout. Growth was evaluated by measuring shell length, shell width, shell height, total weight, biomass wet tissue weight, gonad weight, and adductor muscle weight. Gonad and muscle indices were estimated from days 118 to 382. For both, the index was obtained for each individual by dividing either the gonad weight or the muscle weight by its wet tissue weight and expressing it as a percentage. Until day 205, sampling was made from triploid and control groups without verification of the ploidy of samples. That is, 'triploid'

data T5 comprises both, diploids and triploids. On the final three sampling dates days 205, 280, and 382 , scallops within the 0.5 mg/ltreated group were classified into two groups: 'putative triploids' PTs and 'treated diploids' 2N-T5 . The same was not done for the 0.1 mg/lgroup T1 because of the low percentage of triploids in this group. Putative triploids were those individuals for which the gonad presented a characteristic morphology by visual inspection, where rather than having the common orange-reddish and white-cream colors in the gonads of diploids, it was of a uniform brownish discoloration and the gonadal sac was large.

Treated diploids were organisms from within the 0.5 mg/lCB-treated group with the characteristic gonadal morphology of diploids. Whereas all analysis were done comparing the T5, T1, and control groups, further unplanned analysis were done later on PTs, 2N-T5, and control groups. Verification of the classification method based on gonad morphology as PT or as 2N-T5 was done in a group of individuals from a second triploid induction unpublished results . Scallops were sampled, individually numbered, and classified as either PT or diploid by their gonad morphology. Then they were analyzed by flow cytometry to determine their ploidy status. The certainty in visually classifying scallops as PTs was 91%.

2.6. Gonadal development and histology

Gonadal development was evaluated macroscopically during sampling and data collection following Sastry (1963) criteria; immature, partial maturation, maturity, and spent. For microscopic analysis, the same portion of the gonad was always sampled, fixed with Davidson's fixative, and preserved in 70% alcohol for later histological processing. Sections 5- to 7-mm thick were cut, stained with hematoxylin-eosin, and counterstained with eosin Y.

Table 1
The cytochalasin B effect on survival of catarina scallop

Treatment	Number of eggs± S.E. ($x10^3$)	Number of larvae ± S.E. ($x10^3$)	Survival to D- larvae* (%)	Spat Number± S.E. ($x10^3$)	Survival to spat*	Percent of triploid ± S.E.
Control	668±87	413±69	100% [a]	40.5±17.9	100[a]	0
0.1 mg/l	681±166	63±21	15% [b]	6.5±4.6	16[b]	8±8
0.5 mg/l	659±111	38± 79	9% [b]	0.8±0.04	2[b]	58±20.5%

Percent survival of triploid was calculated relative to the control at that larvae stage±standard error.
Different letters are used when significantly different ANOVA, P<0.05 .

The microscopic scale for classification of gametogenic stages was that of Villalejo-Fuerte and Ochoa (1993) for this same species, but the term 'follicle' was substituted by 'acinus' following the Beninger and LePennec (1991) statement that sex cells in scallops are produced in acini female and male and not follicles: *Stage I= initial gametogenesis* — characterized by abundant interfollicular connective tissue, evident female acini development with 54 acini per mm 2 , and abundant spermatocytes; *Stage II= advanced gametogenesis* — interfollicular connective tissue is scarce, with 32 female acini per mm 2 , oocytes are polygonal and pyriform, and spermatocytes are abundant in the periphery of the male acinus; *Stage III= maturity* connective tissue is absent, 12–16 female acini/mm^2, each full of oocytes, and abundant spermatocytes and spermatides; *Stage IV = spawning* — connective tissue is scarce and scattered, 18 female acini/mm^2 partially empty, and groups of spermatozoids in central position of the male acinus; *Stage V = spent* — abundant interfollicular connective tissue, with 34 female acini/mm^2, and isolated oocytes and spermatozoids. Oocytes of diploid and triploid scallops were measured by using an image analyzer to determine differences between ploidy groups. Two measurements, 'pole to pole', and the largest distance perpendicular to the 'poles' were taken to obtain an average diameter even in those oocytes that were not circular.

Table 2
Means from ANOVA results for each studied growth trait are the average of means at age during growout. Means for derived indices are averages from days 118 to 382 of growout CB-treated groups

Traits	Control	CB-treated groups	
		T1 (0.1 mg/l)	T5 (0.5 mg/l)
Shell length (mm)	43.3 [a]	43.2 [a]	46.2 [b]
Shell width (mm)	21.3 [a]	20.8 [a]	23.2 [b]
Shell height (mm)	42.5 [a]	42.4 [a]	45.3 [b]
Total weight (g)	27.9 [a]	26.3 [a]	34.5 [b]
Tissue weight (g)	10.0 [a]	9.4 [a]	12.8 [b]
Muscle weight (g)	3.6 [a]	3.3 [a]	5.3 [b]
Gonad weight (g)	0.7 [a]	0.7 [a]	0.8 [a]
Muscle Index (%)	25.8 [a]	25.7 [a]	28.1 [b]
Gonad Index (%)	10.3 [a]	10.5 [a]	8.5 [b]

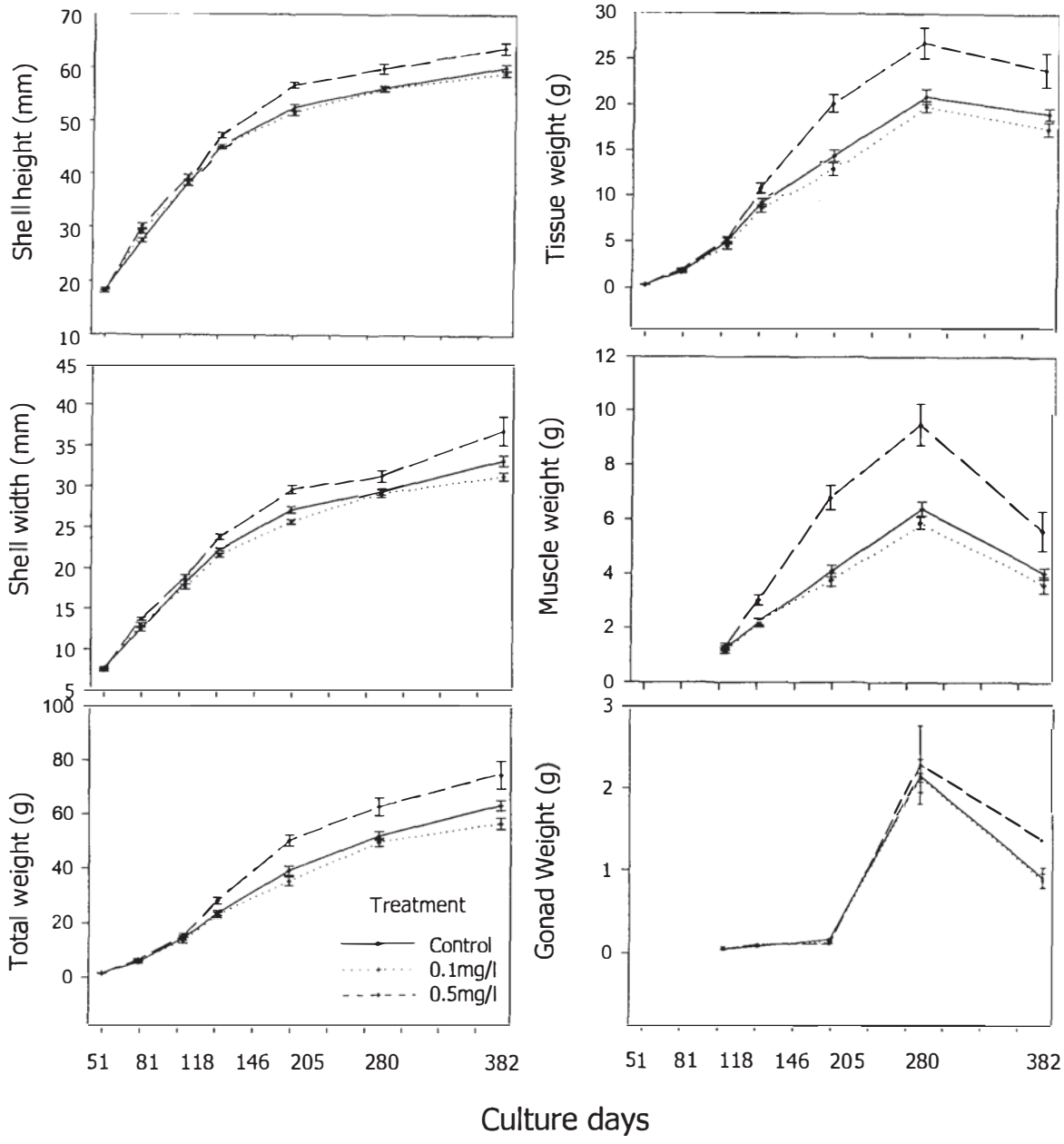

Fig. 1. Growth of control, 0.1 mg /l (T1) , and 0.5 mg/l (T5) groups for the different traits evaluated during growout. Means at age are indicated with their standard errors.

2.7. Statistical analyses

Each of the response variables evaluated for larval stages number of eggs, number of D-larvae, number of eyed-larvae were analyzed using a single factor ploidy condition, model I analysis of variance. For growout data on direct traits and indices, two-factor ploidy and age with interaction analyses of variance were used for each trait. Indices were transformed to arcsin for the analysis. Means were compared using Tukey tests (Sokal and Rohlf, 1981). Significance for all tests was preset at P=0.05.

3. Results

3.1. Triploid induction

Treatment with CB produced triploids of catarina scallop with both CB concentrations, although 0.5 mg/l (T5) was most effective, resulting in an average of 58% triploids among replicates. For 0.1 mg/l (T1) , triploid induction was very low 8% (Table 1) . No triploid spat were detected in the control group. Eggs treated with 0.5 mg/l CB had lower survival generally, but this was not statistically different from 0.1 mg/l; both showed a significantly lower survival than controls (P = 0.007), for both larvae and spat.

3.2. Growth

For the total growout period, all metric traits evaluated indicated significant effects of ploidy condition but only in the T5 group Table 2 . When comparisons were done for each growout age, no differences between groups for any of the traits evaluated were seen up to 81 days of growout, but from 118 days to the end of the experimental period, scallops in the T5 group were significantly larger and heavier than both the control group and the T1 group (Fig. 1). Among the different traits evaluated, the largest differences were seen for weight traits, especially for wet tissue weight and muscle weight. At 205 days, total weight of the T5 group was 28% greater than the control, tissue weight was 37% heavier, and adductor-muscle weight showed the largest gain, 63% heavier than the controls (Table 3: T5 vs. Control). Shell height and shell width increased by 9% and 10% at that same age, respectively. From 280 to 382 days, a reduction of the percent differences between scallops in the T5 group and the control group was seen for tissue weight and muscle weight, but

Table 3
Means and percent differences at age between the T5(0.5 mg/ltreated group) and the control groups, and between PTs and their internal controls or 2N-T5

Trait	Growout days	T5 group	Control group	Percent differences	T5 group		Percent differences
					PTs	2N-T5	
Shell height (mm)	146	47.9	45.3	6	--	--	--
	205	57.3	52.6	9	59.7	54.2	10
	280	60.2	56.4	7	64.2	54.2	19
	382	64.0	60.2	6	67.9	60.7	12
Shell width (mm)	146	23.8	22.3	7	--	--	--
	205	29.7	27.1	10	31.8	26.8	19
	280	31.3	29.4	6	34.2	27.0	26
	382	36.8	33.1	11	44.2	32.5	36
Total weight (g)	146	28.0	23.5	19	--	--	--
	205	50.0	39.0	28	58.1	39.4	48
	280	62.6	51.7	21	76.8	41.3	86
	382	74.4	63.0	18	97.1	62.4	55
Tissue weight (g)	146	10.7	9.3	15	--	--	--
	205	20.0	14.6	37	24.1	15.2	59
	280	26.6	21.1	26	33.7	16.0	111
	382	23.8	19.2	24	33.0	18.4	80
Muscle weight (g)	146	3.0	2.2	36	--	--	--
	205	6.7	4.1	63	8.6	4.2	104
	280	9.4	6.4	47	12.7	4.5	182
	382	5.5	4.0	37	9.2	3.5	161
Gonad weight (g)	146	1.0	1.4	-30	--	--	--
	205	2.01	1.9	8	2.0	1.7	14
	280	2.28	2.2	6	2.8	1.4	101
	382	1.30	0.9	39	1.8	1.1	56

not for gonad weight (Table 3: . T5 vs. control; Fig. 1). Scallops in the T5 group had a gonadal sac of similar size to the control and T1 groups during all the culture time, but gonads of triploid scallops were clearly distinguished from gonads of diploid scallops because of a brownish discoloration, where no eggs or sperm could be seen. This allowed for the separation into PTs and 2N-T5 within the T5 group. By separating diploids from triploids on this basis, PTs were larger than those estimated from the whole T5 group

containing a mixture of both triploid and diploid scallops. At 280 days, when the largest differences between the T5 group and the control group were seen, PTs had a total weight 86% greater than 2N-T5, a tissue weight 111% heavier, a muscle weight 182% heavier, and a gonad weight 101% heavier (Table 3: PTs vs. 2N-T5).

3.3. Gonad and muscle indices

Gonad and muscle indices of scallops in the T5 group were significantly different from both

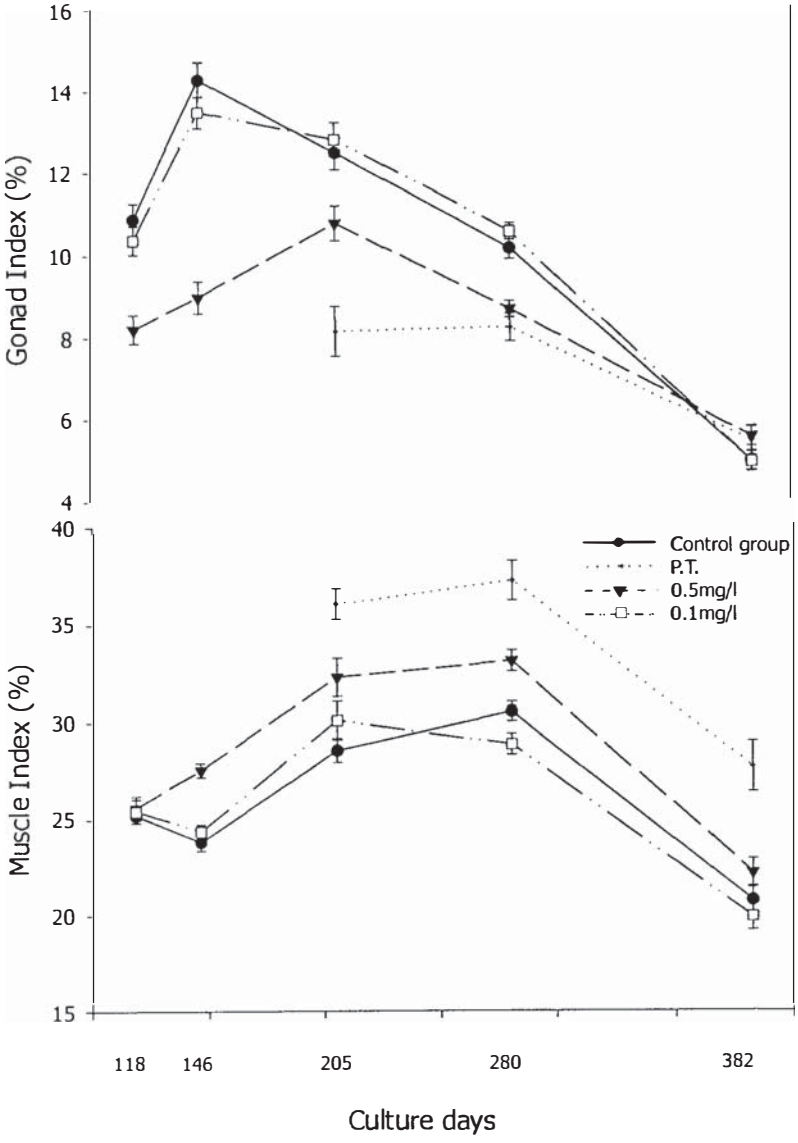

Fig. 2. Gonad and muscle indices for the control, 0.1 mg/l (T1), and 0.5 mg/l (T5) groups

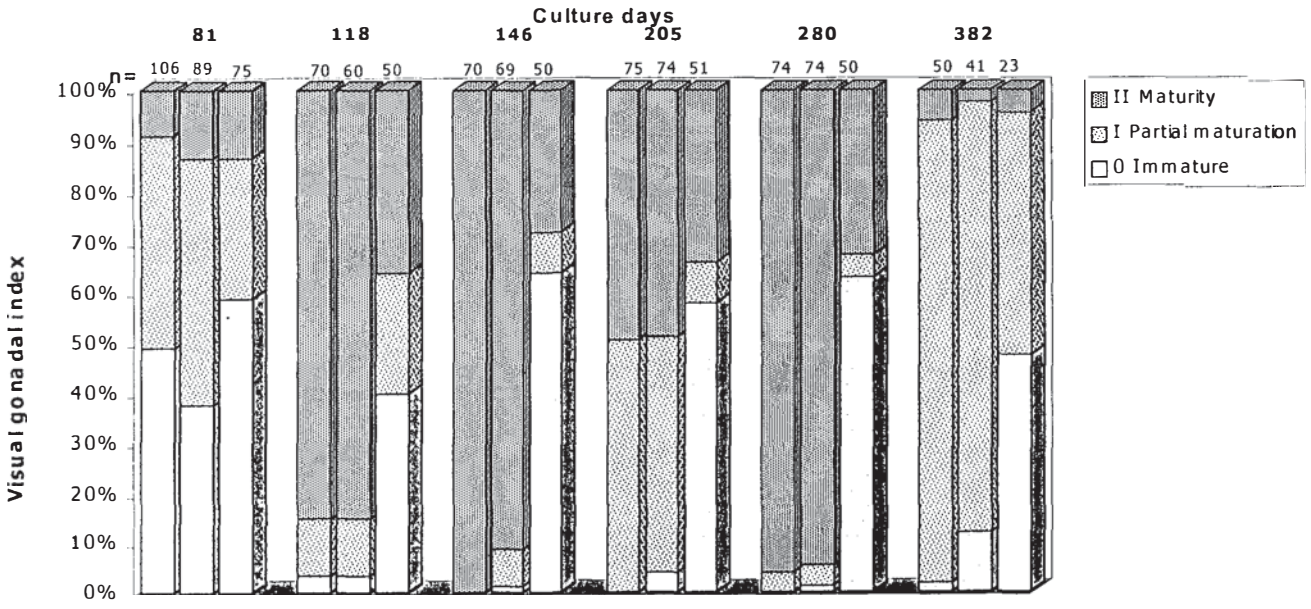

Fig. 3. Visual macroscopic maturation index of gonad development for the control, 0.1mg/l (T1) , and 0.5mg/l (T5) groups during growout. Numbers sampled (*n*) are included on each column.

the increase in muscle index (Fig. 2). When the indices are calculated again with the separated PTs, their gonad index was the lowest when compared with the control, and with the T5 group from which they were separated, and their muscle index was the highest (Fig. 2).

3.4. Visual maturation scale After almost 4 months of growout, beginning days 118 up to 382, visual differences in maturation index were observed between the groups. Gonads of scallops in the control and T1 groups were partially mature or mature (Fig. 3), and egg and sperm portions of the gonadal sac were easily identified. For the T5 group, a larger percentage of the gonads was classified as immature, although a few scallops were mature possibly being diploids, because of the large percent of diploids within the T5 group . For scallops within the T1 group, only a small percentage of gonads were classified as immature or undifferentiated up to day 280. The spent stage was not seen. Some scallops with partial spawns were classified as mature.

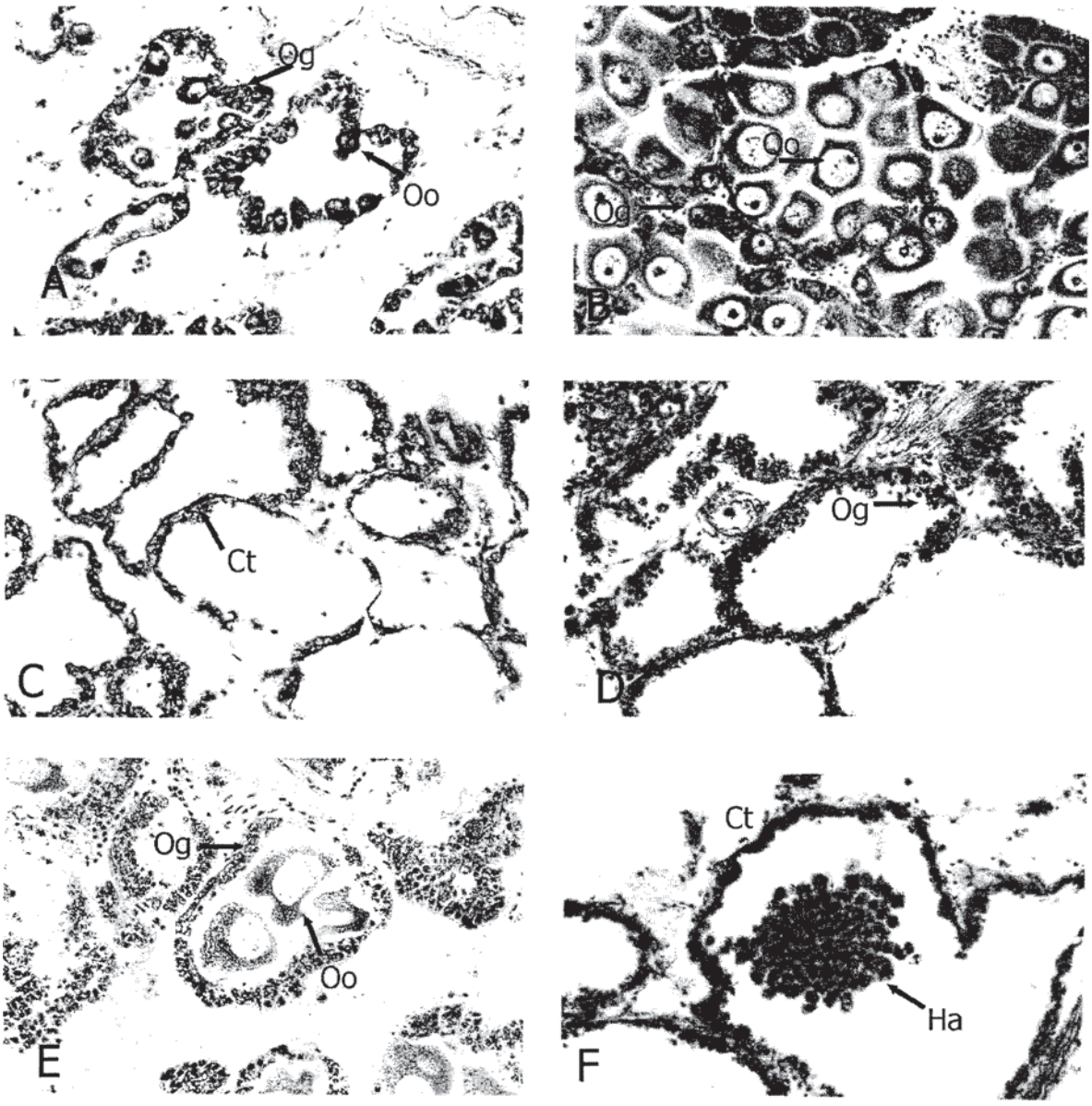

Fig. 4. Female gametogenesis in diploid and triploid catarina scallops. Only the female portion of the gonad for the functional hermaphrodite catarina scallop is shown. A initial stage of diploid, 20=; B maturity stage of diploid, 20=; C inactive stage of triploid, 20=; D early active 'a' stage, 20=; E early active 'b' stage, 20=; F indeterminate stage of triploid female portion, 20=.Og s oogonie; Oo s oocyte; Ct s connective tissue; Ha s haemocyte.

3.5. Gametogenesis

Histological analysis showed that maturation in the control diploid scallops was normal. At 81 days of growout, about 50% of the scallops were either mature or spawned. Maturing

individuals were seen during the whole study period thereafter. The first spent scallops were seen on day 205, indicating that the first massive spawn took place between days 146 and 205. By day 280, a large proportion of the individuals were seen again in an intermediate gametogenesis (Figs. 4A,B and 5A,C,E). All diploid individuals were hermaphrodites. At the microscopic level, triploid gonads were easily distinguished from diploids because gametogenesis in the triploid scallops was abnormal and different from diploids. Most scallops in the T5 group had an abnormal gonad development, but 30–45% had normal development, presumably because they were diploids. This percentage agreed well with what was seen with the macroscopic visual maturation index (Fig. 3), and with the triploid percentage determined by flow cytometry for spat. Because triploids within the T5 group could be easily detected as such at the microscopic level, their gametogenesis was followed separately from that in the diploids within the group. The abnormal development of gametogenesis in triploids was separated into four stages, described in Table 4. Using those stages to define gonad development for triploids, we can see that the female portion of the gonad in triploid scallops was maturing but it was delayed when compared with diploids (Fig. 6). On day 81, most of the triploid PTs gonads were in the inactive stage (Fig. 4C). By

Table 4
Gametogenic stages in triploid scallops, defined from histological data

Stage	Characteristics	Figure
A, inactive	In the female part of the gonad, there were abundant acini, with extensive layers of oogonies in their walls, but no oocytes were present. Acini were retarded showing few spermatogonies with a reduced size.	Fig. 4C
B, early active 'a'	The difference with Stage A was the presence of few oocytes in the female acini, often abnormal, although some oocytes were apparently normal and mature.	Fig. 4D
	In the male portion of the gonad, development of the acini were retarded showing few spermatogonies with a reduced size.	Fig. 5B,D
C, early active 'b'	This stage has the same morphology of Stage B for the female gonad, with the presence of a few maturing and mature oocytes, but the acini were reduced.	Fig. 4E
	The male portion of the gonad was often occupied with female acini, where some oocytes were developing. Scallops in this stage was classified as 'female' sex.	Fig. 5F
D, indeterminate	The gonad was empty, with abundant connective tissue dispersed and the acini filled with abundant haemocytes. There was no evidence of spawning.	Fig. 4F

day 118, a large proportion were in early active 'a' stage Fig. 4D but some still remained in the inactive stage. By day 146, less than 20% of female gonad part were inactive, and the remaining were divided about equally between early active 'a' and early active 'b' (Fig.

4E) stages of development. The male gonad part did not mature further than the early active 'a' stage (Fig. 5B,D). By days 205–280, most triploid female gonads were in early active 'b' stage. By day 382, all triploid scallops were in an indeterminate stage of development (Fig. 4F)..

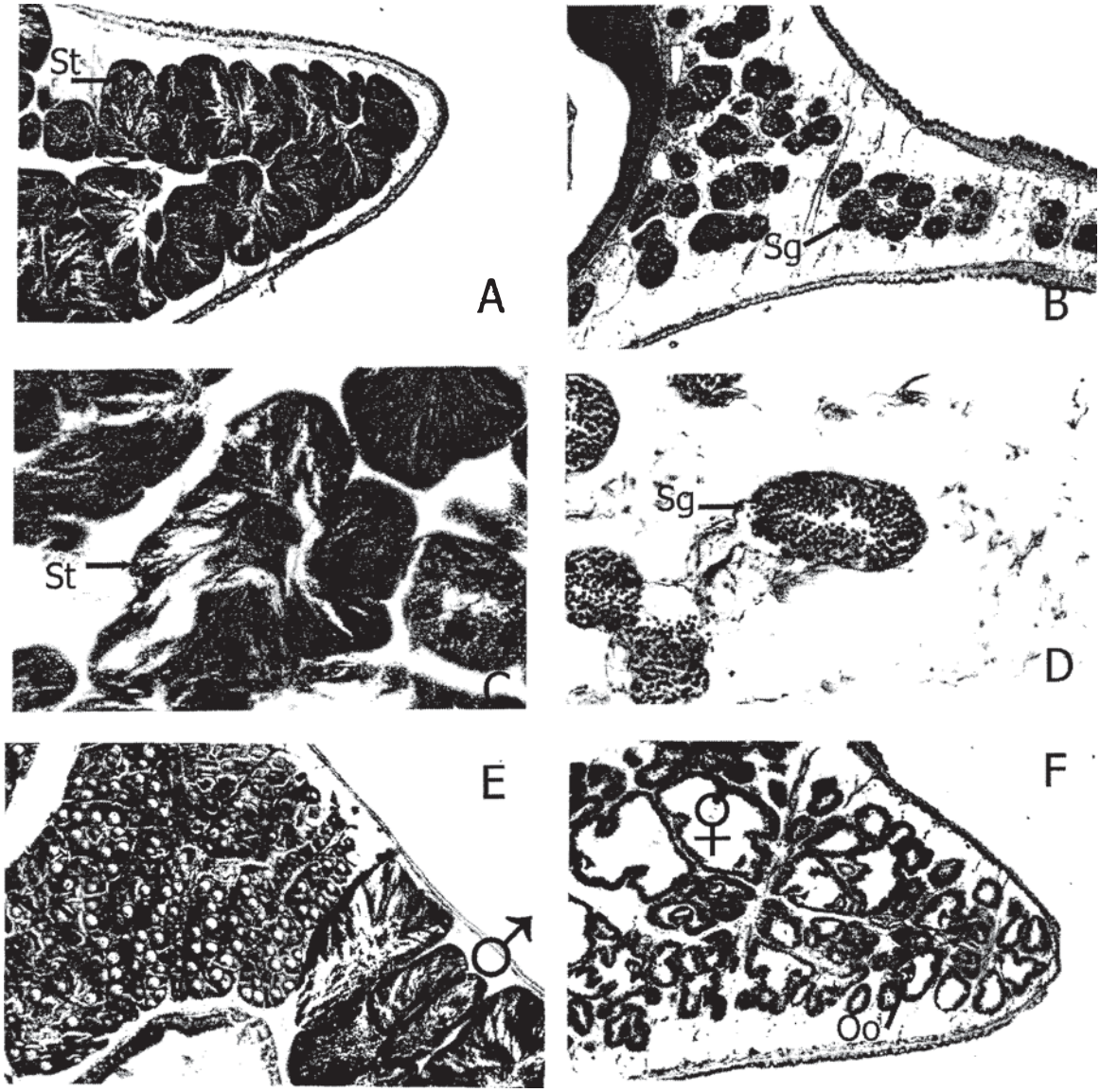

Fig. 5. Male gametogenesis in diploid and triploid catarina scallop. The male portion of the gonad for the functional hermaphroditic catarina scallop is shown in a, b, c, and d. Both, female and male portion of the gonad are shown in e and f. A Advanced stage of diploid, 10=; B early active stage of triploid male gonad part, 10=; C maturity stage of diploid, 20=; D early active stage of triplold, 20=; E maturity stage of diploid male and female portions of the gonad, 10=; F triploid all female morphology in both gonad portions, 10=.St s spermatocytes; Sg s spermatogonies; Oo s oocyte.

When gonads of triploid 3N-T5 were histologically compared with those of diploids from the untreated control control for oocytes, there were differences in mean oöcytes size early in the culture 118 days (Table 5) . At that age, the oocytes of triploids 27.8 mm were smaller than diploid oocytes 40.3 mm (Table 5) . When oocytes of 3N-T5 were compared with 2N-T5, the differences at 118 and 280 days were significant, but only at 280 days were the oocytes of 3N-T5 scallops larger (41.6 mm) than those of 2N-T5 (35.3 mm) . At 146 and 205 days, oocytes of 3N-T5 were not significantly different from oocytes of 2N-T5. By day

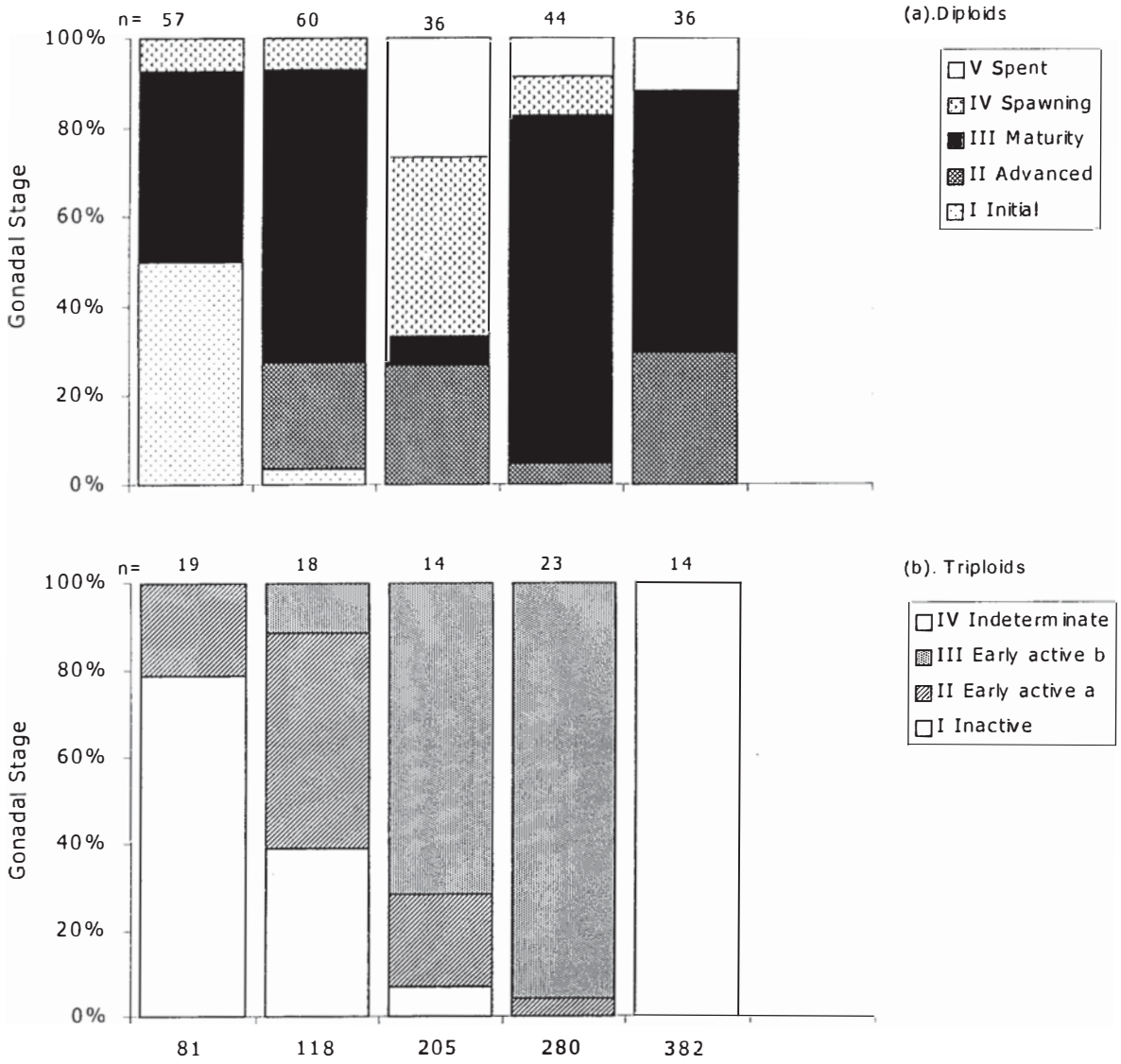

Fig. 6. Frequency of each gametogenic stage for the female part of the gonad in a diploids and b triploids for the growout period. Ploidy condition was detected on the basis of histological inspection of gonadal development. Numbers sampled *n* are included on each column.

382, there were a large number of oocytes in the diploid scallops, but no oocytes were seen in triploid scallops.

Table 5
Oocyte size mm comparisons at age for triploids (3N-T5) and diploids from the control group (Control), and for triploids (3N-T5) and diploids from the treated 0.5 mg/l group (2N-T5) Different letters between columns indicate significant differences between diploid and triploids.

Days of growout	Oocyte size mm		Oocyte size mm	
	Diploid Control	Triploid 3N-T5	Diploid 2N-T5	Triploid 3N-T5
118	40.3^{c}	27.8^{a}	32.1^{b}	27.8^{a}
146	36.5^{b}	37.4^{bc}	39.0^{cd}	37.4^{cd}
205	37.6^{bc}	40.5^{bc}	38.2^{cd}	40.5^{d}
280	41.3^{c}	41.6^{c}	35.3^{bc}	41.6^{d}

3.6. Functional hermaphroditism

Within each sampling period and for the triploid scallops within the T5 group, histological data showed an abnormal decrease in the number of gonads that were hermaphroditic. The frequency of hermaphroditic triploid scallops went from 100% at day 81 to only 4% at day 280 (Table 6). For diploid scallops (within the T5 group as well as within the control group), all gonads were hermaphroditic for all sampling times. There was only one exception within the T5 group, where one 'only male' scallop was seen.

Table 6

Observed sex ratios during growout for triploid scallops within the T5 group, estimated from histological data

Days of culture	Percent hermaphrodites	Percent females only	(χ^2 test significance (P)
81	100	0	1.00
118	79	21	0.03
146	55	45	0.00
205	33	67	0.00
280	4	96	0.00
382	0	0	–

4. Discussion

4.1. Growth

Whereas some differences as early as larval stages have been shown to exist between diploid and triploid (Yamamoto et al., 1988; Beaumont and Kelly, 1989), it is generally accepted that triploid shellfish are not much different from diploids before beginning maturation (Guo and Allen, 1994[a]) . This was true for the catarina scallop, for which the growth advantage was first seen after about 3 months of growout (>118 days), when diploid scallops were largely mature. The largest difference between the control and the T5 groups was seen at 205 days, after the maturity peak at 146 days. At 205 days, tissue weight was 37% larger and muscle weight was 63% larger in the T5 group. Triploid mollusks were significantly larger than diploids in almost all species studied (Guo and Allen, 1994[a]). Triploid advantage was observed in *Argopecten irradians*: tissue weight 36% larger and muscle weight 73% larger (Tabarini, 1984) . Also, in *C. nobilis* triploids, wet and dry tissue weights were 32–52% heavier (Komaru and Wada, 1989); in *Pinctada martensii*, wet tissue weight was between 27% and 58% heavier in triploids (Jiang et al., 1993); in *C. gigas*, tissue weight was 80% heavier (Akashige, 1990), and in *M. lateralis*, tissue weight was 72% heavier (Guo and Allen, 1994[a]) . Another reported characteristic in mollusks associated with triploid sterility is the reduction in gonad size. An exception was noted by Tabarini (1984) who found that triploid scallops actually had a heavier wet gonad weight than diploid scallops. In our results, lack of statistical differences in gonad weight between the treated and the control groups contrasted with visual observations, which indicated that triploid scallops had a gonadal sac larger than diploids, although it had few or no eggs, and no macroscopically visible sperm as diploids did. The significance of the larger gonadal sac in triploids was confirmed only when PTs were compared against diploids from the treated groups treated diploids s 2N-T5 ; gonads of PTs were 100% heavier than those of diploids. In spite of the larger gonadal sac of triploids, gonad index of the T5 scallops was reduced when compared with the control group from 118 to 280 days. Because that was paralleled with a high muscle index, the low gonad index can be attributed to a large muscle weight rendering the wet tissue weight of triploids larger than for the diploid

control. Whereas, Tabarini (1984) found no decreased gonad index for triploid bay scallop, Komaru and Wada (1989) found diploids to have a 62% greater gonad index than triploids of *C. nobilis*, which is very close to our results of a gonad index 61% greater in diploid than in triploid catarina scallops at the peak of maturity 146 days . Up to 280 days of growout, PTs, followed by the T5 group mixture of triploids and diploids , had the lowest gonad indices and highest muscle indices observed. However, for all groups, both indices decreased later during the culture 382 days , probably because of the abnormal environmental conditions present that year of El Nino 1997-1998, when unusually high temperatures were present. High temperatures are known to affect gonad maturation in scallops (Sastry, 1963; Barber and Blake, 1991), which would result in a decreased gonad index, and this is probably associated with the expected decrease in phytoplankton biomass during years of El Niño (Lluch-Cota et al., 1999; Tran et al., 1993 cited by Lluch-Cota et al., 1999) reported a 60% decrease in phytoplankton biomass during El Niño 1982–1983. A similar, or even larger decrease might have occurred during El Niño 1997–1998, which would explain the decrease in muscle index, even in triploids.

4.2. Growth of PTs

When PTs were compared against 2N-T5, the differences in muscle weight and tissue weight between triploid and diploid scallops were striking Table 3 . The catarina scallop is known to have an early sexual maturity when environmental conditions are appropriate (Cruz, Rodriguez-Jaramillo, and Ibarra, unpublished data), with partial spawns all year and a recovery period of 15 days (Villalejo-Fuerte and Ochoa, 1993). The early sexual maturity and concomitant expense of energy could explain the large difference between diploid and triploid scallops for muscle weight, a difference that increased during the culture period. Perhaps in triploids, the adductor muscle is storing energy that is never used for the maturation process, different from diploid scallops that go through the normal maturation process using that energy. A continuous maturation process implies consecutive storage and use of energy, however, for triploids, energy is apparently just stored but seldom used for maturation of gametes because few gametes develop, which could result in a continuous increase in size of the adductor muscle. A hypothesis to explain the large difference found between triploid and diploid mollusks was proposed by Guo and Allen (1994[a]) : the differences are caused by the occurrence of polyploidy gigantism in triploids resulting from

an increased cell volume paired with lack of cell-number compensation. For scallops, particularly for adductor-muscle weight, an additional hypothesis can be proposed to explain the large increase in that tissue over the others. In scallops, one of the main sites for storage of energy glycogen is the adductor muscle (Martinez and Mettifogo, 1998; Barber and Blake, 1991). If glycogen is not used in triploids for maturation, its concentration will increase over time. Glycogen accumulation in cells could result in an increased size of the muscle because of cell gigantism, which will not necessarily be caused only by an additional set of chromosomes, but also by the unused and accumulated glycogen as it is known to occur in the liver of vertebrates, where glycogen is stored (Stryer, 1981). Support for this hypothesis comes from Tabarini's (1984) results, finding glycogen concentrations in adductor muscle of triploid *A. irradians* as high as 135% of that from adductor muscle of diploid scallops. Furthermore, in the present study, muscle weight of triploid scallops also decreased as it did in diploid scallops when both were kept up to 382 days.

Environmental conditions during our study were unusual, because El Niño 1997–1998 was in progress, with increased temperatures and decreased productivity (Lluch-Cota et al., 1999). If polyploidy gigantism was the only cause of larger muscle weight, muscle weight should not have decreased. However, if glycogen reserves in excess were the cause of a heavier weight, then muscle weight will probably decrease as glycogen is used during those harsh conditions.

4.3. Gametogenesis and hermaphroditism

In diploid scallops, gametogenesis was normal and similar to what Villalejo-Fuerte and Ochoa (1993) had previously reported for this functional hermaphroditic species. However, in triploid scallops, both the gametogenesis process and the normal condition of functional hermaphroditism were largely affected by the triploid condition. The effects of triploidy on gametogenesis were similar to that reported for *C. nobilis* (Komaru and Wada, 1989), *M. arenaria* (Allen et al., 1986), and *S. commercialis* (Cox et al., 1996). In contrast to those species, the catarina scallop is a functional hermaphrodite with simultaneous presence of male and female gonadal portions, maturing synchronously. In this study, the effects of triploidy seen in the male and female portion of the gonad resulted in differences in synchronization of the gonad maturation process. The male portion of the gonad, seen only

during the early culture days, was more severely affected by the triploid condition than the female portion; most of the spermatogonia were arrested early in development. This agrees with results reported for males of the sequential hermaphrodite *C. nobilis*, and for non-hermaphrodites such as *M. arenaria*, *S. commercialis*, and *Mercenaria mercenaria* (Allen et al., 1986; Komaru and Wada, 1989; Cox et al., 1996; Eversole et al., 1996), where spermatozoa development was hindered, although not necessarily suppressed. For *Mytilus galloprovincialis*, Kiyomoto et al. (1996) reported that triploids produced an average of 0.8% of the sperm produced by diploids. In contrast, for *C. gigas*, *P. fucata*, and *Crassostrea virginica*, spermatozoa has been produced in triploids (Allen and Downing, 1986; Allen et al., 1986; Komaru and Wada, 1990). Our results were different. Not only was there a lack of further development to form spermatozoa in triploid catarina scallop, but the male portion of the gonad was gradually replaced with female acini (Fig. 5F), such that by day 280, 96% of the triploid scallops were sexed as 'female only'. Though unused or unspawned, mature oocytes are recycled through lysis (Pazos et al., 1996). In our study, the male acinus did not show any evidence of being recycled through lysis. The phagocytic cells observed in Stage D were seen only at the end of the growout but not when the male acini were being replaced by female acini days 118–280 . The mechanism of progressive suppression of the male gonad seen during the culture for the triploid population is unknown, but Sertoli cells in phagocytosis of germ cells as suggested by Pipe (1987 cited by Kiyomoto et al., 1996) might have been involved. Abnormalities in the functional hermaphroditic condition of this species have been observed previously. For example, the occurrence of only female gonads have been observed in natural and hatchery-reproduced populations of this species at a low frequency, whereas the occurrence of only male gonads has also been seen, but at a much lower frequency, indicating the normal hermaphroditic condition can be altered (Ibarra, unpublished data).

Different hypotheses for sex determination in mollusks have emerged as a consequence of abnormal sex ratios observed when triploidy is induced (Kiyomoto et al., 1996), but this is the first case reported for which a functional hermaphrodite bivalve is shown to have an altered sexuality caused by triploidy. The genetics underlying the hermaphroditic condition in bivalves has not been elucidated to this day. For *Crassostrea* oysters, in which protandric sex change, dioecy, and hermaphroditism exist, Guo et al. (1998) concluded that sex is determined by two alleles at a single loci with a dominant 'M' allele and a recessive 'F' allele,

and that the occurrence of rare functional hermaphrodites could be caused by developmental or genetic abnormalities. For other species, for which functional hermaphroditism is the rule, as for example, hermaphrodite nematodes, it is known that both sex chromosomes and autosomal loci sex factors or genetic determinants are involved in determining the fate of sexual development in these organisms. Triploid *Caenorhabditis* sp. individuals XXX AAA are hermaphrodites, whereas incomplete triploids XX AAA are males. Mutations in different genes in autosomes also produce abnormal sex, independently of the genotype of sex chromosomes (Bull, 1983). Whether or not sex chromosomes do exist in catarina scallop, the presence of an extra set of chromosomes does not suppress initial differentiation of the male gonad part, which could indicate a deterministic role of sex chromosomes on sexual differentiation, as was evidenced by the presence of male gonads early during gametogenic development. However, the triploid condition suppresses further male gonad development, resulting in replacement of male germinal tissue by female germinal tissue as evidenced by the presence of some oocytes in the male part of the gonad. This could indicate multipotency of germinal cells in becoming either sex. As stated by Guo et al. (1998), "the primary gonad of dioecious species including humans is hermaphroditic, and sexual differentiation is achieved by selectively suppressing the development of the opposite sex". Though in many protandric bivalves, germinal cells seem to be multipotent because changes in sex are seen during their life cycle, in scallops as the catarina scallops, the gonadal sac is divided into two compartments clearly differentiated as male and female from early in development of the gonad, and both gonadal compartments are always present during the life cycle of the scallop. We can speculate on the presence of genes involved in early differentiation of each compartment, with other genes acting later to maintain a sexual differentiation for each compartment in a multipotent gonadal tissue. Abnormalities in the expression of these later gene products caused by the triploid condition could result, for example, in overexpression of genes conferring female characteristics. Contrary to that seen in the male portion of the gonad of triploids, gametogenesis in the female portion was retarded but similar to diploid scallops, although few oöcytes developed. The early active 'b' stage described here for the female gonad of triploids, in which the walls of female acini were lined with oogonies but oocytes were scarce and abnormal, has been observed in almost all triploid female bivalves (Allen et al., 1986; Komaru and Wada, 1989; Cox et al., 1996; Eversole et al., 1996). The presence of large numbers of phagocytic

cells in triploid gonads by day 382 may indicate reabsorption of eggs. The cause of that possible failure to spawn is not known. We know that triploid catarina scallop can spawn because we have achieved this under laboratory conditions (A.M. Ibarra, unpublished results).

Finally, the non-conclusive results on differences in oocyte size between triploid and diploid catarina scallops deserve further research, which is under way. Differences in egg volume have been reported for other species. For example, Guo and Allen (1994b,c) measured egg diameter in triploid Pacific oyster, and found an increase of 33–54% egg volume in triploids.

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