Description de las Temperaturas Usadas en el Cultivo de Larvas de *Strombus* spp.

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RESUMEN

*Strombus gigas*, *S. costatus*, *S. pugilis*, *S. raninus*, *S. gallus*, and *S. alatus* son especies de importancia comercial en el Caribe. Economicamente *Strombus gigas* es la más importante y consecuentemente la más estudiada. En esta descripción se analizan los efectos de la temperatura en el desarrollo de las etapas de crecimiento de la concha de los veligers, la tasa de metamorfosis, la taza de establecimiento y la taza de supervivencia. Por mucho tiempo se ha sugerido que la temperatura como un factor importante en la regulación de la taza de desarrollo, la duración de la etapa pelágica y de la mortalidad de las larvas de invertebrados marinos bentónicos. Es bien conocido que el establecimiento temprano de las larvas dentro de ciertos límites, se relaciona directamente con la temperatura del agua. El propósito de este artículo es analizar los efectos de la temperatura (24-32 °C) en el desarrollo del veliger hasta metamorfosis. El cual se logra en aproximadamente en 14 días a temperaturas de 29°C y en 60 días a temperaturas de 27°C, Por qué?

PALABRAS CLAVE: Conch, larvas, temperatura

Overview of Temperatures Used in Larviculture of *Strombus* spp.

ABSTRACT

*Strombus gigas*, *S. costatus*, *S. pugilis*, *S. raninus*, *S. gallus* and *S. alatus* are of commercial importance in the Caribbean. Economically, *S. gigas* is the most important and consequently the most widely studied. In this overview is analyzed the effects of temperatures on the development of shell growth stages of conch veligers, metamorphosis rate, settlement rate an survival rate. Temperature has long been suggested as an important factor regulating the developmental rate, length of pelagic life, and mortality of larvae from benthic marine invertebrate organisms. It is known that the rate of early cleavage, within certain limits, is related directly to water temperature. The purpose of
this paper is to analyze the effects of temperature (24 - 32°C) on the veliger’s development until metamorphosis which is attained within 14 days at 29°C to 60 days at 27°C. Why?

KEY WORDS: Conch, larvae, temperature

INTRODUCCION

De Jesús (1999) analizó los temas de los artículos científicos publicados sobre Strombus gigas (n = 230) y encontró que ha habido una evolución de estos desde los años 1950s en que se escribieron los primeros. Así en los años 1960s la mayoría de los artículos fueron sobre la biología genera, en los 1970s el principal tema fue la biología pesquera. Al inicio de los 1980s y como consecuencia de la sobre-explotación del recurso en la mayoría de los países del Caribe, se da una fructífera producción de información sobre el cultivo larvario, producción de juveniles y los primeros ensayos de siembra de éstos en el medio natural con fines de repoblamiento. Posteriormente, en los años 1990s, cuando los trabajos de cultivo no proporcionan los resultados que se habían proyectado, se inicia una novedosa línea de investigación en Ecología de larvas y juveniles con el grupo de trabajo de Stoner y Davis, estudiándose el reclutamiento de la especie (Stoner and Davis 1994, Stoner and Ray 1993, Stoner et al. 1996; Stoner et al. 1998, Stoner and White 1990, Stoner 1990, Ray-Culp et al. 1999).

Los estudios de ecología y biología de larvas son de utilidad para comprender mejor los procesos de reclutamiento en el medio natural, así como la aplicación de esta misma información para mejorar los resultados de las técnicas de acuicultura para la producción de semillas en el laboratorio.

Una utilidad adicional de los experimentos realizados en laboratorio, sobre biología y ecología de larvas, es la ayuda que proporcionan para comprender los procesos de asentamiento, desarrollo y sobrevivencia, dado que el seguimiento de los organismos, en particular de larvas en el océano es difícil. Así, la mayor parte de la información disponible acerca de la vida larvaria proviene de estudios en laboratorio.

Davis, et al. (1996), señalan que el comprender los factores que afectan el crecimiento, desarrollo y sobrevivencia en larvas de invertebrados marinos en el campo, proporciona un conocimiento pertinente para determinar la dispersión larval y el proceso de reclutamiento.

Con el objetivo de incrementar el conocimiento científico sobre la biología de larvas y su aplicación a mejorar las técnicas de cultivo y comprender los procesos de reclutamiento, en el presente trabajo se realizó una revisión del efecto de la temperatura en el cultivo larvario que han sido utilizadas para Strombus gigas, Strombus costatus y Strombus pugilis. Se resume también información
de la posible relación entre la temperatura con los resultados de crecimiento, desarrollo, metamorfosis y sobrevivencia.

**Condiciones de Cultivo y Temperatura para la Crianza de Larvas Veligeras de Strombus gigas**

*S. gigas* es una de las especies más importantes de especies pescadas en el Caribe, y su cultivo ha sido ampliamente estudiado, en menor grado lo han sido *S. costatus* y *S. pugilis*, incluso las técnicas desarrolladas para la primera especie han sido aplicadas indistintamente para las otras dos.

En la Tabla 1 son resumidas las diferentes condiciones que han sido utilizadas para el cultivo larvario de *S. gigas*, los resultados están expresados en términos de tasa de crecimiento, longitud máxima, duración de la metamorfosis y sobrevivencia.

De la revisión de artículos publicados sobre las condiciones de cultivo larvario de *Strombus gigas*, se observa que solamente 3 trabajos han sido específicamente para estudiar el efecto de la temperatura durante el desarrollo larvario (Aldana Aranda et al. 1987, Corral y Ogawa 1985, Bradshaw-Hawkins 1982). Los demás autores han realizado sus experiencias de cultivo larvario bajo un rango de temperatura variable o pocos de ellos bajo una temperatura constante. Incluso el 30% de los autores citados en la tabla anterior, ni siquiera señalan la temperatura que emplearon para correr su experimento. Mucho menos presentan alguna relación entre la temperatura y el crecimiento, desarrollo o sobrevivencia en la vida larvaria de *S. gigas*.

A partir de la Tabla 1, se observa que en la vida larvaria de los Strombídos, en particular de *S. gigas*, la temperatura es un factor que ha sido poco evaluado su efecto en el proceso de crecimiento, desarrollo y competencia para la metamorfosis.

Respecto al rango de temperatura utilizado para el cultivo larvario *S. gigas* este ha sido entre 23 y 31°C. Del análisis visual de los datos de esta tabla se observa que los mejores resultados en términos de crecimiento y metamorfosis son observados en el rango de temperatura entre 27 y 31°C. En este rango Davis et al. (1993) obtiene la metamorfosis de *S. gigas* a los 21 días. En contraste tres décadas antes, D’Asaro (1965), reporta por primera vez larvas metamorfoseadas de esta especie en 60 días con una temperatura promedio de 25°C. Aldana-Aranda and Torentena (1987) trabajando con larvas de *S. gigas* cultivadas a 20 ± 2°C, 24 ± 3°C and, 28°C observaron una mortalidad masiva al quinto día de cultivo para las larvas cultivadas a 20 ± 2°C; y al noveno día para aquellas cultivadas a 24 ± 3°C. Contrariamente, la mortalidad de las larvas cultivadas a 28°C fue observada 10 días después de iniciado su cultivo y la metamorfosis de estas larvas se presento a los 20 días.
Ahora bien, los mismos valores de la Tabla 1 fueron analizados estadísticamente, para establecer posibles correlaciones entre la temperatura y los siguientes parámetros: tasa promedio de crecimiento, longitud máxima alcanzada al momento de la metamorfosis y tiempo en que alcanzan la metamorfosis. Sólo el tiempo que utilizan las larvas para alcanzar la metamorfosis presento un coeficiente de correlación por arriba de 0.75, las otras relaciones de la temperatura no presentaron una correlación alta mayor a 0.80. A continuación las curvas que presentaron la mejor correlación y su ecuación.

Table 1. Results in growth, maximum length (ML), metamorphosis (M) and survival (S) of S. gigas larvae, under different culture conditions. Food is indicated as: a. Enriched natural cultures of phytoplankton, mainly Nitzchia spp., Skeletonema costatum, and Chaetoceros spp., b. Thalassiosira spp., c. Tetraselmis spp., d. Isochrysis spp., e. Chaetoceros gracilis, f. Nanochloris, g. Dunalieilla tertiolecta, h. Platymonas tetraselmis, i. Prorocentrum minutum, j. Emiliana huxleyi, k. Heterocapsa pygmaea.

<table>
<thead>
<tr>
<th>TC</th>
<th>Food</th>
<th>Growth</th>
<th>ML</th>
<th>M</th>
<th>S</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>µm/day</td>
<td>mm</td>
<td>Days</td>
<td>%</td>
<td></td>
</tr>
<tr>
<td>24-27</td>
<td>H</td>
<td>-</td>
<td>60</td>
<td>-</td>
<td>-</td>
<td>(1)</td>
</tr>
<tr>
<td>24-30</td>
<td>A</td>
<td>82*</td>
<td>2.2</td>
<td>28-33</td>
<td>-</td>
<td>(2)</td>
</tr>
<tr>
<td>24-30</td>
<td>A</td>
<td>-</td>
<td>27-35</td>
<td>-</td>
<td>-</td>
<td>(3)</td>
</tr>
<tr>
<td>28 ± 1</td>
<td>b, c, d, g</td>
<td>1.9</td>
<td>28</td>
<td>-</td>
<td>-</td>
<td>(4)</td>
</tr>
<tr>
<td>29</td>
<td>b, d, f, g</td>
<td>-</td>
<td>14-35</td>
<td>-</td>
<td>-</td>
<td>(5)</td>
</tr>
<tr>
<td>26-30</td>
<td>A</td>
<td>-</td>
<td>18-21</td>
<td>-</td>
<td>-</td>
<td>(6)</td>
</tr>
<tr>
<td>23-31</td>
<td>c, d</td>
<td>-</td>
<td>19-30</td>
<td>mortality</td>
<td>-</td>
<td>(7)</td>
</tr>
<tr>
<td>26 ± 1</td>
<td>d, g, i, j, k</td>
<td>13-93</td>
<td>1.2</td>
<td>-</td>
<td>83-96</td>
<td>(8)</td>
</tr>
<tr>
<td>27-29</td>
<td>A</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>(9)</td>
</tr>
<tr>
<td>28</td>
<td>c, d, c+d</td>
<td>40</td>
<td>1</td>
<td>20</td>
<td>-</td>
<td>(10)</td>
</tr>
<tr>
<td>25-31</td>
<td>d, e, c</td>
<td>-</td>
<td>1.1</td>
<td>21-40</td>
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<td>(11)</td>
</tr>
<tr>
<td>28-30</td>
<td>D</td>
<td>24</td>
<td>0.9</td>
<td>Not reached</td>
<td>59 ± 9</td>
<td>(12)</td>
</tr>
<tr>
<td>27</td>
<td>a, d</td>
<td>-</td>
<td>1.3</td>
<td>27 ± 2</td>
<td>-</td>
<td>(13)</td>
</tr>
<tr>
<td>27-30</td>
<td>d, e</td>
<td>39</td>
<td>1.2</td>
<td>21</td>
<td>-</td>
<td>(14)</td>
</tr>
<tr>
<td>29 ± 1</td>
<td>d, c</td>
<td>5-13</td>
<td>0.7</td>
<td>-</td>
<td>21-52</td>
<td>(15)</td>
</tr>
<tr>
<td>28</td>
<td>C</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>(16)</td>
</tr>
<tr>
<td>28-32</td>
<td>b, c, d</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>15-20</td>
<td>(17)</td>
</tr>
<tr>
<td>29 ± 1</td>
<td>b, c, d,</td>
<td>24-37</td>
<td>0.8</td>
<td>-</td>
<td>25-82</td>
<td>(18)</td>
</tr>
<tr>
<td>29-30</td>
<td>d, e</td>
<td>1.3</td>
<td>18-23</td>
<td>-</td>
<td>-</td>
<td>(19)</td>
</tr>
<tr>
<td>26-29</td>
<td>A</td>
<td>-</td>
<td>18-30</td>
<td>-</td>
<td>-</td>
<td>(20)</td>
</tr>
</tbody>
</table>

*Rate calculated for this table from other data in source.
En la Figura 1 se presenta la correlación entre la temperatura y la tasa de crecimiento diaria, con un ajuste polimodal y un coeficiente de correlación de 0.63. La tendencia de estos puntos muestra que la temperatura a la que se obtiene la mejor tasa de crecimiento es a 27°C (60 um por día), luego una zona subóptima con una tasa de crecimiento de 40 um en el rango de temperatura de 26°C y 28°C. Por último una zona de bajo crecimiento a temperaturas superiores a 29°C e inferior a 26°C.

**Figura 1.** Correlación entre el efecto de la temperatura y la tasa promedio de crecimiento diaria de la longitud de la concha de larvas veligeras de *Strombus gigas*. Los datos para establecer la correlación fueron tomados de los autores citados en la tabla 1. \( Y = -13.039x^2 + 709.9x - 9597.2 \) \( r^2 = 0.6313 \)
La Figura 2 muestra la correlación entre la temperatura y la longitud máxima al momento de la metamorfosis no presento correlación alguna, su mejor ajuste fue el exponencial con un coeficiente de correlación bajo de 0.32.

Por último la correlación entre la temperatura y el tiempo en que la larva alcanza la metamorfosis (Figura 3), presento un buen ajuste exponencial con un coeficiente de correlación de 0.80. Los datos muestran una tendencia a disminuir el tiempo de la metamorfosis de 26°C a 29°C, utilizando menos de 25 días a esta última temperatura. De esta misma tendencia se observa que a temperaturas superiores a 30°C, las larvas se salen de su rango óptimo y vuelven a requerir de mayor tiempo para la metamorfosis.

Figura 2. Correlación entre el efecto de la temperatura y la longitud máxima de la concha de larvas velligeras de Strombus gigas. Los datos para establecer la correlación fueron tomados de los autores citados en la tabla 1. \( Y = 138.33x^2 - 9075x + 1189 \) \( r^2 = 0.3195 \)

Por otra parte, la información obtenida de los cultivos larvarios y de sus resultados en términos de crecimiento o duración de la metamorfosis, no es relacionada con la época del año de la cual proviene la masa de huevos. El único trabajo que trata de establecer una relación en este sentido es Ogawa y Corral (1985) quienes durante tres años y de manera mensual (1983 - 1985), estudiaron cultivos larvarios hasta la metamorfosis, sugiriendo que la frecuencia de éxito de la cría de larvas hasta la metamorfosis depende de la estación del año en la que se efectúa el cultivo, con mejores resultados de junio a septiembre. Los autores obtienen un mayor número de cultivos que llegan hasta metamorfosis al aumentar la temperatura, pero si esta sigue incrementándose, las pruebas
positivas hasta metamorfosis vuelven a bajar. Estos resultados llevan implícito el concepto de la calidad del hueso de los progenitores que ha sido estudiado ampliamente en *Pecten maximus* por Dorange (1990).

![Time to Metamorphosis](image)

**Figura 3.** Correlación entre el efecto de la temperatura y el tiempo en que alcanzan la metamorfosis las larvas veligeras de *Strombus gigas*. Los datos para establecer la correlación fueron tomados de los autores citados en la tabla 1. $Y=8.1548x^2-348.86x+4964.9; r^2=0.7975$

De Jesús (1999) en un estudio sobre abundancia y distribución de larvas no encontró correlación significativa entre estas y la temperatura, salinidad o el oxígeno. Sólo observó correlación entre estas y la abundancia de juveniles.

Sin embargo, otros autores como Stoner et al. (1992) y Barilé (1994) encuentran correlación entre la abundancia larval y la temperatura y el fotoperiodo. Lo anterior podría estar asociado con la capacidad adaptativa de la larva para acortar el tiempo hacia la metamorfosis, disminuyendo la probabilidad de depredación en el plancton y evitar la competencia con los miembros del fondo (Stoner 1997, Pechenick 1999).

**DISCUSIÓN**

**Temperatura, Factor Exógeno y su Efecto en las Larvas de Moluscos**

La atemperatura es uno de los factores físicos que son conocidos por afectar el crecimiento de las larvas de moluscos, lo mismo que el alimento (Bayne, 1983) La tasa de crecimiento de las larvas se incrementa al incrementarse la temperatura hasta un óptimo para luego declinar a temperaturas más elevadas.
Ursin (1963) describe la relación entre la temperatura y el tiempo de completar una cantidad de crecimiento como una curva catenaria simétrica definida por:

\[ Y = y_0 \cosh p(x - x_0) \]

Donde y es el tiempo, x es la temperatura, \( x_0 \) es la temperatura a la cual el desarrollo es más rápido, y es el tiempo de desarrollo al tiempo \( x_0 \) y p es el coeficiente de temperatura. Del recíproco de la ecuación anterior 1/y, es la tasa de crecimiento. La simetría alrededor de la temperatura óptima para el crecimiento puede no estar presente, ya que para algunas especies se presenta una transición abrupta desde el óptimo hasta una temperatura superior letal (Lucas and Costlow 1979). Loosanoff (1959) para Mercenaria mercenaria logra describir una relación lineal entre la temperatura y el número de días desde la fertilización hasta el asentamiento, de la siguiente manera:

Días al asentamiento = 37.9 - T (°C)

Sin embargo a 15°C y 33°C el crecimiento y desarrollo fueron anormales y la mortalidad fue alta. Ansell (1968) resume datos sobre la tasa de crecimiento de Mercenaria mercenaria a través de su rango de distribución geográfica y concluye que la temperatura media óptima es de 20°C; lo cual contrasta con el crecimiento de la larva, que tiene su óptimo cerca de los 30°C. La explicación de estas observaciones, y su significado ecológico permanece sin explicación. Algo similar se observó en el análisis de los resultados obtenidos en este trabajo con S. gigas; donde a 26°C se tiene la mejor sobrevivencia, a 27°C la mejor tasa de crecimiento y a 29°C el mejor resultado para la metamorfosis.

Una evaluación de los efectos de la temperatura sobre el crecimiento larval no es completa, si no se considera el período durante el cual la larva es competente para alcanzar la metamorfosis. Lutz y Jablonskib (1978) han sugerido que una correlación negativa entre la temperatura y la longitud de la concha a la que alcanzan la metamorfosis, puede ser de utilidad en paleoclimatología.

En el medio natural las larvas pueden estar expuestas a cambios cortos de temperaturas, sin embargo experimentos del efecto de variaciones de temperatura sobre el crecimiento son escasos.

En larvas veligeras de Nassarius obsoletus, Scheltema (1967) obtiene una alta tasa de crecimiento a la temperatura de 25°C. La temperatura más baja a la cual el desarrollo se completo hasta la metamorfosis fue de 16 a 17°C. Este autor observó 46% de inhibición en el crecimiento de las larvas entre la temperatura óptima y la mínima a la cual el desarrollo es completado. Estos resultados muestran que a bajas temperaturas (< 27°C) se incrementa la tasa de mortalidad, y
el atraso del desarrollo de las veligeras. Por otra parte a pesar de que la fuente de alimento de larvas *S. gigas* son algas unicelulares, cuyo valor nutritivo depende de la digestibilidad de las celulas algas, especialmente de la pared celular, y la producción de toxinas (Lucas 1990). Scheltema (1967) puntualiza que algunos dinoflagelados o diatomeas pueden crecer y sobrevivir igual de bien entre 15 and 30°C, sin embargo tanto el número de celulas como su valor nutritivo puede diferir marcadamente entre estos extremos. Davis y Calabrese (1964) encontraron que *Chlorella* sp. Incrementan su valor nutricional en larvas de almejas y ostiones, cuando la temperatura es incrementada por arriba de 20°C. Estos autores han sugerido que las enzimas necesarias para digerir digerir la pared celular pueden estar activas completamente a altas temperaturas.

La temperatura es un factor que controla el metabolismo del organismo e influye en el crecimiento larval y en la sobrevivencia (Pechenik et al.,1990); por lo que mucha mayor atención debe prestarse a este factor. Este autor con larvas de *Mytilus edulis* y de *M. mercenaria* y *C. Virginica*, encuentra una pobre correspondencia entre el efecto de factores ambientales y la tasa de crecimiento y el desarrollo. Lo anterior es similar a lo que se presenta en este estudio para *S. gigas*. En los estudios de Bayne (1965), el incremento de la temperatura acelera la tasa de crecimiento más en relación a la tasa de desarrollo.

En parte por las implicaciones ecológicas que tienen el desarrollo de los moluscos (Pechenik 1990), los biólogos han examinado los efectos de la temperatura, la concentración de alimento y otros factores ambientales sobre su desarrollo larval, su sobrevivencia y su crecimiento; a través de la longitud de la concha o en el mejor de los casos con el peso seco (Pechenik 1987). Por lo que respecta a los efectos de los factores ambientales sobre las tasas de diferenciación permanecen no explorados para los moluscos, a pesar de que existe una buena razón para pensar que las tasas de crecimiento y diferenciación pueden ser afectadas en diferentes grados por cualquier cambio ambiental. En la medida que diferenciación anatómica y fisiológica puede presentarse en ausencia de crecimiento. En este sentido larvas de opistobranquios, presentan sustanciales cambios anatómicos y desarrollo de competencia para la metamorfosis, posteriores varios días de haber cesado el crecimiento en longitud de la concha. Existen evidencias marcadas de la independencia entre el proceso de crecimiento con la diferenciación durante el desarrollo larvario. Así larvas de *Crepidula fornicata* desarrollan filamentos branquiales a tallas pequeñas de longitud de la concha cuando se cultivan en bajas temperaturas (Pechenik y Lima 1984). Si el tiempo requerido para desarrollar los filamentos branquiales ha incrementado en la misma proporción que la tasa de crecimiento de la concha ha decrecido, las branquias se deberan desarrollar mas tarde, pero a la misma talla; las tasas de crecimiento de la concha aparentemente decrecieron mas por las bajas temperaturas en relación a la tasa de diferenciación de las branquias, de manera que las larvas
desarrollan branquias a tallas en promedio más chicas. De manera similar larvas de C. fornicata cultivadas a altas temperaturas crecen más rápido y alcanzan la metamorfosis a tallas en promedio más chicas (Pechenik 1984), lo que implica un efecto diferencial de la temperatura sobre las tasas de crecimiento y diferenciación. El grado de extensión al cual las tasas de crecimiento larval y diferenciación están unidas no ha sido específicamente estudiada en moluscos. De manera clara este efecto diferencial se presenta en este análisis realizado del efecto de la temperatura sobre el desarrollo y crecimiento larval de S. gigas.

CONCLUSIÓN

El efecto de la temperatura no ha sido estudiada sobre el desarrollo larvario de los Strombidos, ni en particular sobre S. gigas. El conocer el rango óptimo de temperatura para el desarrollo y crecimiento de una especie, tiene una aplicación en mejorar las técnicas de cultivo para la obtención de semilla, pero que también tiene aplicación en comprender el papel de este factor físico en el proceso de desarrollo larvario, metamorfosis y asentamiento de nuevos reclutas, con la implicación que tiene en la dinámica de posblaciones de un recurso pesquero.

Al conocer el efecto de la temperatura sobre el desarrollo larval, sirve para predecir el grado de éxito que tendrá el proceso de reclutamiento. Esto esta siendo ya aplicado en peces con el modelo de Appeldoorn, que estima la mortalidad natural de éstos en base a la temperatura ambiente media.

Desde el punto de vista ecológico, la temperatura presenta un papel estratégico en la sobrevivencia larval, ya que a mayor temperatura el crecimiento y desarrollo se acelera, disminuyendo la duración de la vida larvaria y en consecuencia el tiempo que la larva permanece en el plancton, disminuyéndolo el riesgo de mortalidad por predación.

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KEY WORDS: Juvenile mortality, light attracted plankton, recruitment

ABSTRACT

Settlement stage Lutjanus synagris and Ocyurus chrysurus were caught in light traps set off fore reefs in the British Virgin Islands and raised in floating mesh cages tethered in water 1 - 2 m deep. Lights were designed to attract plankton into the cages and provide live natural food for the fish. Plankton taxonomic composition around lights was compared with samples from plankton tows in adjacent water, as well as with gut contents of cage reared and wild fish. Fish mortality and growth were followed over the first few weeks of life and compared with results from a field trial assessing natural mortality and growth. Cage rearing reduces the exceptionally high early post-settlement mortality typical of Ocyurus chrysurus from approximately 80% to 40% within the first month. We suggest two uses for this technique, a) to help speed recovery of over-exploited stocks within no-take marine reserves b) to rear settlement stage ornamental species until they are large enough to survive on artificial food. Light traps and cage culture in combination could replace destructive fishing practices on reefs exploited for the aquarium trade.

INTRODUCTION

Light attracted plankton has previously been investigated for culture of freshwater pike-perch (Sizostedion lucioperca) fry (Schlumpberger and Ziebarth 1981, Jaeger and Nellen 1983, Jaeger et al. 1984, all cited in Hilge and Steffens 1996). This paper reports preliminary investigations into the feasibility of collecting settlement stage reef fish and rearing them past their initial mortality hurdle by using light attracted zooplankton as food. High post settlement mortality is typical of many coral reef fishes. Shulman and Ogden (1987) found >90% natural mortality in French grunts Haemulon flavolineatum during the month after settlement, and this rate may be characteristic for species that settle in pulses, and/or form schooling aggregations of juveniles (Roberts 1996, and references therein).

We discusses potential uses for this rearing technology both to enhance recovery of recruitment overfished reef fish stocks within protected areas and to provide a non-destructive alternative income for fishers raising ornamental species for the aquarium trade.

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MATERIALS AND METHODS

Settlement stage reef fishes were caught using light traps modified from a design by Stobutzki and Bellwood (1997). The traps consisted of 40 x 40 x 40 cm aluminium ‘angle-iron’ frames supporting transparent plexiglas panels. On each side of the trap a clear funnel ran the height or width of the cube, (two vertical and two horizontal) each with an opening of 12mm. The light was a ‘stand alone’ system inserted into the top of the main trap. It consisted of an O’ring sealed plastic box, housing two six volt rechargeable batteries and connected to a clear plastic tube containing an 8 watt fluorescent bulb. A light sensitive switch mounted on the top of each light box turned the trap on at dusk and off at dawn. An advantage of this modular design is that changing batteries and mending equipment did not require the entire light trap to be taken ashore. Buckets set beneath the main trap retained the fish as traps were hauled. Traps were set at a depth of 2 m, 100 - 200 m offshore of three fringing reefs on Tortola, British Virgin Islands around new moon periods in 1998 and 1999. Commercially important yellowtail and lane snappers (*Ocyurus chrysurus*, *Lutjanus synagris*) caught in light traps were reared in floating cages (125 L, mesh size 3 mm) tethered in 1 - 2 m of water for approximately one month to investigate the feasibility of rearing reef fish on light attracted plankton. At night, each cage was lit with either an 8 watt light module from the light traps or a 9 watt mains powered light sealed with silicone into plastic drinks bottles and appropriately fused. All cages were regularly scrubbed of algae. The order and variety of experiments described below were unavoidably constrained by unpredictable availability of fishes in monthly settlement pulses over the recruitment season.

Early Mortality in Caged and Wild Fish

Survival of *Lutjanus synagris* reared under two different stress regimes was examined to isolate the effect of handling stress on survival. For two cages (both with 45 fish initially) handling stress at first capture from the light trap was minimised by not touching the fish and by keeping them in extra large aerated buckets until placing them in the cage as the earliest opportunity. One group was counted and measured every week, while the other was sampled only fortnightly. *L. synagris* reared in two other cages (with initial numbers of 35 and 40) received no special treatment at capture and were both sampled weekly. Survival of *O. chrysurus* in two cages (initial n = 35 and 63) was recorded weekly to investigate density dependence in mortality.

As part of a parallel study, early juvenile natural mortality of *O. chrysurus* was estimated from daily censuses of an area of seagrass 25 x 50 x 1-1.5 m in depth. The area was divided into 5 x 5 m squares. One observer swum
concentrically around each individual section until the entire area of each square was covered. Each census took a total of 2 - 2.5 hours. Daily censuses were performed during the 11 days leading up to peak settlement in September 1999 and for six days following. Over the next nine days a different observer conducted censuses. Resumption of observations by the original observer identified bias in numbers of fish observed (but not the proportion of size classes - see section on growth below) and nine days data were dropped from the analysis of mortality. Observations then continued every other day for a further 11 days until the pulse of settlement became indistinguishable from other juveniles. Temporal patterns in abundance were assumed proportional to the processes of settlement and natural mortality (as in McGehee 1995). We also assume disappearance of fish represented mortality rather than movement, and that any emigration from the census area would be balanced by immigration. We base this assumption on the enormous size of the census area relative to observed home ranges of settlement stage *O. chrysurus* (Watson and Gell in prep and see discussion), and on results from a preliminary mark recapture experiment which found elastomer tagged *O. chrysurus* moved only a few metres after settlement (Watson and Gell in prep). The day when the number of settlement size fish peaked (91 compared to only four 10 days earlier) was assumed to represent the settlement event, and numbers of fish remaining over time were expressed as a percentage of that number for comparison with mortality in cage reared fish.

**Estimates of Early Juvenile Growth for *L. synagris* and *O. chrysurus*, and Comparison of *O. chrysurus* Growth in Cage Reared and a Natural Population**

Early juvenile growth was estimated from weekly measurements of two groups of *O. chrysurus* (n = 35 and 63) and three groups of *L. synagris* as in the mortality section above (n = 45 ‘low stress’, n = 35 and n = 40). Data from the ‘low stress’ trial measured fortnightly were excluded as there were only two data points. Total lengths of wild fish from the September settlement peak were estimated (to the nearest 0.5 cm) during daily censuses of the seagrass grid until the cohort could no longer be clearly distinguished (about 25 days). Modal length from field observations was plotted against modal length of *O. chrysurus* (grouped to the nearest 0.5 cm) reared in two cages in order to compare growth of captured and wild fish.

**Food Availability and Feeding Preferences**

Zooplankton was sampled from water adjacent to floating cages using a mini-plankton net (30 cm diameter aperture) towed at approximately one knot for five minutes. Zooplankton attracted into cages was sampled with two swipes of
a dip-net in an empty cage every five minutes over a 25 minute period. Gut contents were analysed for seventeen cage-reared \textit{O. chrysura}us (total length 20-40mm) caught at night. Gut contents were also analysed for ten wild \textit{O. chrysura}us (22 - 50 mm total length) caught during the day. Percent composition by volume in the water samples and in fish guts (individuals within each sample pooled) were compared to examine selective feeding behaviour.

RESULTS

Early Mortality in Caged and Wild Fish

Survival of \textit{L. synagris} was greater in 'low stress' higher density cages (n = 45) than in 'high stress' lower density cages (n = 35 and n = 40) (Figure 1.). Two cages where initial handling stress was minimized had approximately 80% survival after three weeks, compared to cages stocked at lower initial densities (23% and 12% less fish) where survival was around 50% within two weeks. After initial capture, weekly measuring did not appear to have a marked affect on survival. Thus a large part of early mortality of \textit{L. synagris} (and probably \textit{O. chrysura}us) in cages appears to be due to initial handling stress.

![Figure 1](image_url)

\textbf{Figure 1.} Percent survival of \textit{Lutjanus synagris} against time in cages for four rearing regimens; triangle, initial n = 40; no special treatment; open triangle initial n = 35, no special treatment; circle initial n = 45, 'low stress' regime, sampled weekly; open initial n = 45, 'low stress' regime sampled fortnightly
Survival of *O. chrysurus* at initial densities of n=35 and n=63 over approximately one month were very similar (Figure 2) suggesting that mortality is not density dependent up to at least 0.5 fish per litre. Survival in these two cages was approximately 30% higher than estimates of survival from wild fish after one month (Figure 2). Daily censuses estimated mortality of *O. chrysurus* to be 80% within one month in the wild. Cage reared fish had a mortality of approximately 30-40% over the same time period. Mortality in all three groups fitted exponential curves (see equations on the graphs), i.e. 'type three' mortality. The curve fitted through the wild population was not forced through an intercept of 100% since the actual number of settlers is not known because settlement took place over a number of days during which mortality was already operating.

![Graph showing percent survival against time for *Ocyurus chrysurus* < 1 month since settlement and reared in floating cages.](image)

**Figure 2.** Percent survival against time for *Ocyurus chrysurus* < 1 month since settlement and reared in floating cages (closed circles initial n = 35, y = 100e^{-0.0182x}, r^2 = 0.71, open circles initial n = 63, y = 100e^{-0.0125x}, r^2 = 0.80) compared with percent survival in the wild estimated from daily field censuses of one monthly cohort (triangles, y = 74.831e^{-0.0445x}, r^2 = 0.83).

**Comparison of Growth in Natural and Cage Reared Fish.**

Early juvenile growth in cages was linear for both *O. chrysurus* and *L. synagris* and shows no signs of density dependence between 0.28 and 0.5 fish per litre (Figure 3 and Figure 4). Modal length of *O. chrysurus* in cages was not significantly different to modal length of the September 1999 cohort censused in the wild over the first month (Figure 5).
Figure 3. Mean total length (mm) of Lutjanus synagris against time in cages for three rearing regimes; closed circle initial n = 45, 'low stress' y = 1.05*X + 19.7, r² = 0.98; triangle initial, n = 35, 'high stress' y = 1.35*X + 18.5, r² = 0.99; open circle initial n = 40, 'high stress' y = 1.275*X + 16.72, r² = 0.99. Error bars represent 95% confidence levels.

Figure 4. Mean total length (mm) of Ocyurus chrysurus against days in cage for two groups; closed circles initial n = 35, y = 1.0383*X = 17.99, r² = 0.99; open circles initial n = 63, y = 1.0081*X + 19.01, r² = 0.99. Error bars represent 95% confidence levels.
Figure 5. Modal total lengths of *Ocyurus chrysurus* (less than one month since settlement) estimated to the nearest 0.5 cm from field observations (open circles) and two cage culture experiments (square and triangle) against time in days from peak settlement and from date of capture in light traps, respectively. Error bars for caged fish indicate size range.

**Food availability and feeding preferences**

Zooplankton samples taken from tows in water adjacent to floating cages and from dip-net sweeps inside lighted cages show zooplankton attracted to the lights is similar to that in open water (Figure 6). Both wild and cage reared *O. chrysurus* fed selectively, and included fish (mostly juvenile ‘bait fish’ - Atherinidae and Clupeidae) in their diet (34% and 13% respectively). These fish, which are attracted to lights, avoided dip-nets and the plankton tow. Malacostraca make up very similar volumes in the diet of both wild and caged fish (43% and 40% respectively). Caged fish also ate annelid worms (23%) attracted up off the bottom by the light. Combined with growth estimates, the data suggest zooplankton provides an adequate food source comparable with a natural diet for early juvenile snappers.

**DISCUSSION**

Our results demonstrate that the ‘low tech’, low maintenance technique of rearing reef fish using light attracted plankton supports growth at least equal to that in the wild, and substantially reduces mortality over the first month. We suggest two potential uses of this technique. In coral reef areas where
recruitment over-fishing has diminished the supply of new recruits, recovery of fish populations may be extremely slow even if fishing ceases. For example, identical studies comparing reef fish settlement to the heavily exploited reefs on the north Jamaican shelf, and to the moderately exploited reefs in the British Virgin Islands found orders of magnitude differences in settlement rates (Munro and Watson 1998; Munro and Watson 1999), implying that stock recovery will take many years. If reefs are self-seeding recovery may not occur without intervention. Light traps may be a means of catching juvenile reef fish for stock enhancement (Doherty 1994). Using the techniques described here, settlement stage fish could be collected from a relatively large area, reared for several weeks, and concentrated within a marine protected area.

Figure 6. Percent composition by volume for plankton sweeps inside floating cages, a plankton tow in water adjacent to floating cages, and cages reared (n = 17, total length 20 - 40 mm) and wild (n = 10, total length 25 - 53 mm) Ocyurus chrysurus. Key: black = Copepoda, grey = Ostracoda, wide hatching = Malacostraca, thin hatchin = Annelida, wavyline = fish, dotted = Crustacean debris.
This technology is intended for heavily exploited populations where even a slight increase in juvenile survival from small releases would be valuable. Stock enhancement has been heavily criticized as uneconomic (Hilborn 1998), inappropriate (Scarnecchia 1988, Meffe, 1992), difficult to evaluate (Leber et al. 1996, Hilborn 1998), and potentially harmful to wild populations through introduction of disease or through genetic selection for inappropriate characteristics (Schramm and Piper 1995). However, most enhancement efforts have been large scale, expensive 'high tech.' programs raising up to several million fish from eggs to fingerlings. The present approach is low cost, low maintenance, and rears only wild caught stocks on site. Fish are fed with natural food before release to an unfished native habitat. The methods might be suitable for small scale marine protected areas projects in developing countries were coral reef fisheries resources are under the greatest threat.

In this study, cage rearing had the potential to decrease mortality in the first month after settlement from approximately 80% to around 40%. This estimate is probably conservative due to inaccuracies in the censuses of wild fish. It was not possible to measure the true natural settlement rates both because visual observations may miss many fish and because numbers of settling fish increased rapidly over several days and mortality before the settlement peaked was ignored in our analysis. For many reef fishes, mortality is thought to be highest soon after settlement (Victor 1986, Doherty and Sale 1986, Sale and Ferrell 1988). Furthermore, we compared natural mortality with O. chrysurus reared in cages before development of faster, low stress handling techniques at capture. Results from L. synagris suggest cage mortality can be reduced to around 20% with careful handling.

Perhaps one of the most important criticisms of stock enhancement is that artificially reared fish lack the behavior necessary to ensure survival in the wild. A parallel study of 'early juvenile' snapper behavior in seagrasses (Watson and Gell in prep) found that recently settled O. chrysurus and L. synagris (approximately 2 - 3 cm) remain almost stationary near the bottom and pick plankton from the water column. They rarely move more than 20cm from their initial position and tolerate conspecifics of the same size. However, from approximately 3 - 3.5 cm their range becomes considerably bigger (several square metres), they switch to feeding on benthic invertebrates and act aggressively towards similarly sized conspecifics. Thus cage rearing is probably most suited to rearing fish to approximately 3 - 3.5 cm (3 - 4 weeks).

The present study, which was designed to test the feasibility of the technology, has not addressed the question of whether cage reared fish subsequently released have a higher overall survival than wild fish from the same cohort. High mortality in the first few days after settlement (or release) may be due to unfamiliarity with the habitat. However, rearing in semi-natural
environments (Masuda and Tsukamoto 1998 and references therein) and conditioning to the habitat (Olla et al. 1994) have been shown to reduce postrelease mortality. An evaluation phase is planned for 2000. Despite his many criticisms, Hilborn (1998) concedes that stock enhancement programs are most likely to succeed where wild stocks are essentially gone. This is the case for commercially fished stocks on many overexploited reefs. Even where high mortality makes cost per surviving individuals substantial, the value should be considered in terms of survivor reproductive potential rather than the contribution to stock numbers (Stoner and Glazer 1998).

The second application we suggest for rearing fish on light attracted plankton is to supply ornamental reef fish to the aquarium trade. Widespread use of destructive collecting techniques, such as sodium cyanide, often leads to high mortality in captured fish, does substantial harm to the reef ecosystem, and has made development of sustainable collection techniques an international concern. However, growing consumer demand for ‘eco-labeled’ fish suggests sustainable aquarium fisheries could provide a valuable alternative income for fishers, particularly where overfishing is currently degrading coral reef resources. We suggest fish could be reared on light attracted plankton from late pelagic/settlement size until they are big enough to thrive on artificial food. Catching fish before they reach the ‘wall of mouths’ (Kaufman et al. 1992) on the reef provides an opportunity to avoid high post-settlement mortality. Where natural populations are already overexploited, a proportion of the fish caught for the aquarium trade could also be grown past their initial ‘mortality hurdle’ and used to enhance natural populations.

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Mitochondrial Control Region of Striped Mullet, *Mugil cephalus*: A Tool to Restore Marine Fisheries Resources

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**ABSTRACT**

Mitochondrial DNA (mtDNA) has been frequently used as a molecular marker in fisheries stock assessment and has potential applications in stock enhancement. In some fish species, sequence data from the mtDNA control region exhibits enough intraspecific variability that the likelihood of two unrelated individuals being identical is very low to nil. Efforts to restore the depleted natural stocks of the striped mullet, *Mugil cephalus*, have been undertaken in the Pacific, through cultured fish release. In such enhancement programs the use of molecular markers, particularly during the initial pilot-scale releases, is a reliable method to estimate contributions of cultured fish to wild stocks. The purpose of this study was to create primers in the flanking tRNAs to amplify the mtDNA control region in *M. cephalus* as a prerequisite to then conduct population genetic studies supporting stock enhancement activities. Further study of the control region of *M. cephalus* will allow separation of individual stocks and the possible utilization of molecular tags to identify genetic contributions of hatchery-reared individuals to the wild population in stock enhancement activities in the Gulf of Mexico and Caribbean.

**KEY WORDS: Mugil cephalus**, control region, mitochondrial DNA

**INTRODUCTION**

The steadily increasing demand for seafood in the United States, together with technological improvements in harvesting methods and natural events have resulted in depletion of marine fisheries resources. About half of the commercially important marine fishes in the United States are currently being harvested beyond their maximum sustainable yield, leading to a steady decline in abundance (National Marine Fisheries Service 1997, Blankenship and Leber 1995). This trend will likely continue into the future as a result of projected worldwide increases in human population, particularly in developing countries (National Marine Fisheries Service 1997).

Restoration of depleted stocks is possible through effective traditional management procedures designed to reduce fish mortality, reduce fishing effort, and restore habitat. Since overfishing of most commercial stocks is inevitable
(Grimes 1998), traditional management procedures, along with non-traditional methods, such as stock enhancement, should be considered before stocks are significantly depleted.

Stock enhancement has been utilized in varying degrees from the mid-1880s to present time in the United States, Canada, France, Australia, United Kingdom, New Zealand, and Norway (Grimes 1998). Early attempts at marine stock enhancement often involved the indiscriminate release of eggs and newly hatched larvae; most of which were considered failures and in some instances were thought to have caused deleterious effects on existing stocks (Grimes 1998). Blankenship and Leber (1995) proposed a more responsible approach to marine stock enhancement which prioritizes and selects a target species, develops stock rebuilding goals, and uses genetic resource management to avoid deleterious genetic effects. Baseline genetic data is necessary to direct restocking efforts and minimize the negative genetic impacts caused by accidental or intentional inbreeding (Hinder et al. 1991, Blankenship and Leber 1995).

The primary objective of this study is to develop the necessary technical procedures for using mtDNA, specifically the control region, as a molecular marker to support stock enhancement activities through the United States Gulf of Mexico Marine Stock Enhancement Consortium Program (Consortium). The goals of this program are the development, refinement, field testing, and demonstration of a successful marine fishery stock management program for the U.S. coastal Gulf of Mexico that blends stock enhancement technology with traditional fishery management practices. When the identified constraints are resolved, the technology will be transferred to local and regional resource management agencies for their consideration as an additional management tool to supplement, and help replenish certain high-priority, declining stocks of marine fishes. The striped mullet, *Mugil cephalus*, was used as a test species in this study because of its initial designation as a prototype target species by the Consortium. These procedures will be used in future papers to discuss the characterization of the control region, as well as facilitation of a population genetic study of *M. cephalus* from three ocean basins. The procedures described in this study will then be adapted to other target species as designated by the Consortium.

MATERIALS AND METHODS

*Mugil cephalus* was collected from Biloxi Bay, Mississippi. White muscle tissue was excised and placed in SED buffer (250 mM EDTA, pH 7.5, 20% DMSO, 3.42 M NaCl; weight to volume ~ 2 - 3 grams to 16 ml). *M. cephalus* was identified using fish keys by Hoese and Moore (1977), Murdy (1983), and Robins and Ray (1986), and taxonomic specialists (Jim Franks, Stuart Poss, and Richard Waller) at the Gulf Coast Research Laboratory, Ocean Springs, MS.
Total genomic DNA was extracted from each sample, under sterile conditions, using a procedure modified from Taggart et al. (1992). DNA was then quantified using fluorescence spectrophotometry, as described in Gallagher (1994). Samples were adjusted to a concentration of 100 ng/μl in TE (10 mM Tris, 1 mM EDTA, pH 8.0) prior to storage at -20°C.

Portions of cytochrome b, 12S rRNA, and the control region, and the entire tRNA threonine (tRNA-T) and proline (tRNA-P) were amplified by PCR using universal primers CB3R (5’ CACATTCAACCAGAATGATATT 3’; Palumbi, 1996; Fig. 1) and 12SA-H1067, referred to in the present study as 12SAR, (5’ ATAATAGGTTATCTAATCCTAGT 3’; Martin et al., 1992; Figure 1). PCR amplification was performed in replicate 25 μl reactions containing 100 ng template DNA, 1.5 mM MgCl₂, 200 μM deoxynucleotide triphosphates (Promega, Inc.), 0.3 μM of each primer, and 1.75 units Taq DNA polymerase with 10X PCR buffer (Amersham Life Science). PCR cycling parameters were 3 min at 94°C, followed by 35 cycles consisting of a denaturing of 0.75 min at 94°C, an annealing of 1 min at 55°C, and an extension of 2 min at 72°C, with a final elongation of 7 min at 72°C. After visualization on an agarose gel, the appropriate PCR product was excised, purified using the QIAquick Gel Extraction Kit (Qiagen, Inc.), quantified, and direct sequenced. Species-specific primers in the genes coding for tRNA-P (MulPro, 5’ CCAAGGCCAGGATTTTACGT 3’; Figure 1) and 12S rRNA (Mul12S, 5’ CACGAGATTTACCGGCCCTATTAG 3’; Figure 1) were then designed.

These primers, MulPro and Mul12S, were then utilized to produce a PCR fragment using the previously described cycling conditions. Purified PCR products were cloned using the pGEM®-T Easy Vector System (Promega, Inc.). Ligated vector DNA was transformed into competent JM109 cells that were then cultured on Luria-Bertani (LB)/ampicillin plates with x-gal and IPTG. Colonies containing inserts were identified by blue/white selection and used to inoculate 5 ml minipreps. The cloned plasmid DNA was isolated using the Wizard® Plus DNA Purification System (Promega, Inc.). Plasmid DNA was then purified using the PEG method (Nicoletti and Condorelli 1993), quantified, and sequenced.

A species-specific primer in the tRNA-phenylalanine (tRNA-F; MulPhe, 5’ CTCTTGAATCTTACCGTCGTCG 3’; Figure 1) was subsequently designed and used with the primer MulPro to amplify the entire control region with the above PCR cycling parameters. The appropriate PCR-product was gel-purified, quantified, and direct sequenced. All DNA sequencing was done with an ABI model 373A automated sequencer at the University of Maine DNA Sequencing Facility.
RESULTS

Total genomic DNA was extracted, and a piece of DNA approximately 2000 base pairs (bp) in length was produced by amplification with the universal primers CB3R and 12SAR (Figure 2a). Approximately 640 bp of sequence was obtained from the CB3R priming site and 400 bp of sequence from the 12SAR site. Results using the NCBI’s BLAST WWW Server (Basic Local Alignment Search Tool; Altschul et al. 1990) on the resulting sequences indicated the products obtained closely matched portions of the 12S rRNA, control region, and cytochrome b, as well as tRNA-T and tRNA-P. This sequence information was used to design species-specific primers located in the tRNA-P (MulPro) and in the 12S rRNA (Mul12S) located approximately 180 bp downstream of the 12SAR site. The species-specific primers, MulPro and Mul12S, produced a 1,300 bp PCR-product (Figure 2b). This DNA fragment was subsequently gel purified, T/A cloned (Figure 2c), and completely sequenced, producing the entire control region, tRNA-F, and a partial 12S rRNA. A species-specific primer located in the tRNA-F (MulPhe) was designed from this available sequence data. Finally, MulPro and MulPhe primers produced a DNA-fragment of approximately 880 bp (Figure 2d), which contained the entire control region. BLAST searches identified these sequences as the mtDNA control region and it was most homologous with Xiphias gladius (swordfish). The entire 1694 bp sequence is deposited in GenBank, accession number AF108270.
Figure 2. PCR products of *Mugil cephalus* mitochondrial control region and flanking genes produced, using the primers: (A) CB3R/12SAR; (B) MulPro/Mul12S; (C) MulPro/Mul12S, Lanes 2 and 4 contain uncut clones, Lanes 3 and 5 contain clones from which the insert was cut out with EcoRI enzyme; (D) MulPro/MulPhe (see Materials and Methods for details). Lane 1 in all gels is the ladder of sizes 2000, 1500, 1000, 750, 500, 300, 150, and 50 base pairs (Amresco PCR marker, Amresco, Inc.).
DISCUSSION

The purpose of this study, providing the needed technical background for the eventual development of genetic tags to support stock enhancement activities in the Gulf of Mexico and Caribbean, was accomplished by sequencing the portion of the mtDNA between the cytochrome b and 12S rRNA. It was necessary to create species-specific primers in the flanking tRNAs because tRNAs are conserved within species and amplification of the smallest sequence, including the entire control region, is needed to obtain the greatest overlap in the 5' and 3' sequences. Further utilization of these primers with an increased number of samples allowed for characterization of the control region and a population genetic study of M. cephalus between three geographically distant populations: Gulf of Mexico, Atlantic, and Pacific Basins (Garber 1999). This thesis research determined the genetic differences within the Gulf of Mexico and between the three Basins, as well as determining if these differences constituted genetically distinct populations. The sequences could then also be utilized to evaluate whether or not a partial or entire control region can be used as a genetic tag.

Stock enhancement is considered a non-traditional option for restoration of marine fisheries, but it may become an extremely useful alternative management tool in the near future. Genetic considerations need to be defined and maintained, such as: identifying the genetic risks and consequences of enhancement, defining an enhancement strategy with adequate genetic controls, implementing those genetic controls in the hatchery, and monitoring and evaluating effects/impacts of hatchery-reared fish on wild stocks. Utilizing mtDNA, specifically the control region, may be extremely useful in assessing adverse impacts of releases and possibly provide solutions necessary to circumvent many of these problems before they occur. A population-genetics study using control region sequence data (Rocha-Olivares et al. submitted) as well as a complete characterization of the control region (Garber et al. in preparation) will be presented elsewhere. Appropriate genetic monitoring will result in preservation of fisheries resources and will protect the natural genetic population structure of marine species worldwide. If our fishery stocks are to ever recover, fisheries management entities must work together to combine the available biological, technological, economical, and political information in an effort to identify the best possible solutions.

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LITERATURE CITED


Induction of Ovulation in Common Snook, *Centropomus undecimalis* [Bloch], using Human Chorionic Gonadotropin (HCG) and Gonadotropin-Releasing Hormones (GnRH)

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ABSTRACT

Common snook, *Centropomus undecimalis*, is one of Florida's most popular inshore sport fish and the subject of an ongoing stock enhancement program. In the development of hatchery techniques for spawning this species, both human chorionic gonadotropin (HCG) and gonadotropin-releasing hormones (GnRH) were investigated. Doses of HCG were compared to determine the smallest dose that would induce ovulation and produce optimum egg and larval survival. Doses of 50, 100, 250, 500, 1,000, and 2,000 IU/kg body weight (BW) were used. A dose of 500 IU/kg BW of HCG produced consistent ovulation, good egg quality and larval survival. Secondly, four analogs of GnRH were each administered in time-release pellets at a dose of 10 μgm/kg/day over five days. These were salmon (sGnRH), chicken (cGnRH-II), seabream (sbGnRH), and mammalian (mGnRH) gonadotropin-releasing hormones. With the exception of sbGnRH which was ineffective, the time to ovulation was similar with all hormones, and viable larvae were obtained. Control fish did not ovulate.

KEY WORDS: Common Snook, induced ovulation, spawning

INTRODUCTION

Common snook is a popular inshore sport fish in southern Florida. This species is an important candidate for both aquaculture and stock enhancement (Chapman 1982). The procedure to induce final oocyte maturation and ovulation in common snook has been to use HCG at a dose of 1,000 IU/kg BW. In an effort to improve spawning methods, six doses of HCG were compared to determine the lowest dose that would induce ovulation and provide the best egg quality and larval survival. In addition, four forms of gonadotropin-releasing hormone were evaluated for their ability to induce ovulation in common snook. Two of these were known forms (cGnRH-II and sGnRH) that occur in common
snook (Sherwood et al., 1993). A third form of GnRH was also isolated from common snook in insufficient quantity for sequence determination. However, sbGnRH has since been sequenced (Powell et al. 1994) and may be the same as the unknown form in common snook. The three GnRH forms that have been isolated from fish and the mammalian analog were compared for their ability to induce gonadotropin-releasing activity in the pituitary of seabream, Sparus aurata (Zohar et al. 1995). We compared their ability to induce ovulation in common snook.

MATERIALS AND METHODS

Common snook were collected during their spawning season using either a seine or trammel net (Taylor et al. 1998). To compare doses of HCG, females were divided into six groups of five fish each and injected with: 50, 100, 250, 500, 1,000, and 2,000 IU/kg BW of HCG. Controls were injected with the carrier, 0.9% sodium chloride. Each form of GnRH was administered to groups of four female common snook as intraperitoneal, time-release GnRH pellets (10 µg/m/kg/day). Controls were untreated or implanted with a placebo.

Individual fish were kept in soft-mesh net pens in recirculating tank systems where they were biopsied to follow final oocyte maturation. Percent of fertilized eggs, percent hatch, and percent survival to first feeding (72 hour post-hatch) were evaluated to determine spawn quality (Neidig et al. 2000).

RESULTS

The dose of HCG and number of fish injected versus the number that ovulated were as follows: controls 5:0; 50 IU 5:0; 100 IU 5:1; 250 IU 5:2; 500 IU 5:5; 1,000 IU 5:5; and 2,000 IU 5:5. Control fish did not ovulate. The percent fertilization and percent survival of larvae to first-feeding from fish injected with 250, 500, 1,000, and 2,000 IU/kg BW HCG were within acceptable ranges (Neidig et al. 2000).

In the GnRH study, the control, placebo, and sbGnRH-treated fish did not ovulate. Most (75%) of these fish had no advance in oocyte maturation. Three of four mGnRH-treated fish ovulated, and the ovaries of the fourth reached a prevulatory stage. All of the fish implanted with sGnRH and cGnRH-II ovulated and produced viable eggs.

DISCUSSION

The dose of HCG that produced consistent ovulation and good egg quality was 500 IU/kg BW. Implants of sGnRH, cGnRH-II, and mGnRH induced ovulation and produced viable eggs. The GnRH study was performed in August at the end of the reproductive season, and this may account for one of the fish (mGnRHa) failing to ovulate.
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LITERATURE CITED