Diferenciación Sexual e Inicio de la Madurez de la Cachicata Blanca, *Haemulon plumieri* (Pisces: Haemulidae), en La Parguera, Puerto Rico

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La cachicata blanca, *Haemulon plumieri*, es una especie dominante y un componente importante de las pesquerías deportivas y comerciales en la región del Caribe. Por ser muy susceptible a ser capturada por todas las artes predominantes (nasas, redes, arpones, compresores) esta especie está en riesgo de ser sobrepescada. Los estudios reproductivos necesarios para el manejo de la especie son escasos y generalmente dirigidos hacia individuos adultos. Con el fin de caracterizar las etapas tempranas del desarrollo reproductivo y determinar la talla en la cual se inicia la diferenciación y la madurez sexual en *H. plumieri*, se realizó un estudio de febrero a mayo de 1997. Se anotaron detalles individuales de comportamiento, talla, ubicación dentro o fuera de la costa y se determinó el estadio gonádico correspondiente. Se encontró que la talla mínima de diferenciación sexual fue de 72 mm LH en las hembras y de 86 mm LH en los machos, mientras que la madurez sexual se inicia a 91 mm LH en las hembras y 86 mm LH en los machos. El cincuenta por ciento de las hembras maduran a 120 mm LH y los machos desde 80 mm LH. Estos valores son menores que los reportados anteriormente para la misma especie.

PALABRAS CLAVE: *Haemulon plumieri*, diferenciación sexual, primera madurez.

Sexual differentiation and First Maturation of the White Grunt, *Haemulon plumieri* (Pisces: Haemulidae), In La Parguera, Puerto Rico

The white grunt, *Haemulon plumieri*, is a dominant species and an important component of the sport and commercial fisheries in the Caribbean region. Because it is very susceptible to capture by all dominate gears (traps, nets, spears, hooks) this species is at risk of being overfished. The reproductive studies needed for management of this species are scarce and generally directed toward larger adults. A study was conducted from February to May of 1997 to characterize the early stages of reproductive development and to determine the lengths at differentiation and first maturation in *H. plumieri*. Individual details of behavior, length, and inshore-offshore distribution were recorded and the corresponding gonadic stage was determined. The minimal observed lengths at sexual
La cachicata blanca (*Haemulon plumieri*) es un pez arrecifal de gran importancia comercial y recreacional en las pesquerías de mar del oeste del Atlántico y en las aguas del Caribe (Manooch 1976; Dammann 1980; McFarland et al., 1979). Es explotada en Puerto Rico e Islas Virgenes (McFarland, 1979 Darcy 1983); en la República Dominicana (Aquino e Infante 1990) y en toda la plataforma cubana (Claro et al. 1994). Por ser abundante y alcanzar una de las mayores tallas dentro de su familia, la especie se encuentra bajo riesgo de sobre pesca (Appeldoorn y Lindeman 1985). Los estudios reproductivos necesarios para su manejo adecuado son escasos y orientados generalmente a descripciones histológicas.


Teniendo en consideración la escasez de datos en relación al tamaño en que *H. plumieri* comienza a madurar y la necesidad de actualizar y enriquecer la información existente sobre el desarrollo sexual de la cachicata blanca, los objetivos particulares de este trabajo fueron:

i) determinar la talla a la cual *H. plumieri* se diferencia sexualmente y,

ii) registrar la talla de primera madurez sexual en hembras y machos de la especie.

**METODOLOGIA**

Se colectaron doscientos ocho especímenes de *H. plumieri* de varios tamaños en La Parguera, suroeste de Puerto Rico desde Febrero hasta Mayo de 1997. Los peces fueron capturados con arpon, y el comportamiento previo a la captura fue anotado para cada individuo y sus gónadas, así como los valores de longitud y peso.
Las gónadas fueron procesadas histológicamente siguiendo la metodología propuesta por Luna (1968) y ligeramente modificada por Polofski (c.p.) que conlleva deshidratación e inmersión en parafina y luego aclaramiento y tinción con hematoxilina de Harris y eosina. Los cortes histológicos se analizaron por triplicado y aquellas en donde no se pudo establecer el sexo fueron catalogadas como indeterminadas. En la identificación de las distintas etapas celulares, del sexo y grado de desarrollo se siguió a Román Cordero (1991), Asoh (1991), Claro (1996) y Posada (1996). La característica principal que permitió clasificar los especímenes en los inicios del desarrollo fue la presencia de ovocitos primarios en las hembras y en los machos, los espermatocitos primarios (Figura 1). Las hembras con proliferación de ovocitos terciarios y los machos con espermatocitos terciarios y espermatozoides fueron catalogados como maduros inactivos.

RESULTADOS

Treinta y tres de los individuos colectados no pudieron ser identificados debido al mal estado de las gónadas o al sexo irreconocible por estar en una etapa muy temprana del desarrollo gonádico. La proporción total fue de machos:hembras =1:2.27 (N = 160). En el caso de los especímenes maduros la proporción sexual hallada fue de machos:hembras = 1:2.94 (N = 69). La talla mínima de diferenciación sexual encontrada para la cachicata blanca fue de 86 mm LH en los machos y de 72 mm LH en las hembras, mientras que el tamaño mínimo en el cual los primeros alcanzaron la madurez fue a los 86 mm LH y las hembras a los 91 mm LH.

Al tomar en consideración las diferentes etapas de desarrollo y madurez de las gónadas en este trabajo, se encontró que el 50% de las hembras de H. plumieri estaban en período de madurez inactiva a partir de 120 mm LH; maduras activas desde 160 mm LH y desovadas desde 190 mm LH. Se halló un 100% de individuos inmaduros entre 70 y 80 mm LH; maduros activos a partir de 180 mm LH y desovados a 210 mm LH. En el caso de los machos, los resultados de este trabajo indican que el 50% de los mismos están maduros inactivos desde los 80mm LH, pero que es posible encontrar especímenes inactivos con tallas por encima de los 220 mm LH. El 50% de especímenes maduros activos solo fue encontrado a partir de 230 mm LH.
Figura 1. A) Primeras etapas en la formación de los ovocitos en una hembra inmadura de *Haemulon plumieri* (Ov1 = ovocito primario, longitud horquilla = 114 mm, aumento = 40x). B) Macho en los inicios del desarrollo sexual (Esp1 = espermaticitos primarios, longitud horquilla = 129 mm, aumento = 100x).
DISCUSION

La razón sexual total y en especímenes maduros hallada en el presente estudio, difiere de la proporción encontrada por Román Cordero (1991) para la especie en Puerto Rico (machos:hembras = 1:1.05) y del valor teórico esperado en las poblaciones (1:1). Estos resultados sugieren que las hembras en la población no son un recurso limitado. Diferentes autores han propuesto que la diferencias entre proporciones de sexo son debidas a diferencias sexuales en el crecimiento, mortalidad o disponibilidad de alimento. García Cagide et al. (1994) plantean que la proporción en que se encuentran los individuos está sujeta a gran variabilidad y está relacionado con el carácter adaptativo de la especie y con el hábitat ocupado por cada sexo y este puede variar durante el ciclo anual y la ontogenia.

Las tallas mínimas de diferenciación sexual encontradas para la cachicata blanca en el presente trabajo (hembras = 72 mm LH, machos = 86 mm LH) son ligeramente menores que las indicadas por otros autores (Tabla 1). En estos trabajos se evidencia una ligera tendencia en las hembras a diferenciarse sexualmente a una talla menor que en los machos, aunque éstos alcancen tamaños mayores. Nagahama, (1983) indica que, usando las células germinales en meiosis como criterio, se ha hallado que la diferenciación sexual ocurre primero en hembras. Esto ha sido demostrado en el pez medaka, Oryzias latipes y en la tilapia, Sarotherodon mossambicus (Hoar et al. 1983).

**Tabla 1.** Tallas de diferenciación e inicio de la maduración en la cachicata blanca, Haemulon plumieri en La Parguera, Puerto Rico. Los valores representan las tallas de longitud horquilla en milímetros (DS= diferenciación sexual, IM= inicio de la madurez, L50= longitud de clase en la cual el 50% de los especímenes están maduros). M = machos; H = hembras

<table>
<thead>
<tr>
<th>Autores</th>
<th>DS H</th>
<th>IM (Lmin)</th>
<th>L50</th>
</tr>
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<tbody>
<tr>
<td>Mateo (1999)</td>
<td>h = 72, m = 86</td>
<td>h = 91, m = 86</td>
<td>h = 120, m = 80</td>
</tr>
<tr>
<td>Román Cordero (1991)</td>
<td>h = 74, m = 97</td>
<td>h = 165, m = 160</td>
<td>h = 210, m = 165</td>
</tr>
<tr>
<td>García Cagide (1987)</td>
<td>90 ??</td>
<td>h = 110, m = 120</td>
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<td>García Cagide y Claro</td>
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<td>140 - 160</td>
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<td>Gaut y Munro (1983)</td>
<td></td>
<td></td>
<td>h h = 220, m = 200</td>
</tr>
<tr>
<td>Billins y Munro (1974)</td>
<td></td>
<td></td>
<td>h = 143, m = 145</td>
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<tr>
<td>Mota y Pessoa (1973)</td>
<td></td>
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<td>h = 110, m = 120</td>
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</tbody>
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La definición de las tallas de diferenciación y de maduración sexual pueden ser elementos importantes al momento de clasificar las especies e individuos de acuerdo a su estádio ontogenético, en donde, usualmente, sólo se toman en cuenta el tamaño promedio de la especie y su conducta. Nuestros resultados, al ser comparados con otros sugieren que la cachicata blanca obtiene su diferenciación sexual aún en tallas propias de juveniles.

El proceso de diferenciación sexual de las gónadas ha sido estudiado en diferentes peces teleósteos y se ha encontrado que este período varía de acuerdo a la especie y al sexo de los individuos. Redding y Páinio (1993) explican que la diferenciación sexual en peces es un proceso controlado por genes específicos en donde las células germinales bipotenciales podrían diferenciarse como espermatogonias en respuesta a andro-inductores y otras como ovogonias en presencia de gino-inductores, sin embargo la naturaleza de esos agentes no se conoce de manera definitiva.

Las tallas de inicio de la madurez en hembras y machos de *H. plumieri* registradas en este estudio fueron 91 y 86 mm LH, respectivamente, y son menores que las indicadas por otros autores para la misma especie (Tabla 1).

Al calcular la talla en la cual el 50% de los individuos alcanzan la madurez sexual, se encontró que los machos pueden estar maduros inactivos a partir de los 80 mm LH, mientras que las hembras maduraron a una talla más grande (120 mm LH). Estos valores de tamaños de maduración son mucho menores que los obtenidos por Román Cordero (1991) y Gaut y Munro (1983). Las tallas más pequeñas observadas en este estudio podrían estar relacionadas con varios factores. Primero, en este estudio se hizo énfasis en colectar los individuos dentro de la amplitud de tamaño en en la cual se espera que tengan lugar la diferenciación y maduración. Segundo, los peces fueron colectados en áreas diferentes. En los estudios previos la mayoría de las muestras fueron tomadas de la pesquería y, por lo tanto, estuvieron prejuiciadas hacia los individuos más grandes de las áreas de pesca comercialmente importantes. De esta manera, nuestro estudio ha debido tener una mayor facultad para determinar con más precisión los límites de tallas a las cuales el desarrollo gonádico se inicia.

Por otro lado, estos tamaños menores pueden ser el resultado de diferencias en las condiciones ambientales entre los estudios. Numerosos estudios (Pauly 1984, Stearns y Crandall 1984) han mostrado que las variaciones en los factores ambientales pueden afectar el tiempo de desarrollo gonádico en peces. Se ha sugerido (Roff 1984, Stearns y Crandall 1984, Stearns y Koella 1984, Schaefer 1987, Appeldorn 1989) que tales variaciones son adaptaciones (genéticas o fenotípicas) hacia condiciones ambientales que afectan diferencialmente los patrones de crecimiento y mortalidad. En particular, cuando la mortalidad en adultos es alta y el crecimiento en las etapas tempranas es rápido, se debería esperar una maduración temprana. Esto ocurre en condiciones de elevada presión de pesca.
García Cagide et al. (1986) sugieren, específicamente, que esto podría afectar el desarrollo gonádico en las cachicatas. Así, es también posible que las tallas menores de diferenciación y maduración observadas sean un reflejo de que, al momento de nuestro estudio, la cachicata blanca haya estado sometida a una mayor presión pesquera en relación con los estudios previos. Es de notar que los estudios pasados en Puerto Rico han mostrado que el recurso está siendo sobreexplotado (Stevenson 1978, Appeldoorn y Lindeman 1985, Dennis, 1988), y no hay una base para esperar que la presión por pesca haya hecho otra cosa que incrementarse.

En un estudio asociado, 35 cachicatas blancas fueron mantenidas en un tanque grande con fondo natural y alimentadas diariamente. Bajo examinación histológica estos individuos presentaron un desarrollo gonádico más avanzado que otros individuos de tallas similares en el campo (Mateo Pérez 1999). Esto demuestra claramente el potencial para la variabilidad inducida por el ambiente en el inicio y tasa de desarrollo gonádico en esta especie. García Cagide y Claro (1983), García Cagide (1986b, 1987) argumentan que en peces de pequeño a mediano tamaño como son las cachicatas en su mayoría, la diferenciación sexual ocurre antes de la madurez sexual, la cual tiene lugar a temprana edad y esto provee un mecanismo de respuesta a las condiciones ambientales lo cual que se traduce como un mayor avance en el desarrollo gonádico y en la talla de madurez.

Si se toman en consideración los resultados obtenidos por diferentes autores (Román Cordero 1991, Billings y Munro 1974, Mota y Pessoa 1973, García Cagide y Claro 1983, García-Cagide 1987) respecto al tamaño mínimo de maduración en hembras y machos de la cachicata blanca, es difícil asegurar con exactitud cuál de los sexos madura primero ya que las tallas obtenidas varían de lugar a lugar. Es importante destacar, sin embargo, que los tamaños indicados para Puerto Rico (Román Cordero 1991, el presente trabajo) permiten sugerir que los machos inician la madurez sexual a tallas ligeramente inferiores que las hembras; mientras que en los informes de Brasil, Jamaica y Cuba, son las hembras las que parecen madurar primero.

Los resultados de este trabajo pueden ser aplicados en el diseño de planes de manejo de la especie y deben ser tomados en consideración a la hora de establecer tallas mínimas de captura con fines de evitar la sobrepesca del reclutamiento.

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LITERATURA CITADA


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Methods and Lessons Learnt in the Application of Ultrasonic Telemetry to Coral Reef Fish Movement Studies

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ABSTRACT

There is increasing interest in the use of acoustic telemetry to track the movement of medium-sized marine fishes, particularly coral reef species, as a result of recent improvements in technology which have allowed reductions in the size of both receivers and transmitters. However, acoustic tagging and tracking techniques are still at a developmental stage. In this study we use six medium-size (12 - 46 cm FL) reef fish species (Clepticus parrae, Cephalopholis cruentata, C. fulva, Lutjanus mahogani, Ocyurus chrysurus and Kyphosus sectatrix) to investigate appropriate non-injurious capture and in situ retention methods, examine suitable transmitter tag placements (internal versus external) using dummy tags, and test the feasibility of simultaneously tracking up to 20 individuals in a reef environment. The most appropriate capture methods were species-specific and included use of an Antillean fish trap, barbless hook and line, and small mesh net. Clove oil proved to be an effective and fast-acting anaesthetic. Surgical implantation of transmitters into the body cavity proved more successful than external attachment to the dorsal musculature. Super Glue® was found to be effective in closing the incision wound, with full healing in as little as five days. The use of coded transmitters operating on the same frequency allowed for tracking of many individuals simultaneously. However, when several transmitters were in close proximity, the ability of the receiver to decipher individual transmitter codes was greatly reduced.

KEY WORDS: Acoustic tagging methodology, reef fish movement

INTRODUCTION

Patterns of movement and space utilisation by fishes are important to the understanding of population distribution and community structure (Zeller 1997). Such information is also critical in predicting and understanding the effectiveness of marine reserves (MRs) in conserving adult fish biomass, and in providing adult fish biomass to adjacent fisheries (Russ and Alaca 1996).
Information on the pattern of movement and home range size of adult fish is also important for the successful design of MRs. For example, placement of the reserve relative to the position of different habitats; reserve size; and area to boundary ratio will all affect the degree to which adult fish are conserved or 'leaked' to adjacent fishing areas (Holland et al. 1996, Kramer and Chapman 1999). Furthermore, movement patterns are likely to vary among different species.

The need for information on adult fish movement is particularly urgent in coral reef areas where the use of MRs is now being favoured over the more traditional management tools for the effective long-term management of sustainable coral reef fisheries (e.g. Bohnsack 1993, Alaca and Russ 1990, Rowley 1994, Russ and Alaca 1996). However, the majority of information on fish movement and habitat utilisation, by coral reef fish species of commercial importance to fisheries, is inferred from conventional mark and recapture studies which involve the use of fishing gear to recapture marked individuals, or rely on visual observations of marked individuals by SCUBA (visual recaptures) (e.g. Corless et al. 1997, see Appeldoorn 1997 for review). The numerous constraints of conventional mark and recapture studies are well documented (e.g. Parker 1990, Watson et al. 1993, Appeldoorn 1997). Observer presence and experience may greatly effect the data by means of failure to notice, recognise and record the correct code and position of both the tag and/or the individual fish. Additionally, the recapture of a tagged individual in a particular area at a particular point in time does not preclude its presence elsewhere at any time between release and recapture (Winter & Ross 1982). Consequently this method will always provide an underestimation of actual movement (Appeldoorn 1997). Perhaps because of this constraint, adult reef fish are generally considered to be site-attached with low potential for supplementing fished areas adjacent to MR boundaries (Bardach 1958, Corless et al. 1997, Holland et al. 1996). Clearly, more complete data on fish movements are needed before any firm conclusions can be drawn on the extent to which MRs will supply adult fish biomass to adjacent fisheries.

Ultrasonic telemetry, which allows continuous tracking of marked individuals, is an ideal tool with which to address the movement and activity patterns of fishes (Winter and Ross 1982, Zeller in press). Until relatively recently, this technique was used exclusively on large pelagic species because of the large size and high cost of the early acoustic transmitter tags (Nelson 1990; cited in Zeller in press). However, improved technology allowing the production of much smaller and relatively less expensive transmitters now means that this technology can be used on much smaller species, including a wide range of reef fishes. Recent studies have successfully applied ultrasonic telemetry to a number of medium-sized coral reef fishes including Mullolides flavolineatus (Holland et al. 1993), Heamulon plumieri (Tulevech and Recksiek 1994),

This paper outlines the methods and the lessons learned in the application of ultrasonic telemetry to the study of movement of several other medium-sized coral reef fish species.

METHODS AND RESULTS

Capture Methods

Successful tagging studies require efficient, non-injurious methods of capture. Several methods were investigated in this study to capture medium-sized (12 - 46 cm FL) reef fishes (i.e. creole wrasse, Clepticus parrae; grasby, Cephalopholis cruentata; coney, C. fulva; mahogany snapper, Lutjanus mahogani; yellowtail snapper, Ocyurus chrysurus; and Bermuda chub, Kyphosus sectatrix) with the least damage and stress to both the specimen and the environment. The capture methods and results are described below.

Traps — Traditional un-baited Antillean Z-shaped traps (2 m long x 1 m wide x 0.6 m high, with 1.5” wire mesh) set on sand patches within the reef (5 - 15 m deep) were used in an attempt to capture the target species.

This passive mode of capture was unsuccessful for C. parrae, which tend to school above the reef, but was successful for the more benthic reef dwellers C. cruentata, C. fulva, L. mahogani, and K. sectatrix and was also occasionally successful for O. chrysurus in areas where densities were high. However, repeated soaks in the same area (i.e. > 4 times in 7 days) resulted in O. chrysurus and K. sectatrix displaying an avoidance behaviour towards the traps.

Once avoidance behaviour was observed, a more active mode of trap fishing was employed for these species. This involved baiting the trap underwater with stale bread placed close to the entrance, and having a diver lying as still as possible, approximately 2 m from the trap. Bread was then crushed and allowed to float in the water near the trap to attract the fish. Once attracted, the diver then slowly moved closer to the trap, eventually rushing the final 0.5 m with arms wide open. This presented the opening of the trap as an avenue of escape, into which the fish fled. This method was very successful for K. sectatrix but did not work for O. chrysurus.

Fish were removed promptly from traps, since prolonged retention caused additional stress and often resulted in injury.

Nets — A miniature cast net (2 m diam. 1 cm mesh size) was spread in an area in which the targeted fish were frequently seen. The net was either held by two divers or was left hanging at the mouth of a trench in the reef or between two large coral heads. Two to three divers using SCUBA then attempted to shepherd
the fish in the direction of the net. Once a fish swam into the net, the net was folded over by a diver to prevent escape. This method was successfully used to capture C. parrae and L. mahogani in highly rugose areas of the reef. The rate of success of this method decreased markedly as rugosity of the reef declined. Attempts to capture K. sectatrix, O. chrysurus and C. cruentata using this method were unsuccessful.

Hook and line — Small barbless No. 9 hooks (2/0 O'Shaughnessy) were baited with strips of fresh ballyhoo (hemirampus sp.), flyingfish (Hirundichthys sp.) or clupeids, and dangled near the target species by divers equipped with SCUBA. This was the most successful method of capture for C. cruentata. Little success was achieved when this method was employed in the capture of the other target species, and the bait was often rapidly removed from the hooks by schools of Abudefulf saxatilis (sergeant major) and Melichthys niger (black durgon).

Suction gun — A suction gun was constructed from the cylindrical barrel of an old Van Dorn sampling device (10 cm diam, 75 cm length) fitted with a manual plunger constructed from a circular Perspex disc (9.3 cm diam.) and a 1/2” (1.25 cm) PVC pipe handle that fitted snugly to the interior of the tube, but was free to move up and down. The mechanism of operation is such that when the plunger is depressed and then pulled underwater, a strong suction force is produced which draws the fish into the barrel, facilitating capture. This device, although successful in capturing small-sized benthic reef fishes, was not successful in the capture of any of the target species in this study because of the difficulty in getting close enough to the medium-sized fish for the suction force to be effective.

Retention of Fish

Ex situ retention — Following initial trials of different capture techniques, fish were transported from the reef in sampling coolers containing 40 L seawater and placed in concrete flow-through seawater tanks (0.43 m³) for investigations of anaesthetic and transmitter tag attachment.

Fish were held without feeding for 24 - 48 hr prior to tagging. During this period they were observed for signs of injury from capture and handling, and a crude index of stress was measured by dividing the concrete tank into 4 imaginary sectors and recording the degree of movement between sectors during periods of 20 minutes for each fish, before and after tagging.

None of the three species investigated (C. cruentata, C. parrae and L. mahogani) showed significant differences in their behaviour before and after tagging. However, the death of two L. mahogani and two C. parrae before
tagging was attributed to stress-related trauma which could not be visually detected.

**In situ retention** — Once initial trials had been completed, fish were no longer removed from the field, but were retained in underwater shade cages (Figure 1) placed in the reef, for a period of observation and starvation (of at least 24 hrs) prior to tagging. Only one fish was retained in each shade cage at any one time.

Two different sized shade cages (small or large) were used depending on the size of the fish to be held. These followed the basic design of P. Sikkel\(^1\) and were constructed of green or black shade cloth (the type used to provide 80% shade in plant nurseries) covering a nylon rope and 1/2" (1.25 cm) PVC pipe frame measuring 0.5 m x 0.35 m x 0.35 m for small cages and 1 m x 0.70 m x 0.70 m for large cages. A door comprising black Perspex\(^\circledR\) was placed in the centre (measuring 0.38 m x 0.18 m for small cages and 0.76 x 0.36 m for large cages) of the top face of the cage and this was hinged with plastic cable ties and fastened with nylon rope.

The shade cage served as a means of isolating the fish from its environment (ensuring a period of starvation) as well as providing a safe and secure place to rest (thereby allowing recovery from the stress of capture). The soft material also served to reduce abrasions or injury to the fish when moving suddenly. The shade cages were weighted down with four 1 kg lead weights as well as tied to the traps to prevent relocation by strong currents, and kept in the reef during use. The shade cages worked well for all species tried (i.e. *K. sectatrix*, *O. chrysurus* and *C. cruentata*). However, removal of *O. chrysurus* from the cages without losing them proved to be very difficult, even with the use of a safety net placed around the door, owing to their powerful acceleration and strong swimming ability.

**Anaesthetic Procedure**

**Ex situ trials** — Two anaesthetics, Quinaldine and clove oil (active ingredient 4-ally-2-methoxy-phenol; Anderson *et al.* 1997) were used in *ex situ* trials with *C. cruentata, C. parrae* and *L. mahogani* in the laboratory holding tanks. Quinaldine was used at a concentration of 0.1 ml/L (2 ml Quinaldine to 20 L of seawater). Clove oil was first dissolved in 70% ethanol (2.4 ml clove oil to 24 ml ethanol) to give a 10% stock solution. This was then added to 20 L of seawater.

Both clove oil and Quinaldine performed equally well at these concentrations in inducing a rapid (in < 2 min) deep anaesthesia in fish of size range 12 – 46 cm FL, from which they recovered in less than 3 minutes.

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\(^1\)Dr. Paul Sikkel, Department of Biology, Murray State University, KY
However, clove oil was chosen for use in the field because of its non-toxic nature to the environment.

![Diagram showing design of the shade cage used for in situ retention of reef fish (after P. Sikkel)](image)

**Figure 1.** Diagram showing design of the shade cage used for *in situ* retention of reef fish (after P. Sikkel)

*Field procedure* — Two species, *K. sectatrix* and *C. cruensiata* were routinely anaesthetised in the field using the following procedure. The shade cage was first slowly brought to the surface (from depths not exceeding 15 m) by a SCUBA equipped diver, and placed on board the tagging boat (a 5 m inflatable dingy). The fish was then removed from the shade cage and immediately exposed to the anaesthetic by placing it in a sampling cooler containing 20 L of seawater and 24 ml of 10% Clove oil stock solution.

The three induction stages described by Hikasa et al (1986) in Anderson et al (1997) (stage 1: onset of rapid opercula movement; stage 2: erratic swimming and partial loss of equilibrium; stage 3: total loss of equilibrium) were clearly observed in both species and the onset of each stage was recorded by stopwatch. The time to full induction (stage 3) varied among individuals but was negatively correlated with weight in *K. sectatrix*, $\log_{10}$ induction time (sec) = 4.035 -
0.802 \log_{10} \text{weight (g)}; r^2 = 0.542, P = 0.006) and not significantly correlated to length or weight in *C. cruentata* (P > 0.05 in both cases) (Figure 2).

**Bermuda chub (Kyphosus sectatrix)**

**Grasby (Cephalopholis cruentata)**

**Figure 2.** Relationship between fish weight and anaesthetic induction time for the Bermuda chub (*Kyphosus sectatrix*) and grasby (*Cephalopholis cruentata*).

**Tag Attachment and Recovery**

*Dummy tag trials* — Both external attachment, through the dorsal musculature, and internal surgical implantation methods of tag attachment were investigated using dummy transmitter tags in initial laboratory-based trials. Dummy tags were made from PVC plastic tubing in two sizes (8 and 11 mm diam., and 18 and 35 mm long respectively) weighted with linseed oil putty and capped at both ends with epoxy resin. The size and weight of the dummy tags approximately
mimicked those of the Lotek CAFT8-1 (8 mm) and CAFT11-2 (11 mm) transmitter tags.

After induction to the anaesthetic, external attachment was tried using L. mohagani, C. cruentata, and C. parrae. This involved strapping the tag, with 1 mm (diam.) nylon thread, flush to the body of the fish parallel to the dorsal fins as described by Holland et. al. (1996). Total time for both external and internal tag placements never exceeded seven minutes, inclusive of induction time to anaesthetic.

Based on both laboratory and field observations of L. mohagani, swimming ability was not diminished by the externally attached dummy transmitter tag. However, repeated attempts were made to remove the tag by chaffing against rocks, and we recorded a 50 % tag loss rate in as little as four days after release into the field. C. cruentata showed obvious signs of distress from external attachment of the dummy transmitter tag, manifested by rapid swimming movements and repeated attempts to remove the tag by chaffing it along the side of the holding tank. C. parrae showed good recovery from the handling and tagging procedure, but displayed great difficulty swimming. Although they quickly rejoined a school on release into the field, they were unable to keep up with the rest of the school and were soon left behind.

Surgical implantation of dummy transmitters in the body cavity was tried using L. mahogani, C. fulva and C. cruentata (C. parrae were considered too small for internal implantation). This procedure involved making an incision immediately posterior to the right pelvic fin, through which the tag was inserted into the body cavity followed by closure of the wound using Super Glue®.

Internally tagged L. mahogani, and C. cruentata showed good recovery after surgery, with full healing of incision wounds observed in as little as five days. Swimming ability and feeding both appeared unaffected by the operation. The single C. fulva used in these trials survived only for a few hours after the surgery owing to the rupture of an extended stomach during implantation. Internal implantation of transmitters was therefore selected for the field study of fish movement.

Field procedure — Acoustic transmitter tags were surgically implanted into 11 individuals of C. cruentata and 11 individuals of K. sectatrix in the field using the following procedure.

Firstly, the anaesthetised fish was placed on a clean, flat, shaded surface in preparation for the surgery. Then one end of a 3 m long transparent plastic hose (1 cm diam.) was inserted into the mouth and held in place. The other end of the hose was connected to the drain of an elevated cooler containing fresh seawater, thereby allowing a continuous flow of seawater over the gills during the surgical procedure. The fish was also periodically bathed in seawater to prevent
dehydration. In the event that the fish showed signs of recovery before the end of the operation, it was re-exposed to the anaesthetic until complete loss of equilibrium was again observed. The cooler was kept full and details of the operation were recorded.

An incision, slightly larger than the diameter of the transmitter tag, was made equidistant from the ventral fins and the anus and slightly to one side of the central line. This was done with the aid of a size 22 scalpel and a blunt ended seeker. The transmitter was then coated with BNT antibiotic cream and inserted into the body cavity through the incision. The wound was closed with a small quantity of Super Glue®. The fish was subsequently externally marked with a Floy® anchor tag (inserted into the dorsal musculature and anchored between the dorsal spines, midway along the dorsal fin) for easy recognition by divers.

After surgery, the tagged fish was placed in a cooler containing fresh seawater to recover from the anaesthetic and finally transferred to the shade cage, which was subsequently replaced on the reef, using SCUBA. This procedure required a team of three people for smooth running of the operation. Surgical latex gloves were always worn when handling the specimens.

The tagged fish was kept in the shade cage for a further 24 hr during which time the health of the fish could be monitored and the functioning of the transmitter verified. After this the fish was released and tracked.

The species tagged using this method (K. sectatrix and C. cruentata) showed no apparent discomfort, exhibited fast recovery and retained the transmitters for the duration of the experiment (maximum observed 70 days).

Electronic Equipment

The electronic equipment used in this field study was manufactured by Lotek Marine Technologies Inc., Newmarket, Ontario, Canada.

Transmitter tags — The acoustic transmitter tags were cylindrical-shaped Lotek CAFT 11-2 tags weighing 7.5 g and measuring 11 x 42 mm. Each tag was factory programmed, with a distinct electronic numeric identification code and set to emit an electronic micro pulse every four seconds, once the magnet switch is activated. Lotek estimated transmitter longevity (battery-life) at 90 days.

Receivers — The pulses (acoustic signals) from the transmitter tags were picked up by a directional HPA-O hydrophone fitted with a DAB 45 noise baffle and converted into 76.8 kHz radio frequency signals via a 150 MHz ultrasonic upconverter (UUC). A SRX 400 manually operated telemetry receiver then deciphered these radio signals and displayed signal strength data.

The noise baffle consisted of a windowed sleeve attached over the end of the hydrophone and tightened by two screws. The exposed portion of the
hydrophone was sensitive to acoustic signals while the covered portion was dampened by the sleeve. The noise baffle served to focus the sensitive portion of the hydrophone in a cone-shaped pattern, providing directionality to the otherwise omnidirectional hydrophone, thereby increasing the ability of the observer to determine the position of the transmitter tag.

There was little problem in following instructions for the assembly of the equipment, but the operations manual for the equipment could only be deciphered by persons knowledgeable in acoustic technology. However, the customer relations department of Lotek Engineering proved excellent, with a trained technician on call to give a step by step tutorial in the operation of the equipment.

Release and Tracking

Following surgical implantation of the transmitter tag and the recovery period, the fish was released, and tracking commenced immediately. Tracking was conducted from the same 5 m inflatable dinghy that the implantation surgery was performed in. The electronic equipment was assembled in the dingy prior to commencement of tracking (as shown in Figure 3), and disassembled and taken ashore at the end of each tracking session.

The hydrophone was operated by securely fastening it to an oar, which was in-turn then secured to the starboard pontoon of the dingy. The attachment was such that it allowed the hydrophone to be manually rotated 360 degrees when held vertically or 90 degrees when held horizontally. Thus giving maximum directional coverage for detection of acoustic tag signals both in mid-water (vertical position) and within the reef structure (horizontal position).

Once the receiver equipment was set up and the hydrophone was in place, the SRX receiver was set at five gain steps for initial detection of transmitter signals. When a signal was detected, the hydrophone was rotated to find the direction of maximum signal strength (determined either by loudspeaker output or by the signal strength meter on the SRX receiver). The boat was then maneuvered in the direction of the signal whilst the gain was gradually reduced towards zero. A reduction of the gain effectively reduced the maximum detectable range of the tag. Increased signal strength allowed for distance to an individual fish to be determined so that its position could be recorded within a few metres accuracy.

During tracking sessions, the geographical location of the fish was recorded every 15 minutes using visual hand-held compass fixes on at least three known landmarks. In the event that a fish spent a long time at one position a visual check on the habitat was made using snorkel gear or SCUBA. For the more sedentary *C. cruentata*, the fish position was recorded directly onto a field map of the study area. The field map was divided into 20 x 20 m grid squares and
showed landmarks, reef depth contours and subsurface features which allowed easy recognition of the different grid squares. For night tracking, landmarks that remained lit throughout the night were used as reference points for compass and sextant readings. Light sticks attached to marked reef features were also used to help in the estimation of fish positions. The initial use of a hand-held Magellan GPS was discontinued since the error incurred by selective satellite availability proved to be approximately 20 m (a distance that could place a transmitter in a separate grid square).

**Figure 3.** Diagrammatic representation of the assembly arrangement for telemetric receiver equipment used in tracking reef fishes.
Fish were tracked continuously during the first 24 hours after their release. This labour intensive technique involved using two separate teams of researchers, each undertaking six hour shifts.

Subsequently, each of up to 22 fish were tracked for a minimum of four hours every 24 hours. Those four hours were split between two tracking sessions occurring randomly throughout any 24 hour period. Several different fish were often tracked simultaneously on the same frequency, since each transmitter had a unique identification number.

The tracking procedure generally worked well and signals were detected from tags at depths of up to 30 m and over distances of up to 250 m. This was however considerably less than the 500 m horizontal distance given in equipment specifications. Signal strength was found to diminish drastically when fish entered crevasses in the reef.

Simultaneous tracking of several fish on the same transmitter frequency allowed for the investigation of schooling behaviour. However, signal overlap during schooling was high. The acoustic receiver was often unable to identify individual signals and as a result a large number of error readings were displayed. Another constraint was that signal strength appeared to degrade with battery age. In cases where a transmitter tag was activated three to four weeks before another, operation on the same frequency resulted in the signal of the newer tag masking the signal of the older tag even when separated by distances of up to 100 m.

CONCLUSIONS

Effective, non-injurious methods of capture were species-specific, with baited hook and line being most effective for *C. cruentata*, while Z-shaped Antillean traps were more effective for *K. sectatrix*, *L. mahogani* and *C. fulva*, and a hanging cast net was most effective for the more pelagic *C. parrae*.

Clove oil proved to be an effective anaesthetic with the option of immediate release into the marine environment. Full recovery from the anaesthetic occurred in less than three minutes as reported by Anderson et al. (1997).

External attachment of transmitter tags has been used successfully in several studies (e.g. Tulevech and Recksiek 1994, Begout and Legardere 1995, Holland et al. 1996, Josse et al. 1998). However, it was rejected in favour of surgical implantation in this study after laboratory trials of both methods. Surgical implantation into the body cavity has the advantages of greatly reduced tag loss compared with external attachment, allows a better distribution of the weight near the fish’s centre of gravity, and does not create drag forces when the fish is swimming (Mellas et al. 1985). Surgical implantation of transmitter tags into the body cavity proved to be effective in this study for long-term tag attachment in *K. sectatrix* and *C. cruentata*. Only one incident of tag induced mortality was observed out of the 22 fish in which transmitter tags were implanted, although a
number of individuals were lost in the latter stages of the tracking study (i.e. after more than 21 days at large). The probability of stomach and/or swim bladder rupture during tag implantation was significantly decreased by ensuring a period of starvation, and a slow accent to the surface prior to tagging.

Successes with surgical tag implantation have been reported for several fresh water and diadromous species (e.g. Mellas et al. 1985, Moore et al. 1990); and for coral reef-associated species (e.g. Colton et al. 1983, Zeller in press). However, in the reef-associated species, immediate release of fish after surgery resulted in mortality due to predation, and therefore a 2-3 week post-surgery recovery period was suggested to reduce this risk (Zeller in press). The predation-mortality may have occurred because of the relatively substantial incision wounds (2-3 cm long) closed by sutures or staples. In this study we found Super Glue® to be effective in closing the small (1 cm) incision wounds without the aid of sutures, and suggest that fish may be safely released 24 hours after tagging. This surgical procedure avoids the problem reported by Tulevech and Recksiek (1994) of exhausting the battery of short-life transmitter tags during post-surgery recovery. Placement of the transmitter tags in the gut was not considered in this study as a result of reported tag losses of up to 60% with this method (Colton and Alevison 1983, Moser et al. 1996).

The use of a mesh shade cage, as suggested by Moore et al. (1990), proved to be a successful means of short-term fish retention. This eliminates the need for seawater flow-through aquarium facilities while in the field and also allowed in situ testing of the transmitter tag before release of the fish.

Tracking large numbers of acoustically tagged fish simultaneously proved a monumental task in terms of manpower. Furthermore, transmitter tag signal interference was common with so many tagged fish in relatively close proximity. Reliability of the electronic receiver equipment was also a major issue. Working from a small open boat presented significant challenges for keeping the sensitive equipment dry. Mounting the SRX receiver and UUC in a protective box was not always successful in protecting the equipment from rain and sea spray. This emphasises the need for back-ups at this stage of developing technologies and the need for development of waterproof units.
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LITERATURE CITED


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The Age and Growth of Southern Flounder, *Paralichthys lethostigma*, in Louisiana Waters

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**ABSTRACT**

Morphometric measurements and otoliths were collected from southern flounder *Paralichthys lethostigma* from a variety of sample sources from Louisiana waters. Transverse sections of otoliths (n = 1286) were examined and opaque zones validated to form once a year in the winter months. Maximum observed age for males was 4 years while that among females was 8 years. Growth was expressed by Von Bertalanffy's growth model as $L_t = 325.65 \{1 - e^{-1.33(t + 0.01)}\}$ for males and $L_t = 520.14 \{1 - e^{-0.74(t + 0.14)}\}$ for females where $t$ is age in years and $L_t$ is total length at age $t$. Growth was shown to be significantly different between males and females with males displaying a faster growth rate than females but a much smaller $L_{\infty}$.

**KEY WORDS:** Otolith, growth model, southern flounder

**INTRODUCTION**

The southern flounder, *Paralichthys lethostigma*, is the largest member of the family Paralichthyidae (Hensley et al. 1984) in the Gulf of Mexico (Henderson-Arzapalo et al. 1988). Southern flounder are distributed from Albemarle Sound, North Carolina, to the Loxahatchee River on the lower eastern coast of Florida. They are absent on the southern peninsular tip of Florida, but occur in the Caloosahatchee River and up the western coast of Florida and around the Gulf of Mexico to northern Mexico (Hoese and Moore 1998, Manooch 1984).

Southern flounder is an important species throughout Gulf coast region. Commercial and recreational landings of *Paralichthys* along Louisiana coasts for 1997 were estimated to be 94,898 lbs. and 319,607 lbs. (personal communication from the National Marine Fisheries Service, Fisheries Statistics and Economics Division). It is the dominant targeted flatfish in the region and is fished mainly using hook and line, gigging, and by trawl. Commercial landings for southern flounder in Louisiana have fluctuated since the 1950s with the highest landings in the mid-1990s at 0.97 million pounds (Louisiana Department of Wildlife and Fisheries 1998). Substantial restrictions have been put on the southern flounder fishery in recent years leading to a decrease in those landings.
The age structure and longevity of a species is important in formulating a proper fisheries management strategy. It is essential to obtain the age structure of fish populations being harvested to effectively monitor the status of those stocks (Williams and Bedford 1974). Age estimation can be accomplished through the use of otoliths. The sagitta, the largest of the three pairs of teleost otoliths is arrowhead shaped in the flounder. Although growth of the otolith is not uniform along all axes, it grows in a radial fashion forming layers of opaque and translucent zones. These zones, or annuli, are often utilized for age estimation; but in order to do so, the periodicity of the annuli must be validated. Validation of this periodicity of annuli formation is as important in fisheries biology as standardizing solutions or calibrating instruments are in other sciences (Beamish and McFarlane 1983).

The few studies conducted on the age and growth of southern flounder suggest that they are a short-lived species. Nall (1979) reported a maximum age of 10 years using whole otoliths, but did not validate his methods. Stokes (1977) used whole otoliths and reported a maximum age of five years for southern flounder. Wenner et al. (1990) used whole otoliths and reported a maximum age of seven years using length frequency data and marginal increment analysis to validate the use of whole otoliths. Interpretation of length frequency data for validation may be suspect, however, because overlapping size classes of cohorts beyond two years (Ross 1988) can complicate age-class designations. Music and Pafford (1984) assigned a maximum age of six years using scales. They attempted to use otolith counts to document the validity of increment counts on scales, although it is unclear whether they used whole or sectioned otoliths.

It appears that a number of flatfish exhibit sexual dimorphism in age and growth rates. Solomon et al. (1987) found that the growth of male and female Limanda yokohamae differed; females exhibited a higher growth rate than males of the same age. The same observation was reported for stone flounder, Kareius bicoloratus (Uehara and Shimizu 1996) with females reaching a greater size and living longer. Lux (1973) reported that female winter flounder, Pleuronectes americanus, grew faster than males after the second year. Lux and Nichy (1969) also stated a similar pattern of growth in the New England yellowtail flounder, Limanda ferruginea. Gilbert (1986) reported that there is evidence that Paralichthys females reach a larger size than males. Stokes (1977) stated that male southern flounder grew slower than females and did not exceed 320 mm total length where Miller et al. (1991) reported a difference in maximum size between male and female southern flounder with male maximum size at only 68% of females at the same age. Therefore, it may be necessary to generate separate growth curves by sex to properly manage the fishery.
The objectives of this study are to describe the age and growth of southern flounder in Louisiana waters through examination of sagittal otoliths. Growth will be modeled using the Von Bertalanffy growth model. Males and females will be compared to determine if southern flounder displays sexual dimorphism in age and growth and if separate models are required.

METHODS AND MATERIALS

Southern flounder used in this study came from a variety of sample sources from Louisiana waters or the Gulf of Mexico off the coast of Louisiana. Multiple sources provided the most reasonable cross section of the estuaries near shore population. Samples were collected at commercial docks in Grand Isle and Leeville, LA from October 1997 to January 1998 (n = 146). The Louisiana Department of Wildlife and Fisheries at the St. Amant Marine Laboratory also collected samples from Grand Terre with the use of a pound net during November and December 1997 (n = 125). An existing flounder data set compiled by Dr. Bruce Thompson of the Coastal Fisheries Institute of Louisiana State University was also used in the analysis (n = 1,134). This data set contains samples from 1987 to 1998 from a variety of sources with the large majority from commercial fish docks (n = 565) and hook and line fishing rodeo tournaments (n = 421).

Fish were weighed (mg), measured (total and standard lengths in mm), sex determined, and otoliths removed. Otoliths were stored in ethyl alcohol to preserve until they were returned to the laboratory for analysis. Otoliths were cleaned of any extraneous tissue and air dried for at least twenty-four hours. Right and left otoliths were then weighed (± 0.01 mg).

A length – weight regression was calculated on log_{10} transformed data using the model log_{10} (weight, g) = slope log_{10} (TL, mm) + intercept. Linear regressions were also calculated for otolith weight (mg) – age using the model otolith weight = age (slope) + intercept. Analysis of Covariance was used to compare sexes for both regressions.

Fish were aged through processing and analysis of 1,286 sagittal otoliths. A number of fish in the data set were not aged due to missing or broken sagittae. Due to the morphological differences between right and left sagittae, The left was chosen for embedding. Otoliths were embedded in a mixture of five parts araldite 8702 epoxy resin to one part hardener 8,700 and left to harden for twenty-four hours. Two transverse sections near the core of the otolith were taken and glued on to glass slides. The better of two sections was polished and inscribed with an identification number, and aged. Sections were read along the medial side of the section along the ventral side of the sulcus groove (Figure 1).

Reader variability was also evaluated; otoliths were viewed and aged by of two independent readers without the knowledge of the date of capture or sample source. Ages were assigned based on annulus count and edge condition.
Edge condition was recorded as opaque or translucent using the criteria of Beckman et al. (1991). Ages were assigned based on a January 1 birth date from Wenner et al. (1990) and data from this study.

![Image of a transverse section near the core of a southern flounder otolith. Arrows point to opaque zones or "annuli" counted for age estimation.]

Figure 1. Photomicrograph of a transverse section near the core of a southern flounder otolith. Arrows point to opaque zones or "annuli" counted for age estimation.

Marginal increment analysis and a plot of edge condition by month were used to determine the periodicity of annulus formation in southern flounder otoliths. In addition, length-frequency distributions were plotted by month for young of the year (YOY) and yearlings with and without opaque zones on their otoliths to determine age of first annulus formation.

Length frequency distributions were examined for males and females. Distributions were plotted in 20mm intervals. A Komolgorov-Smirnov two-sample test (Tate and Clelland 1957) was used to test for differences between sexes.

Sex specific VonBertalanffy growth equations were derived from total lengths using nonlinear regression (SAS Institute Inc., 1985) based on the formula:

\[ L_t = L_\infty \{1 - e^{-k(t-t_0)}}\]

where \( t \) is age in years, and \( L_t \) is total length at age \( t \), \( L_\infty \) is the theoretical maximum length, \( k \) is the growth coefficient, and \( t_0 \) is age at which length is zero. Individual fish were not included in the analysis if age or length data was not available. Each model also included 22 unsexed juveniles to provide points at the lower end of the curve. These juveniles ranged in size from 68 mm to 214 mm total length.

The resultant models fitting parameters for both males and females were then combined into one full six-parameter model and compared to a reduced model on the pooled data in which sex was not considered. A likelihood ratio test of the six-parameter and the pooled data models was used to test for differences in the models. Plots of residuals were used to test for normality of the data.

RESULTS

Fourteen hundred and five southern flounders (139 males, 1,201 females, 22 juveniles, and 43 unsexed) were sampled from August 1987 through January 1998. Fish came from numerous sources including commercial and recreational catches from the northern Gulf of Mexico, Louisiana Offshore Oil Port (LOOP) trawls, and the Louisiana Department of Wildlife and Fisheries which provided samples of flounder taken from Barataria Bay. Because not all parameters could be measured for each fish, the numbers of fish included in the different analyses vary.

Total length (TL) frequency distributions plotted by sex were significantly different (p < 0.05) (Figure 2). Males ranged in size from 68 mm to 414 mm TL. Males were most abundant at the 280 mm interval with fifty-three percent of all males ranging from 260 mm to 300 mm TL. Females were more abundant at much larger sizes ranging from 189 mm to 764 mm TL and were most abundant at the 390 mm interval. Fifty-three percent of all females ranged from 380 mm to 440 mm TL. As expected, males also had a much lower range in body weight than females ranging from 19 g to 936 g. Females ranged in weight from 61 g to 5,953 g.

Regression equations of log_{10} transformed data were calculated to predict total weight at total length for males and females. Analysis of covariance (ANCOVA) showed no statistical difference between sexes (p > 0.05) for slopes; p > 0.05 for intercepts). Therefore, a combined length-weight regression was fit for males and females:

\[ \log_{10} \text{weight (g)} = 3.21 \log_{10}(\text{TL, mm}) - 5.46 \quad (r^2 = 0.98; \ n = 1236) \]

The slope of 3.21 was significantly different then 3 (p < 0.0001).

Opaque rings are easily distinguishable on both the ventral and dorsal sides of the sulcus groove in cross section of southern flounder otoliths. Marginal increment analysis and a plot of otolith edge condition were used to determine the seasonal periodicity of annulus formation. Opaque margins were found in fish caught from the months of January and May and the margins of nearly all samples taken from August through December were translucent. The plot of edge condition corresponded with marginal increment analysis showing the progression of opaque zones from January through May and translucent zones from March through December. Length-frequency distributions for young of the
year (YOY) and yearlings indicated first annulus formation as early as 200 mm in length and up to 330 mm. The first annulus appeared on YOY otoliths between the months of January and March.

Figure 2. Length frequency distribution of male, female and juvenile southern flounder caught from 1987 to 1998. Males range in size from 68mm to 414mm and are most abundant at 280mm interval. Females range in size from 189mm to 764mm and are most abundant at 390mm interval. Juveniles range from 68 to 309mm and are most abundant at 100mm interval. Males are shown in shaded pattern, females are shown in white, and juveniles are shown in solid black.
Ages were assigned through analysis of 1,286 otoliths. Seven otoliths were excluded from the analysis due to lack of agreement between the two readers. Each of the seven age estimates differed by one year. The two readers agreed on all other otolith annulus counts (N = 1279) or 99.5% of age estimates. The mean coefficient of variation (V) was 0.0011. The mean index of precision (D) was 0.00081 indicating an average error of 0.08 annuli per one hundred counts (Beckman 1989).

A large number of the fish collected were estimated to be two years of age (Figure 3). Forty-six percent of females and thirty-six percent of males fell into this age class. The oldest female was 8.5 years in age and the oldest male was found to be 4.13 years.

![Graph showing age frequency distribution for male and female southern flounder sampled from 1987 through 1998. Males reached a maximum age of 4 years and females reached a maximum age of 8.5 years.](image)

**Figure 3.** Age frequency distribution for male and female southern flounder sampled from 1987 through 1998. Males reached a maximum age of 4 years and females reached a maximum age of 8.5 years.

Data were fit to a Von Bertalanffy growth model and compared. A likelihood ratio test indicated that there was a significant difference between a full six-parameter Von Bertalanffy growth model and the pooled data growth model (p <0.0001). Therefore, separate growth models were fit for each sex (Figure 4). The Von Bertalanffy growth models derived from total lengths are:

- Male: \( L_t = 325.65(1 - e^{-1.33(t + 0.01)}) \) \( (r^2 = 0.68) \)
- Female: \( L_t = 520.14(1 - e^{-0.74(t + 0.14)}) \) \( (r^2 = 0.52) \)

Plots of residuals indicated normal distribution of the data.
**Figure 4.** Von Bertalanffy growth models fit for male and female southern flounder sampled from 1987 through 1998. Each model includes 22 unsexed individuals ranging from 68 mm to 214 mm. Gray crosses represent females and hollow circles represent males.

**DISCUSSION**

The hypothesis that southern flounder display sexual dimorphism in age and growth is supported by length frequency distributions for males and females. Females had a distribution mode of 390 mm and reached up to 764 mm TL while males had a mode of only 280 mm and reached a maximum size of 414 mm. Shepard (1986) reported similar modal lengths with female mode at 358 mm and a male mode of 247 mm. Wenner et al. (1990) did not report modal lengths by sex but stated that forty-four percent of aged females were greater than 300 mm and few aged males were above 300 mm.

The length-weight regression of \( \log_{10} \) transformed data indicated the slope was significantly different from 3.0 \( (p = 0.0001) \) indicating growth is allometric; weight of the fish increases in relation to its size as length increases. Length-weight regressions on southern flounder produced slopes of 3.14 in South Carolina, 3.09 in Georgia, 3.10 in Florida, and 3.13 in Texas. These slopes were not tested to see if they were significantly different from 3 so it is unclear if there is significant allometric growth in southern flounder from different regions. The higher slope suggests Louisiana southern flounder are more robust than those in previous studies.

Marginal increment analysis and plot of edge condition indicate that one opaque zone is deposited on the otolith between the months of January and May and that annuli on sectioned otoliths may be utilized for accurate age estimation (Barger

1985). A peak of fifty-six percent of individuals with an opaque zone at the growing edge of the otolith is consistent with that of Beckman and Wilson (1995). In a review of 49 studies on north latitude temperate populations using sectioned otoliths, Beckman and Wilson (1995) reported a mean percentage of 65% of individuals with an opaque zone at the growing edge of the otolith.

Analysis of sectioned otoliths of young of the year (YOY) and yearling southern flounder indicated they formed their first annulus as early as 200 mm and up to 330 mm TL. The first annulus began to form in January with all yearlings completing their first annulus by March. These findings are consistent with Powell (1982) who found first annulus formation in the summer flounder to take place between January and March. Stokes (1977) also reported lengths of up to 300 mm by first annulus formation in southern flounder. Wenner et al. (1990) detected no delayed or “lost” first annulus. Therefore this variability in size at first annulus formation is most likely due to differential growth among individuals, which Fitzhugh et al. (1996) found accounted for the broad dispersion of lengths occurring in the first year.

Females live longer than males. Females reached a maximum age of just over eight years while males reached only four years. These findings are close to Wenner et al. (1990) who reported a maximum age of seven years for females and three years for males. Although they employed the use of whole otoliths, their validation techniques of evaluating the edge condition of whole otoliths were similar to this study producing similar maximum age estimates for each sex. Music and Pafford’s (1984) maximum age of six years for a female came from a data set of only 198 fish. It is not unexpected that they found a lower maximum age considering that only 11 out of 1,286 (0.009%) aged fish in our data set were five years or older. Nall (1977) reported a maximum female age of 10 years. However, this age estimate seems unlikely when taking into account his invalidated use of whole otoliths. Williams and Bedford (1974) stated that the main source of difficulty in using whole otoliths to age fish is the presence of secondary checks or rings that could be perceived as additional annuli and thus increase your age estimation. In all studies on southern flounder cited here, males have never been aged above three years.

Growth parameters from sex specific curves suggest rapid growth to age two for males and to age three for females. Maximum theoretical size was calculated at 326 mm for males and 520 mm for females. This study predicts more rapid growth and smaller maximum sizes for males and females than reported on southern flounder from South Carolina by Wenner et al. (1990). These parameters and the contrast of the Louisiana and world record suggest that Southern flounder occupying the cold temperate waters of the Atlantic appear to reach greater maximum sizes than those in the warm temperate waters of the Gulf of Mexico. These differences in sizes between the Atlantic and Gulf of
Mexico populations suggest zoogeographic variation in population dynamics of southern flounder. Such variation has been suggested for red drum (Matlock 1987) and Atlantic croaker (White and Chittenden 1977).

These sex specific growth models included 22 unsexed young of the year fish ranging from 68 mm to 214 mm total length. Music and Pafford (1984) stated that sex could not be determined before 130 mm for females or 232 mm for males. Stokes (1977), however, reported that sexual differentiation was not possible for either sex before 170 mm. Juvenile southern flounder have exhibited a capacity for high growth rate relative to other fishes (Fitzhugh 1993). The addition of unsexed juveniles into the growth models may account for the high growth coefficient (k).

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