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POSGRADO EN BIOCIENCIAS

ESTUDIOS NUTRICIONALES ENCAMINADOS AL DESARROLLO DE ALIMENTOS BALANCEADOS PARA EL CULTIVO COMERCIAL DE LA CURVINA GOLFINA (*Cynoscion othonopterus*), DE LA CURVINA DE ALETA CORTA (*Cynoscion parvipinnis*) Y DE LA TOTOABA (*Totoaba macdonaldi*).

TESIS

Para obtener el grado de:

DOCTOR EN BIOCIENCIAS

Presenta:

CHRISTIAN MINJAREZ OSORIO

Hermosillo, Sonora, México **15 de Diciembre de 2015**

Universidad de Sonora

Repositorio Institucional UNISON

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APROBACIÓN

Los miembros del comité designado para revisar la tesis intitulada "ESTUDIOS NUTRICIONALES ENCAMINADOS AL DESARROLLO DE ALIMENTOS BALANCEADOS PARA EL CULTIVO COMERCIAL DE LA CURVINA GOLFINA (*Cynoscion othonopterus*), DE LA CURVINA DE ALETA CORTA (*Cynoscion parvipinnis*) Y DE LA TOTOABA (*Totoaba macdonaldi*)" presentada por Christian Minjarez Osorio, la han encontrado satisfactoria y recomiendan que sea aceptada como requisito parcial para obtener el grado de Doctor en Biociencias con especialidad en acuacultura.

Dr. Martín Pérez Velázquez
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Dr. Gerardo Navarro García
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Dr. Alejandro Varela Romero
Sinodal interno

Dr. Delbert M. Gatlin III
Sinodal externo

DEDICATORIA

A mis padres:

Luis Alejandro Minjarez Urrea y María del Carmen Osorio Encinas

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RESUMEN

En México, el cultivo de peces marinos luce como una actividad prometedora. Sin embargo, el desconocimiento de los requerimientos nutricionales de estos organismos ha evitado que la actividad se desarrolle plenamente, por lo que continúan los esfuerzos por elucidar estos aspectos en especies con potencial en acuacultura. Actualmente se evalúa el cultivo de tres especies de esciénidos del Golfo de California, la curvina golfina, *Cynoscion othonopterus*, la totoaba, *Totoaba macdonaldi* y la curvina de aleta corta, *C. parvipinnis*. Sin embargo, la falta de información concerniente a sus requerimientos nutricionales ha impedido la elaboración alimentos específicos, por lo que el objetivo de la presente investigación fue realizar estudios nutricionales encaminados al desarrollo de alimentos balanceados para su cultivo comercial. Se realizaron tres experimentos con duración de 56 días cada uno utilizando dos sistemas de recirculación donde se mantuvieron en óptimas condiciones las variables fisicoquímicas del agua de cultivo. Al final de los experimentos, se observó que la curvina golfina alimentada con una dieta con un contenido de 11% de grasa cruda mostró los mejores índices de crecimiento y utilización del alimento, mientras que en la totoaba no se observaron diferencias significativas en crecimiento utilizando dietas con contenidos de grasa cruda desde 8 hasta 22%, con excepción del nivel de 14%, en donde los organismos tuvieron un menor desempeño que aquellos que recibieron un nivel de 20%. Adicionalmente, los tejidos analizados mostraron mayor acumulación de lípido a medida que se incrementó el nivel de lípido dietario. Para la curvina de aleta corta, se logró sustituir satisfactoriamente hasta el 75% de harina de pescado utilizando concentrado de proteína de soya y de maíz. Se concluye que el requerimiento de lípido dietario de la curvina golfina es de 11.4%, mientras que la totoaba puede tolerar desde 8 hasta 22% de inclusión de lípido dietario sin afectar su crecimiento, aunque sí influyó en la composición proximal de sus tejidos. Finalmente, la curvina de aleta corta presentó una amplia tolerancia a la sustitución de harina de pescado con harinas de origen vegetal. En su conjunto, estos hallazgos representan un avance significativo en el desarrollo de alimentos balanceados específicos para el cultivo comercial de estas especies.

ABSTRACT

Due to the significant losses in shrimp production, Mexican aquaculturists are interested in the diversification of aquacultural species. In this regard, marine fish culture rises as a promising option. Nevertheless, the lack of knowledge of nutritional requirements is a significant constraint to the development of this activity. For this reason, continued efforts are being made to elucidate nutritional aspects of marine fish species with potential for aquaculture. Currently, three sciaenid species from Gulf of California are being evaluated as candidates for aquaculture: the Gulf corvina, *C. othonotperus,* totoaba, *T. macdonaldi*, and the shortfin corvina, *C. parvipinnis*. However, lack of knowledge of nutritional requirements has prevented the development of commercial balanced feeds that suit the specific needs of these species. Therefore, the objective of the present study was to conduct nutritional studies aiming at the development of commercial aquafeeds for the profitable culture of these species. Three 56-day experiments were conducted under standardized conditions in indoor tanks operated as recirculating culture systems with optimal water quality At the end of the experiments, Gulf corvina fed a diet containing 11% of crude fat (CF) showed the best performance values, in terms of growth and feed utilization, while totoaba did not show significant differences when fed with diets containing CF from 8 to 22%, except for fish fed 14% CF, which had lower growth response than fish receiving 20% CF. In addition, body lipid content increased with increasing dietary lipid. On the other hand, it was observed that soybean and corn protein concentrates can replace up to 75% of fishmeal protein in the diet of the shortfin corvina. According to these results, the dietary lipid requirement of Gulf corvina was estimated to be 11.4%, while totoaba can be fed diets containing from 8 to 22% CF without compromising fish growth performance, although its body proximate composition was significantly influenced by dietary lipid. Finally, shortfin corvina exhibited a wide tolerance to plant-based protein sources as replacements for fishmeal protein. As a whole, these findings represent significant advancements in the development of species-specific formulated feeds for the commercial culture of these fish species.

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I. INTRODUCCIÓN Y ANTECEDENTES

I.1. Panorama actual de la acuacultura en México

México se ha consolidado como un importante productor acuícola en Latinoamérica, siendo el cultivo de camarón su principal referente en cuanto a producción, la cual alcanzó un máximo histórico de 132,788 toneladas (t) en el año 2008. Sin embargo, el constante ataque de patógenos como virus, hongos, bacterias y protozoarios, causaron que la producción disminuyera drásticamente a 107,893 t en el 2011 (CESASIN, 2012; FAO, 2010). Principalmente por esta razón y en conjunto con otros factores, ha surgido entre los productores un gran interés en diversificar las especies de cultivo en el país, con el fin de generar actividades rentables adicionales al cultivo de camarón.

A este respecto, el cultivo de peces marinos luce como una opción interesante, tanto económica como ecológicamente, ya que esta actividad podría disminuir en gran medida la presión pesquera a la que actualmente se encuentran sometidas muchas de las especies de peces de interés comercial. Según documentos oficiales de la FAO (2014), el volumen de producción de peces marinos mediante acuacultura alcanzó los 5.6 millones de t en el año 2012, presentando una tasa de crecimiento anual de 7.6%. Lo anterior representó cerca del 12% de la producción total de peces cultivados a nivel mundial, lo cual generó un aporte aproximado de 23.5 millones de dólares estadounidenses (USD). Los datos anteriores indican tanto la gran aceptación de los peces marinos en el mercado, debido a su rápida expansión, como la rentabilidad de su cultivo comercial, ya que su valor unitario es mayor en comparación con el valor generado por los peces dulceacuícolas.

En México, algunas instituciones de educación superior y centros de investigación principalmente localizados en la región noroeste y sureste del país, como lo son el Centro de Investigación Científica y de Educación Superior (CICESE) en Ensenada, Baja California Norte, el Centro de Investigaciones Biológicas del Noroeste (CIBNOR) unidad La Paz, Baja

California Sur, el Centro de Reproducción de Especies Marinas del Estado de Sonora (CREMES), el Departamento de Investigaciones Científicas y Tecnológicas de la Universidad de Sonora (DICTUS) unidad Bahía de Kino, el Centro de Investigación en Alimentación y Desarrollo (CIAD) unidad Mazatlán, la Universidad Juárez Autónoma de Tabasco (UJAT), entre otras, han evaluado diversas especies de peces marinos para su cultivo y producción a nivel comercial. Lamentablemente, en la actualidad son pocas las especies a las que se han logrado llevar a este nivel de producción (Chávez-Sánchez *et al.*, 2008; Zacarias-Soto *et al.*, 2006). Lo anterior se reflejó claramente en las escasas 1,629.8 t que registró México como producción de peces marinos en el año 2012 (CONAPESCA, 2012), una cifra muy baja si la comparamos con los grandes países productores como China, Noruega, Chile y Brasil, entre otros (FAO, 2014). Por esta razón se deben continuar los esfuerzos en la evaluación del potencial de cultivo de especies nativas de peces marinos y consiguientemente, evaluar sus requerimientos nutricionales con el fin de formular alimentos balanceados, facilitando de esta forma su cultivo a escala comercial. De acuerdo con lo anterior, un grupo de peces marinos que recientemente ha generado un gran interés debido a sus características biológicas deseables en la acuacultura, es el de los esciénidos, ya que a este grupo pertenecen algunas especies de gran importancia pesquera y comercial del noroeste de México. El grupo de los esciénidos es un grupo de peces muy diverso, en su mayoría marinos, los cuales se encuentran representados en 70 géneros y alrededor de 270 especies distribuidas a través de las regiones templadas y tropicales alrededor del mundo (Farias *et al.*, 2006; Nelson, 1994). Los esciénidos, comúnmente conocidos como curvinas, tambores o roncadores (por el sonido tan característico que realizan por acción de su vejiga natatoria bien desarrollada y músculos especializados), habitan zonas someras con fondos arenosos y/o lodosos, aunque algunos estudios demuestran que pueden frecuentar áreas mixohalinas, principalmente con fines reproductivos (CITES, 2005; Jiménez *et al.*, 2005; Paredes *et al.*, 2010).

La pesquería de esciénidos representa una de las actividades de mayor importancia comercial dentro de los recursos demersales a nivel mundial y diversos estudios han demostrado que algunas especies de esciénidos se pueden adaptar a condiciones de cautiverio e incluso ser cultivadas exitosamente (Chao y Musick, 1977; Parades *et al.*, 2010; Vergara *et al.*, 2007; Villamer, 1972). El interés mundial en el cultivo de esciénidos inició cuando se

logró madurar, reproducir y criar exitosamente a la curvina roja, también conocida como curvina ocelada, *Sciaenops ocellatus*, bajo condiciones controladas de acuacultura. Estos estudios sirvieron como base para aplicar técnicas similares en otras especies de esciénidos, lo que contribuyó en gran medida a que la producción por acuacultura de este grupo de peces se incrementara significativamente en años recientes (Holt, 2000).

I.2. Los esciénidos: un grupo de gran importancia en la acuacultura

De acuerdo con cifras oficiales de la FAO (2012), en el año 2010 el grupo de los esciénidos fueron el tercer grupo de peces marinos que más se cultivaron a nivel mundial, con una producción cercana a las 150,000 t (Figura 1), sólo detrás del grupo de peces marinos sin identificar y del grupo que abarca a los jureles, pámpanos y caballas. En México, el grupo de los esciénidos se encuentra representado por 15 géneros y tan sólo en el Golfo de California, se han descrito alrededor de 30 especies, algunas de ellas con gran potencial de cultivo (Castro-Aguirre, 1999; Van der Heiden, 1985). Entre las especies de esciénidos que actualmente están siendo evaluadas por diversos centros de investigación localizados principalmente en el noroeste de México se encuentran la curvina de aleta corta *C. parvipinnis*, la curvina golfina *C. othonotperus* y la totoaba *T. macdonaldi*. La curvina de aleta corta es una especie carnívora, la cual habita en aguas costeras y suelos arenosos a través Golfo de California hasta el estado de California en E.U.A (Figura 2) (Chao, 1995). A su vez, la curvina golfina y la totoaba son especies endémicas del Golfo de California, las cuales presentan hábitos demersales y un amplio espectro trófico de alimentación. Algunos reportes de captura señalan que la totoaba puede alcanzar los dos metros de longitud y cerca de los 130 kg de peso (Figura 3), mientras que la curvina golfina puede alcanzar hasta 92 cm de longitud (Figura 4) (CITES, 2005; Parades *et al.*, 2010; Román-Rodríguez, 1990). Una de las principales características de estas especies de esciénidos es la realización de un viaje migratorio, el cual llevan a cabo en el mes de octubre, finalizando hasta junio del siguiente ciclo, con la finalidad de alcanzar los lugares de reproducción ubicados en el Delta del Río Colorado. En este sitio, la curvina golfina, pero no la totoaba, es capturada en grandes cantidades por embarcaciones pesqueras de la región (CITES, 2005; Cudney y Turk, 1998; Encinas-Rivera, 2008; Parades *et al.*, 2010; Román-Rodríguez, 2000; Rowell *et al.*, 2005).

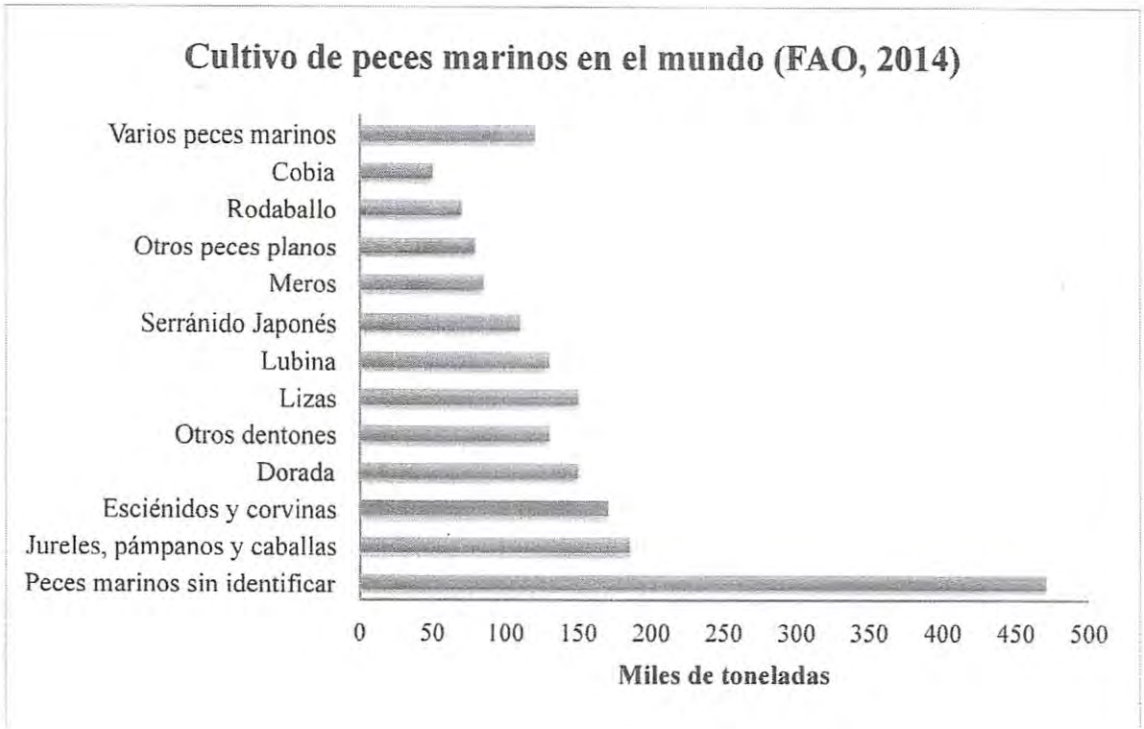

Figura 1. Producción de grupos de peces marinos a nivel mundial (FAO, 2012).

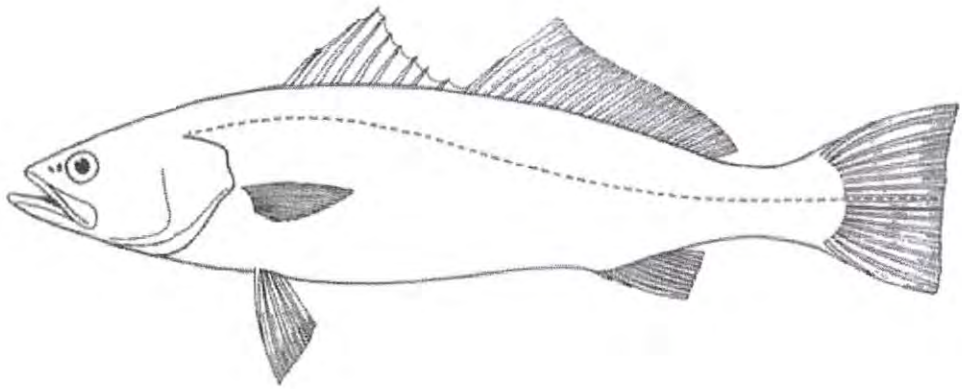

Figura 2. Morfología externa de la curvina de aleta corta, *C. parvipinnis.* (Extraído de Chao, 1995).

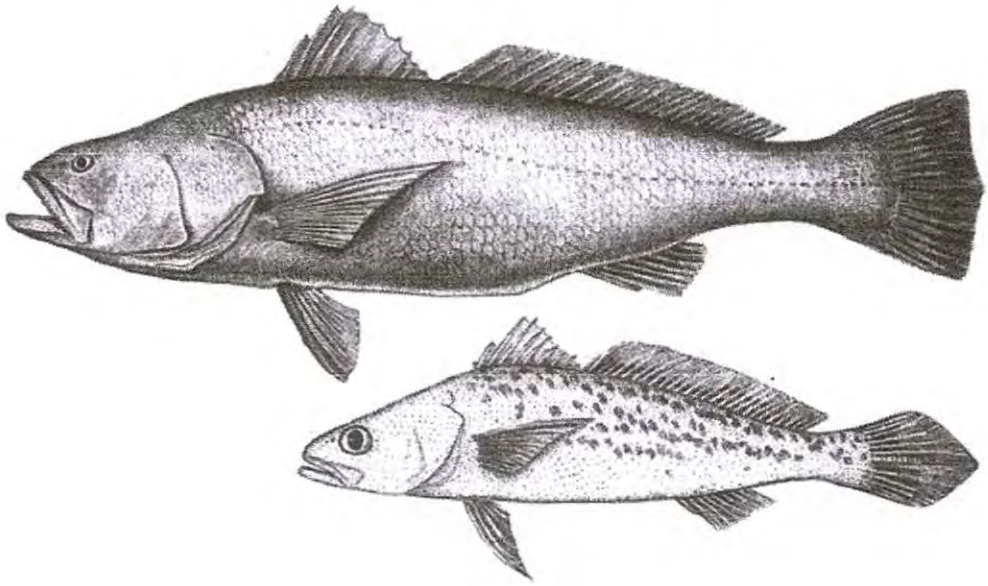

Figura 3. Morfología externa de la totoaba, *T. macdonaldi* en su fase juvenil y adulta. (Extraído de Chao, 1995).

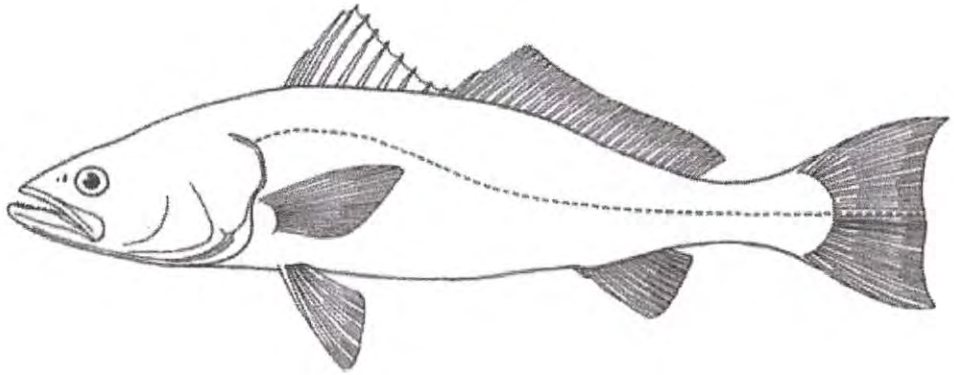

Figura 4. Morfología externa de la curvina golfina *C. othonopterus.* (Extraído de Chao, 1995).

I.3. Situación actual de la pesquería de esciénidos en México

La pesquería de curvinas o roncadores es una de las actividades más importantes de la región noroeste de México, siendo Sonora el estado que aportó el mayor volumen promedio de captura, que fue de 3,104 t durante el periodo 2000-2007 y lo que, a su vez, representó cerca del 90% del volumen total de captura en la zona (Carta Nacional Pesquera, 2012). Sin embargo, la sobrepesca, la pesca en época reproductiva y las alteraciones al curso del Río Colorado han afectado significativamente a las poblaciones naturales de los peces marinos que habitan en la región, incluyendo las de los esciénidos (Carbajal *et al.*, 1996; CONANP, 2004; Flanagan y Hendrickson, 1976).

I.3.1. La curvina golfina *C. othonopterus* y curvina de aleta corta *C. parvipinnis*

La curvina golfina ha sido una de las especies mayormente afectadas tanto por la acción de la actividad antropogénica en la región del Golfo de California, como por la sobrepesca de esta especie en época reproductiva. Una vez que la especie ha completado el proceso de migración hacia el Alto Golfo de California, los organismos comienzan el proceso de alimentación y con ello, el fin del proceso de maduración. De esta forma, a finales del mes de marzo se observa un gran porcentaje de organismos maduros y ya para el mes de abril se observa un gran pico de desove (Román-Rodríguez, 1990). Durante el proceso reproductivo, la curvina golfina tiende a formar agregaciones reproductivas en un espacio reducido y es en este momento, en donde las embarcaciones las capturan con relativa facilidad, impidiendo que muchos de los organismos se reproduzcan. El esfuerzo pesquero para esta especie rebasa las 300 embarcaciones por día, capturando cada embarcación entre los 300 y los 500 kg de producto por viaje, dependiendo de la marea (Paredes *et al.*, 2010).

Existen reportes históricos de que la especie fue fuertemente explotada de 1917 a 1940, sin embargo su pesquería colapsó en la década de 1960, lo cual se ha relacionado específicamente a las modificaciones al flujo del Río Colorado y a la sobrepesca (Román-Rodríguez, 2000). De acuerdo a los reportes pesqueros existentes, la pesquería de la curvina golfina reapareció en la década de 1990 y desde entonces las cifras de captura han fluctuado

de las 1,500 t hasta las 3,700 t presentando su máximo histórico en el año 2002 con 4,300 t (Aragón-Noriega, 2014). Sin embargo, la captura de la especie ha fluctuado desde entonces con tendencia a disminuir, es por esta razón principalmente, que la pesquería de la curvina golfina se reguló en la región del Alto Golfo de California bajo la norma oficial mexicana NOM-063-PESC-2005, la cual define aspectos relacionados con su pesquería, como la talla mínima de captura, tamaño de malla y temporada de pesca (Solana-Sansores *et al.*, 2012). Aún con estas medidas de protección anteriormente descritas, la especie se enfrenta a diversas problemáticas ecológicas en torno a su pesquería, como se revisa a continuación (Paredes *et al.*, 2010):

a) La época de captura de la curvina golfina coincide con el inicio y transcurso de la cuaresma lo que aumenta su demanda y con ello, el esfuerzo pesquero sobre la especie.
b) La rápida disminución del precio, el cual en cuestión de horas puede ir desde los 25 pesos hasta los dos pesos dependiendo de la demanda.
c) La escasa vigilancia en la zona núcleo (lugar donde se encuentra prohibida la pesca de la curvina golfina) ha incrementado la tasa de pesca furtiva en esta región impidiendo de esta forma que los organismos maduros alcancen a reproducirse.

Con el fin aliviar esta problemática, el gobierno mexicano ha tratado de concientizar a los pescadores de la región mediante la creación de programas, con la finalidad de preservar este recurso pesquero, el cual sin duda es uno de los pilares económicos del Golfo de California.

Para otras especies de esciénidos de la región, como es el caso de la curvina de aleta corta la información ecológica, reproductiva y pesquera es muy limitada. La Carta Nacional Pesquera (2012) señala que si bien no se han realizado los estudios necesarios para evaluar sus poblaciones naturales, el estado su pesquería, al igual que para otras especies, es catalogada como al máximo sustentable, recomendando llevar a cabo los estudios poblacionales correspondientes. Sin embargo, algunos estudios coinciden en que cada año se observa un volumen menor de captura de estas especies con respecto a ciclos de pesca anteriores, lo cual podría indicar un efecto nocivo en sus poblaciones naturales (CONANP, 2004; Paredes *et al.*, 2010).

I.3.2. Pesquería y veda de la totoaba, *T. macdonaldi*

La pesquería de totoaba fue una de las actividades económicas más importantes en el noroeste de México a principios de 1900, siendo principalmente capturada para la extracción de su vejiga natatoria comúnmente conocida como "buche", el cual era principalmente exportado, en su mayoría a Estados Unidos y Asia (CITES, 2005; Pedrín-Osuna *et al.*, 2001). Los artes de pesca utilizados normalmente para capturar a la totoaba iban desde la utilización de redes de un grosor de luz de malla aproximado de 10 pulgadas y una longitud de 200 brazadas hasta la utilización de anzuelos y dinamita. Durante la noche los pescadores dejaban "tendidas" las redes en los lugares donde se tenía conocimiento del paso de los organismos, mientras que durante el día la especie era rastreada a través de los lugares frecuentados por la totoaba o bien, por la presencia de "comederos", los cuales eran fácilmente detectados por la abundante presencia de aves marinas (Arvizu y Chávez, 1972).

La totoaba, al igual que la curvina golfina se caracteriza tanto por la realización de un viaje migratorio a través de las zonas de reproducción encontradas través de la región del Alto Golfo de California como en la formación de agregaciones reproductivas, época donde se encuentra susceptible para ser capturada fácilmente mediante la utilización de redes agalleras (Paredes *et al.*, 2010). Según algunos registros disponibles, la pesquería de totoaba alcanzó un máximo histórico de captura cercano a las 2,261 t en 1942, disminuyendo significativamente a tan sólo 59 t en 1975 (Lercari y Chávez, 2007). Es por lo anterior que el gobierno mexicano adoptó diferentes medidas de protección para la especie. La primera de ellas se llevó a cabo de 1940 a 1955 con el fin de proteger su reproducción en el Delta del Río Colorado. En 1974 se establecieron diversas zonas de protección a través del Alto Golfo de California donde se prohibió su pesca, entre las zonas se encontraban la desembocadura del Río Santa Clara y el Faro del Cerro el Machorro, en Sonora y la Reserva Punta Zacatoza, en Baja California Norte. Finalmente, en 1975 el gobierno mexicano vedó definitivamente la pesquería de totoaba en todo el Golfo de California bajo la norma oficial mexicana NOM-059-SEMARNAT. Adicionalmente, en 1991 se declaró como especie en peligro de extinción, y en 1992, se prohibió la utilización de redes con una luz de malla de 10 pulgadas para tratar de evitar capturas accidentales (Pedrín-Osuna *et al.*, 2001). Pese a los esfuerzos realizados por el

gobierno mexicano a favor de la conservación de la especie, existe la impresión generalizada de que las medidas anteriormente descritas no han sido tan efectivas o no han tenido el efecto deseado a favor de la recuperación de las poblaciones naturales de totoaba. Lo anterior, se puede atribuir principalmente a la pesca clandestina, cuyo volumen de captura se ha estimado en 400 t por temporada, a la pesca incidental de juveniles por barcos camaroneros y pesca deportiva y a la reducción del caudal del Río Colorado, lo cual ha afectado las zonas de crianza de la especie, sin embargo no se han realizado los estudios necesarios para relacionar este factor con la disminución de sus poblaciones naturales (Flanagan y Hendrickson, 1975; Martínez-Delgado y Corona-García, 1992; Molina *et al.*, 1988; Pedrín-Osuna *et al.*, 2001). Sin embargo, desde que la especie se declaró en veda, hace más de 40 años, no se han realizado estudios ecológicos necesarios para determinar el estado y salud de sus poblaciones naturales, por lo que el estado de amenaza actual podría resultar inadecuado (Valenzuela-Quiñones, 2014).

I.4. Cultivo de totoaba *T. macdonaldi*: una medida de protección y aprovechamiento

Con el fin de coadyuvar a la recuperación total de las poblaciones de la totoaba, el gobierno mexicano lanzó una iniciativa de cultivo experimental de totoaba con fines de repoblamiento en el medio natural. El proyecto estuvo a cargo de la Unidad en Biotecnología de la Facultad de Ciencias Marinas, Unidad Ensenada, donde después de 20 años de investigar el ciclo reproductivo de la especie bajo condiciones de acuacultura, lograron más de dos ciclos reproductivos consecutivos por hembra, lo cual es de gran importancia, ya que hasta ese momento se consideraba a la totoaba como un reproductor anual. Como resultado de estas investigaciones, se han obtenido una gran cantidad de huevos fértiles por puesta, ya que año con año se ha llevado de manera más eficiente el proceso, lo que ha permitido que una gran parte de la producción se destine para su repoblamiento. Así mismo, se han realizado cultivos experimentales de la especie en granjas de camarón de la región del Alto Golfo de California, con el fin de evaluar su potencial de cultivo. De lo anterior, se han logrado obtener después de dos años de cultivo, alrededor de 150 organismos de una talla y peso de 50 cm y 2 kg, respectivamente (SEMARNAT, 2010).

Por otra parte, en octubre del 2012 y noviembre del 2013 al Centro Reproductor de Especies Marinas del Estado de Sonora (CREMES) se le otorgaron los permisos necesarios para capturar ejemplares de totoaba a través del Golfo de California. Dicha captura fue realizada específicamente en el archipiélago de las Islas Encantadas, en la costa oriental de la península de Baja California, entre Puertecitos y Bahía San Luis Gonzaga abordo de la embarcación "Bura" con puerto base en la Paz, Baja California Sur. Los organismos fueron colectados mediante la técnica de pesca de anzuelo y sedal. Una vez capturados los organismos, se procedió a subirlos lentamente (de una profundidad de entre 30 y 40 m), con el fin de evitar una drástica descompresión. Al final de la captura se colectaron 18 ejemplares a los cuales se les aplicó la técnica de punción en la vejiga natatoria utilizando para ello un estilete quirúrgico hipodérmico de 1.5mm de diámetro. Una vez realizado el procedimiento, cada organismo fue colocado cuidadosamente en los viveros previamente preparados con agua de mar limpia y a temperatura ambiente para su traslado a las instalaciones del CREMES en Bahía de Kino, Sonora. Una vez que los organismos se adaptaron a las condiciones de cautiverio, se dio inició con el proceso de maduración mediante la suministración de sardina enriquecida con un aditivo de vitaminas, aceite de pescado y DHA (ácido docosahexaenoico, 26:6n-3) para ayudar a la maduración rápida de los organismos. Una vez que los organismos alcanzaron la madurez sexual se llevó a cabo el desove y la fertilización. La alimentación durante el cultivo larvario consistió en la administración de rotíferos, nauplios de *Artemia* spp y alimento microencapuslado, logrando el "destete" alrededor del día 60 de cultivo, sin embargo en ciclos posteriores este período se ha reducido considerablemente. Adicionalmente, el CREMES también ha realizado esfuerzos en capturar, madurar, reproducir y criar otras especies de esciénidos como la curvina golfina, *C. othonopterus* y la curvina de aleta corta, *C. parvipinnis*, de las que se ha logrado completar exitosamente su ciclo larvario (IAES, 2013; SAGARPA, 2015). Sin embargo, pese al dominio de los aspectos reproductivos y ciclo larvario de estas especies, no se han logrado producir de forma comercial debido al desconocimiento de sus aspectos nutricionales. Como consecuencia de lo anterior, se han estado utilizando alimentos para otras especies de peces, en su mayoría para trucha, con los cuales no se ha logrado obtener el máximo crecimiento de las especies cultivadas al no satisfacer plenamente sus requerimientos nutricionales (Rueda-López *et al.*, 2011). Es por lo anterior principalmente, que la investigación de los requerimientos nutricionales de estas

especies de peces debe continuar con el fin de lograr su cultivo a escala comercial en el país y específicamente en la región noroeste.

I.5. Requerimientos nutricionales de peces marinos

Existen cinco grupos de nutrientes principales considerados en la formulación de alimentos balanceados utilizados en acuacultura, estos son: proteínas, lípidos, carbohidratos, vitaminas y minerales. Dentro de estos grupos de nutrientes, las proteínas y los lípidos son sin duda los nutrientes primeramente evaluados en experimentos de nutrición (NRFS, 2011).

I.5.1. Proteínas

Las proteínas son polímeros de aminoácidos (conformados por varios cientos) unidos mediante enlaces peptídicos. Estas moléculas llevan a cabo funciones metabólicas vitales como procesos enzimáticos, transporte de nutrientes, almacenamiento, revestimiento, movilidad, estructura, mensajeros, inmunidad y también como fuentes de energía, entre otras. Es por esta razón que los organismos deben obtener un aporte diario de este nutriente para satisfacer su requerimiento y así, mantener el equilibrio de sus funciones vitales (Halver y Hardy, 2002; Lovell, 1998; Watanabe, 1988).

En acuacultura, la proteína representa el ingrediente de mayor costo en la formulación de alimentos acuícolas, los cuales pueden alcanzar hasta el 70% de los gastos operacionales. Es por ello que se debe procurar el determinar el requerimiento de este nutriente de la forma más acertada posible, ya que una incorrecta estimación podría afectar a los organismos cultivados en términos de crecimiento, o bien, afectar significativamente la rentabilidad de la actividad. Además, cabe señalar que el amonio es el principal metabolito de la degradación de las proteínas, el cual es un compuesto sumamente tóxico para la mayoría de los organismos acuáticos (Lovell, 1998). Es por esta razón que la reducción de los niveles de proteína en las dietas para peces marinos es de gran importancia tanto de una perspectiva económica como ambiental (Moreira *et al.* 2008). Algunos estudios de nutrición se han llevado a cabo en una

gran variedad de especies de peces marinos con la finalidad de investigar su requerimiento de proteína obteniéndose resultados muy diversos. Para la lubina, *Dicentrarchus labrax*, se ha reportado que el requerimiento de proteína puede ser de hasta el 60% (Metailler *et al.*, 1981), aunque de acuerdo con otros autores, los organismos alimentados con una dieta de 44% de proteína provista en su mayoría de harina de arenque mostraron los valores más altos de retención proteica y lipídica (Ballestrazzi *et al.*, 1994). Sin embargo, actualmente aún existe discrepancia entre el nivel óptimo de proteína de la especie (Oliva-Teles, 2000). Para el jurel cola amarilla, *Seriola quinqueridiata* y para el pargo rojo, *Pagrus major* el requerimiento de proteína se ha estimado en 50% mientras que para la dorada, *Spaurus aurata* el requerimiento de proteína se estimó aproximadamente en el 40% (Sabaut y Luquet, 1973). Para el lenguado Chileno, *Paralichthys adspersus* mostró un requerimiento cercano al 54% (Piaget *et al.*, 2011) mientras que para la curvina roja, *S. ocellatus* se ha determinado el requerimiento de proteína entre 40 y 45% (McGoogan y Gatlin, 1998).

Por otra parte, algunos estudios han demostrado que un efecto "ahorrador" de proteína puede ser logrado en los peces marinos utilizando una cantidad adecuada de lípido o de carbohidratos (Watanabe, 2002). Sin embargo, debido a la escases de carbohidratos en el ambiente marino, los peces por lo general se encuentran mejor adaptados para utilizar de mejor manera los lípidos y a las proteínas como fuentes de energía, lo cual podría servir como base para formular alimentos económicamente rentables.

I.5.2. Lípidos

Los lípidos son un grupo muy variado de sustancias, los cuales aportan una gran cantidad de energía (9 Kcal/g) debido a que los grupos carbono (CH_2) que conforman a los ácidos grasos se encuentran en su mayoría reducidos, lo que produce mayor cantidad de energía al ser metabolizados. Los lípidos comparten la característica general de ser solubles en solventes orgánicos no polares como el cloroformo, el éter o el benceno. Estas moléculas altamente energéticas se clasifican de acuerdo a la función que desempeñan en el organismo, siendo los lípidos neutros como los triglicéridos, los encargados del almacenamiento de energía y los

lípidos polares como los fosfolípidos y el colesterol, los que forman parte estructural de las membranas como los fosfolípidos y el colesterol (Curtis y Barnes *et al.*, 2001).

Dentro de los lípidos polares más importantes se encuentran los ácidos grasos altamente insaturados de la familia n-3 como el DHA y EPA (ácido eicosapentaenoico, 20:5n-3) y por otra parte el ARA (ácido araquidónico, 20:4n-6) de la familia n-6 (NRFS, 2011). En los peces marinos, estos ácidos grasos juegan un papel muy importante tanto en la permeabilidad de las membranas celulares, respuesta enzimática, síntesis de compuestos, maduración, así como en el desarrollo y mantenimiento del sistema nervioso (Bell *et al.*, 1986; Lee, 2001; Li *et al.*, 2005). Sin embargo, la mayoría de los teleósteos marinos carecen de la habilidad de sintetizar estos ácidos grasos altamente insaturados a partir del ácido linoleico y linolénico debido a la aparente deficiencia de la Δ5-desaturasa, la cual es requerida para llevar a cabo la elongación y desaturación de los ácidos grasos (Bruce *et al.*, 1999; Sargent, 1995).

Algunos estudios en reproductores de peces marinos han demostrado que el contenido de ácidos grasos esenciales en los huevos y las gónadas está fuertemente relacionado a la cantidad de ácidos grasos presentes en el alimento. De igual forma se ha reportado la importancia del DHA durante el desarrollo embrionario de los organismos en comparación con otros ácidos grasos esenciales. Por otra parte, se ha determinado que el ARA también juega un papel fundamental en la maduración final de los organismos, ya que a través de este ácido graso se sintetizan los esteroides y prostaglandinas necesarias para que este evento se lleve a cabo (Bell *et al.*, 1997; Navas *et al.*, 1997; Watanabe, 1993). De acuerdo con la literatura, el requerimiento de lípido de los peces marinos puede variar dependiendo de la especie, el hábitat, distribución, sexo y estado de madurez en el que se encuentren. Bruce et al. (1999) determinaron que el requerimiento de lípido en la dieta especialmente los ácidos grasos altamente insaturados DHA, EPA y ARA incrementan en el esturión de Siberia, *Acipenser baeri* cuando la especie se encuentra en fase reproductiva y estadio larval, lo cual incrementa significativamente las tasas de viabilidad y supervivencia, respectivamente. Pérez *et al.* (2006) determinaron que los machos de *Diplodus sargus* requieren de una mayor proporción de lípidos polares para llevar a cabo el proceso de maduración en comparación con las hembras. A su vez, Thoman *et al.* (1999) recomendaron la inclusión de un nivel de lípido de 9.2% para

la curvina roja, *S. ocellatus*, la cual es una especie de aguas templadas, mientras que Arzel *et al.* (1993) reportó que la trucha café, *Salmo trutta*, una especie de aguas frías presenta un mejor crecimiento con el 29% de inclusión de lípido en las dietas. Es por esta razón que se deben de tomar en cuenta los estadios de crecimiento y maduración en el que se encuentren los organismos al momento de ser cultivados, con el fin de satisfacer sus requerimientos nutricionales en términos de lípido durante su ciclo de vida y con ello maximizar su crecimiento.

I.6. Formulación de alimentos en acuacultura

El proceso de formulación de alimentos acuícolas implica el hecho de combinar diversos ingredientes con el fin de satisfacer en forma adecuada el requerimiento nutricional de los organismos que se estén cultivando, y con ello, asegurar su crecimiento y bienestar. La formulación de dietas para un organismo comienza con el establecimiento de sus requerimientos nutricionales, principalmente en términos de proteína y energía. En el caso particular de organismos marinos, estos nutrientes son principalmente satisfechos mediante la utilización de harina y aceite de pescado debido a su excelente perfil nutritivo. Una vez seleccionados los ingredientes destinados para la elaboración de las dietas, estos son procesados mediante técnicas de compresión y calor principalmente (NRFS, 2011). Entre los procesos más comunes de elaboración de alimentos se encuentran los procesos de peletizado y de extruido. En el proceso de peletizado, una vez que se encuentran mezclados los ingredientes, en su mayoría en forma de polvos, estos se someten a condiciones de calor, humedad y presión mecánica ejercida por la acción de una peletizadora para convertirlos a compuestos de naturaleza más estable. Sin embargo, la capacidad de estabilidad en el agua y tamaño de los pellets es limitado con lo que se puede perder hasta el 20% de la proteína, 50% de los carbohidratos y más del 50% de las vitaminas y minerales antes de ser consumido. A su vez en el proceso de extrusión, las condiciones de presión, temperatura y presión mecánica son mayores que las alcanzadas en el proceso de peletizado mediante la utilización de un extrusor, permitiendo que los almidones sean gelatinizados casi por completo, lo cual mejora las

condiciones físicas del alimento como la flotabilidad, tasa de hundimiento, entre otras (Muñoz-Latuz, 2004).

I.6.1. Harina y aceite de pescado

La harina de pescado es un polvo color café claro, el cual es elaborado a partir de los peces pelágicos menores o también conocidos como peces forrajeros, como sardinas, anchovetas, arenques, menhaden (Género *Brevoortia* conocidos como sardinas), entre otros. La harina de pescado se caracteriza principalmente por su alto contenido proteico (60-75%) y excelente variedad de aminoácidos, ser fuente de ácidos grasos esenciales (DHA y EPA), su amplia disponibilidad comercial, su alto coeficiente de digestibilidad y su buena proporción de vitaminas y minerales. A su vez, el aceite de pescado es la principal fuente de lípido dentro de la formulación de dietas, aportando una buena cantidad de ácidos grasos altamente insaturados, los cuales son de suma importancia en el crecimiento y supervivencia de los organismos cultivados (Gatlin *et al.*, 2007; Lie *et al.*, 1988; Shepherd y Jackson, 2013).

La producción mundial promedio de harina de pescado se encuentra cerca de las 7.05 millones de t por año, a excepción de los años considerados como "niños" en donde la producción por lo general disminuye a 5.7 t (Hardy y Tacon, 2002). Esto debido principalmente a que durante este fenómeno, la temperatura promedio del agua se incrementa provocando que los peces migren mar adentro en busca de aguas más frías, lo que dificulta significativamente su captura (Hardy, 2006).

La harina y el aceite de pescado son los ingredientes mayormente escogidos por los nutricionistas para la formulación y elaboración de dietas para peces marinos debido a sus características deseables anteriormente descritas. Sin embargo, actualmente existe una gran dependencia en estos insumos de origen marino, lo cual se ha considerado como una práctica muy nociva para el medio ambiente. Anualmente, se extraen alrededor de 20.8 millones de t de peces para producir harina y aceite de pescado y la tasa de conversión es muy alta para producir estos insumos. De acuerdo a Shepherd y Jackson (2013), señalan que para producir un kg de harina de pescado se requieren entre 4 y 4.5 kg de peces capturados y a su vez, para

producir un litro de aceite de pescado se requieren entre 10 y 50 kg de peces capturados, lo cual ha mermado las poblaciones naturales de peces forrajeros a nivel mundial. Debido a la creciente demanda por estos insumos marinos su precio se ha incrementado drásticamente disminuyendo en muchos casos, la rentabilidad de la actividad acuícola.

Específicamente en el año 2006, la acuacultura consumió alrededor de 3.7 millones de t de harina de pescado y 0.86 t de aceite de pescado, lo cual ocasionó que el precio de estos insumos se triplicara en la pasada década, y de acuerdo con algunos estudios, la demanda internacional excederá su producción a corto plazo. En años recientes, la producción de harina y aceite de pescado se ha mantenido estable, pero con tendencia a disminuir, lo que se debe principalmente a la sobrepesca de muchas de las especies de peces forrajeros que abastecen a la industria productora de estos insumos de origen marino (Naylor *et al.*, 2009; Shepherd y Jackson, 2013; Tacon y Metian, 2008). Por esta razón, la producción y expansión de la acuacultura dependerá de la búsqueda y desarrollo de alternativas que sean rentables y ecológicamente amigables para satisfacer la creciente demanda por los insumos de origen marino y de esta forma, lograr la optimización de dietas para las especies cultivadas (Gatlin *et al.*, 2007).

I.7. Ingredientes alternos a la harina de pescado

Debido a la problemática anteriormente descrita con respecto a la utilización de la harina y aceite de pescado en la formulación de dietas para organismos marinos, se han investigado algunas fuentes alternas a estos ingredientes, obteniéndose por lo general buenos resultados. Dentro de los ingredientes mayormente investigados se encuentran los descritos a continuación (Ayadi *et al.*, 2012).

I.7.1. Subproductos pesqueros

Este tipo de productos se origina a partir del procesamiento de organismos capturados mediante la pesca, o bien, como producto secundario del procesamiento de organismos

cultivados, ya que durante la extracción del filete más del 50% se descarta en vísceras, cabeza, piel, huesos, aletas y escamas, los cuales en su mayoría no son consumidas por el ser humano (Yano *et al.*, 2008). Adicionalmente, esta alternativa contempla a los peces capturados mediante la pesca incidental, los cuales son demasiado pequeños y/o no tienen mercado para comercializarse (Harrington *et al.*, 2005). Se han realizado algunas investigaciones en la curvina roja, *S. ocellatus*, donde se ha evaluado la sustitución de porcentajes de harina de pescado utilizando sub productos pesqueros, obteniéndose resultados aceptables (Li *et al.*, 2004, Whiteman y Gatlin, 2005). Sin embargo, algunas de las principales limitantes en la utilización de estos productos son la elevada cantidad de cenizas, en algunos casos superior al 30% y a la inconstante presencia y/o variabilidad de algunos nutrientes, lo que dificulta la formulación de alimentos para peces marinos.

Los subproductos pesqueros en específico las vísceras, presentan un gran potencial para ser utilizadas como ingredientes en ensilados o bien, para la extracción de su fracción lipídica, la cual se presenta en la mayoría de las ocasiones, en una gran proporción. Por ejemplo las vísceras de la carpa común, *Cyprinus carpio* pueden almacenar hasta 43.8% de lípido (Mondal *et al.* 2006) mientras que el bagre de canal, *Ictalurus punctatus* puede almacenar hasta el 35%. De forma similar, la utilización de los subproductos provenientes de la industria del salmón luce como una alternativa potencial a la sustitución de la harina de pescado. Existen algunos reportes donde se han utilizado subproductos de salmón en la alimentación del bacalao Murray, *Maccullochella peelii peelii* (Turchini *et al.*, 2003), mientras que la utilización de subproductos de la evisceración en la alimentación del bagre también se considera como una práctica común (Paripatananont, 2002). Sin embargo, a pesar del potencial que esta fuente alterna pudiera representar en la industria de elaboración de alimentos acuícolas, no existe información disponible acerca de la producción, disponibilidad y precios por estos productos (Turchini *et al.*, 2009).

Los subproductos generados por la industria camaronícola representan otra alternativa potencial a tomar en cuenta para ser utilizada en la elaboración de dietas para los organismos marinos cultivados. Mayers (1986) encontró que la proporción de desecho en una planta de procesamiento de crustáceos puede ir desde el 30 al 40% conformados en su mayoría por la

cabeza y las vísceras, y a su vez Heu *et al.*, (2003) determinaron que aproximadamente el 52% del peso del camarón procesado era descartado. Estos desperdicios generados por la industria camaronícola pueden servir como fuentes valiosas de nutrientes para algunos organismos inclusive para complementar dietas para organismos marinos.

I.7.2. Harina de subproductos avícolas

La producción de aves de corral es una de las actividades de mayor crecimiento dentro del sector pecuario (Zielinska *et al.*, 2007). Es por ello que la generación de subproductos derivados de esta actividad (mollejas, vísceras, cabezas y huevos inviables) se ha incrementado en años recientes (FAO, 2010b). El contenido de proteína cruda de este tipo de productos puede variar entre 58 y 64%, dependiendo de las fuentes con que se elaboran. Sin embargo en su mayoría, los desperdicios de la industria avícola proveniente de los Estados Unidos son generalmente consistentes en frescura, calidad y digestibilidad. México por su parte, importa subproductos avícolas de diferente grado de calidad, lo cual se basa principalmente en la cantidad de cenizas presentes durante el análisis. Se ha demostrado que la utilización de subproductos avícolas con baja cantidad de ceniza resultan mejores para las especies de peces carnívoras debido a su palatabilidad, digestibilidad y energía disponible similar a la contenida en la harina de pescado, lo que convierte a este tipo de harina en una opción costo-efectiva para ser utilizada en la formulación de dietas para diferentes especies acuáticas (NRC, 2011; Zhou *et al.*, 2004).

Recientemente, algunos estudios se han llevado a cabo en peces marinos donde se ha evaluado este tipo de harina como alternativa a la utilización de harina de pescado. Zapata *et al.* (2014) determinaron que es posible sustituir hasta el 67% de harina de pescado utilizando harina de subproductos avícolas en juveniles de totoaba, *T. macdonaldi,* mostrando buenas tasas de crecimiento, mientras que Hernández *et al.* (2014) reportaron que el pargo rosado, *L. guttatus*, puede tolerar altos porcentajes de sustitución de harina de pescado utilizando harina de subproductos avícolas, sin mostrar efectos negativos en su crecimiento. Flower (1991) reportó que es posible reemplazar hasta el 50% de la harina de pescado por harina de

subproductos avícolas en el salmón Chinook *Oncorhynchus tshawytscha* sin comprometer su crecimiento. Steffens (1994) reportó que es posible reemplazar hasta el 50% de harina de pescado en dietas para la trucha arcoíris con una combinación de harina de subproductos avícola y harina de plumas sin comprometer su crecimiento, siempre y cuando se adicionara la formulación con metionina y lisina. Sin embargo, Emre *et al.* (2003) reportaron que la inclusión de harina de subproductos avícolas aún en baja proporción, comprometían el crecimiento de alevines de carpa común, mientras que Hasan y Amin (1997) reportaron que las técnicas de procesamiento ejercidas sobre este tipo de harina presentaban un efecto significativo en el rendimiento de alevines de *Cirrhinus mrigala* en términos de crecimiento. Recientemente, se ha demostrado que las condiciones de procesamiento no solo afectan la calidad del producto, sino la disponibilidad de ciertos nutrientes, lo que podría causar un efecto negativo en el rendimiento de los organismos cultivados (Thompson *et al.*, 2008).

I.7.3. Harina de plumas

La harina de plumas proviene del proceso de crianza de aves como pollos, pavos, patos, gansos, entre otros. Las plumas que conforman a este tipo de harina deben ser procesadas mediante aplicación de calor, presión, secado y molienda antes de ser utilizada como ingrediente en la formulación de dietas, ya que de otra manera sería indigerible. El contenido de proteína en este tipo de harina puede oscilar entre 89 y 92%, dependiendo de la fuente de elaboración (Chandler, 2009). La harina de plumas es un subproducto de la industria avícola, la cual ha sido implementada como complemento en dietas para diferentes organismos acuáticos desde hace ya más de 15 años, principalmente en Norteamérica y Sudamérica donde de forma común las dietas para salmón, tilapia y carpa presentan entre el 3 y el 7% de harina de plumas para lograr el correcto balance de nutrientes. Sin embargo en la actualidad, ha surgido el interés en incrementar la proporción de harina de plumas dentro de las formulaciones para peces marinos, lo cual es principalmente debido a la problemática actual en torno a la utilización de ingredientes de origen marino como la harina y aceite de pescado. Algunos esfuerzos se han realizado en determinar el perfil nutritivo de la harina de plumas obteniéndose resultados inconsistentes, es por ello que se requiere de un mayor número de

estudios con el fin de presentar una aproximación real y con ello, incrementar la utilización eficiente de este tipo de ingrediente dentro de la elaboración de alimentos acuícolas (Boreau, 2009).

Algunos estudios han demostrado que la harina de plumas puede sustituir de forma eficiente a la harina de pescado y a la harina de soya en dietas para algunas especies de peces (Eyo, 1999). Hasan *et al.* (1997) reportaron que para dietas para *Labeo rohita* se puede sustituir hasta el 20% sin comprometer su crecimiento y utilización del alimento. Run-ji *et al.* (2010) determinaron que se puede sustituir hasta el 80% de la harina de pescado utilizando una combinación de harina de plumas, harina de sangre y harina de subproductos avícolas en dietas para la cabrilla malabar, *Epinephelus malabaricus*, sin afectar su crecimiento y supervivencia, mientras que Bureau *et al.* (2000) determinaron que en dietas para larvas de trucha arcoíris, *Oncorhynchus mykiss,* es posible reemplazar el 15% de la harina de pescado utilizando harina de plumas sin mostrar efectos negativos en crecimiento, supervivencia y producción de amonio.

I.7.4. Harina de sangre

La harina de sangre proviene del procesamiento del ganado, cuya sangre es drenada del cuerpo entero y después centrifugada para eliminar impurezas. Este tipo de harina se caracteriza por su alto contenido proteico, el cual puede variar entre 80 y 98.8% (Ingredients101, 2010; Martinez-Llorens *et al.*, 2008). En cuanto a la digestibilidad de este tipo de harina, se han obtenido resultados muy variables (Bureau *et al.*, 1999; Cho *et al.*, 1982). Al parecer, la harina de sangre parece ser muy sensible a los procesos de calentamiento y secado, lo cual podría de alguna forma explicar la variabilidad en su coeficiente de digestibilidad. Cho *et al.* (1982) reportaron que la harina de sangre secada mediante flama presentaba solo un 12% de digestibilidad en comparación con la harina de sangre secada mediante spray, que presentó casi una completa digestibilidad. Sin embargo, el desbalance y deficiencia de algunos aminoácidos esenciales como la metionina, han causado que el porcentaje de sustitución de harina de pescado logrado en peces marinos con este insumo sea relativamente bajo, en

comparación con la utilización de otros ingredientes alternos. Otro factor importante que ha frenado la utilización de la harina de sangre, al menos en la región europea ha sido la inminente asociación de este tipo de harinas con la transmisión de enfermedades al consumidor (Martínez-Llorens *et al.*, 2008). Algunos estudios de nutrición se han llevado a cabo con el fin de evaluar este ingrediente en dietas prácticas para diferentes especies de peces. Martinez-Llorens *et al.* (2008) reportaron que para la dorada, *Sparus aurata,* es posible sustituir entre el 10 y 15% de harina de pescado utilizando harina de sangre sin mostrar efectos negativos en términos de crecimiento y supervivencia. Para juveniles de cabrilla, *Epinephelus coioides*, se ha observado que con una combinación de harina de sangre y harina de carne es posible sustituir satisfactoriamente cerca del 80% de la harina de pescado en la dieta, mostrando valores de crecimiento similares a los peces alimentados con la dieta control (100% harina de pescado) (Millamena, 2002).

I.7.5. Harinas de origen vegetal

Los insumos de origen vegetal son un grupo de ingredientes que actualmente están siendo investigados con el fin de sustituir a la harina y aceite de pescado dentro de la elaboración de dietas para organismos marinos (Ayadi *et al.*, 2012). Dentro de este grupo de ingredientes alternos, la harina de soya es el ingrediente mayormente estudiado y continuamente evaluado en nutrición acuícola, por lo que actualmente es utilizada como complemento en la formulación de dietas para diferentes especies (Wang y Johnson, 2001). Sin embargo, la presencia de compuestos antinutricionales, la deficiencia de aminoácidos esenciales, la gran proporción de carbohidratos y la menor cantidad de proteína en comparación con la harina de pescado, han limitado el nivel de inclusión de harina de soya en dietas para organismos acuáticos, especialmente para especies carnívoras (Gatlin *et al.*, 2007; Naylor *et al.*, 2009).

El avance tecnológico en las técnicas de procesamiento y el desarrollo de mejores programas de manejo reproductivo en plantas, han facilitado el desarrollo de nuevas variedades de soya, las cuales están actualmente disponibles para ser evaluadas en dietas para distintos organismos. Los concentrados de proteína basados en ingredientes de origen vegetal,

los cuales contienen una mayor cantidad de proteína y menor cantidad de antinutrientes que las harinas convencionales, lucen como una opción interesante y factible para lograr reemplazar a la harina de pescado en la formulación de dietas para acuacultura (Baker y Stein 2009; Baker *et al.*, 2009).

I.7.5.1. Concentrado de harina de soya (CPS) y de maíz (CPM)

Diversas variedades de concentrados de proteína de soya con un contenido aproximado de 60% de proteína cruda y menor proporción de compuestos antinutricionales y carbohidratos, han sido evaluadas en la formulación de dietas experimentales para el salmón del Atlántico, *Salmo salar*, la trucha arcoíris, *O. mykiss* (Burr *et al.*, 2012), la cobia, *Rachycentron canadum* (Salze *et al.*, 2010), el lenguado Japonés, *Paralichthys olivaceous*, (Deng *et al.*, 2006), el pargo, *P. major*, (Takagi *et al.*, 2001) y la curvina roja, *S. ocellatus* (Rossi *et al.*, 2013), obteniéndose resultados satisfactorios.

El maíz es otro de los ingredientes actualmente evaluados en estudios nutricionales. La harina de maíz se produce cuando el grano de maíz es separado en sus componentes principales: fibra, germen, gluten y almidón. Después, el aceite es extraído del germen dejando así la harina de germen de maíz. La proteína de gluten es concentrada, filtrada y secada hasta obtener el concentrado de proteína de gluten (CPG), el cual puede contener 60% de proteína cruda como mínimo. Aunque refinada y purificada, la proteína de gluten ha alcanzado un nivel de proteína cercano al 74%; sin embargo, este porcentaje no siempre es alcanzado debido a algunas restricciones en el proceso de producción (AAFCO, 2007; Ayadi *et al.*, 2012; Gatlin *et al.*, 2007).

El concentrado de proteína de maíz (CPM) contiene una cantidad menor de compuestos antinutricionales y una mayor cantidad de metionina y cisteína, en comparación con la soya; sin embargo, es deficiente en lisina (Hardy, 2010; Phillips y Sternberg, 1979). Algunos estudios han evaluado exitosamente el reemplazo parcial de harina de pescado utilizando diversos productos derivados de maíz en dietas para el lenguado Japonés (Kikuchi, 1999), cobia (Luo *et al.*, 2013), turbot, *Psetta máxima* (Regost *et al.*, 1999), *Diplodus vulgaris*

(Bulut *et al.*, 2014) y la curvina roja, *S. ocellatus* (Rossi *et al.*, 2013). Los resultados de estas investigaciones sugieren que el nivel de reemplazo de harina de pescado, utilizando harinas de origen vegetal como fuente proteica principal, puede variar ampliamente en los peces marinos dependiendo principalmente de la especie, hábitos alimenticios y tolerancia a estos insumos. Es por esta razón que cada especie deberá ser evaluada individualmente con el fin de determinar el porcentaje de reemplazo de harina adecuado para cada una de ellas, sin provocar efectos negativos en el crecimiento y/o utilización del alimento.

II. JUSTIFICACIÓN

El presente estudio plantea realizar estudios nutricionales encaminados al desarrollo de alimentos balanceados para el cultivo comercial de la curvina golfina, *C. othonopterus*, de la totoaba, *T. macdonaldi* y de la curvina de aleta corta, *C. parvipinnis*. La justificación del presente estudio se basa principalmente en el hecho de que aún se desconoce el requerimiento lipídico de la curvina golfina y de la totoaba, lo cual ha obstaculizado la elaboración de dietas específicas y consecuentemente, su cultivo a escala comercial. Por esta razón, que el presente estudio coadyuvará en la determinación de los aspectos nutricionales de estas especies y con ello, promoverá la formulación y elaboración de alimentos balanceados, los cuales se pretende se encuentren disponibles comercialmente, con el fin de incrementar el volumen de producción de peces marinos en el país.

Por otra parte, con base en la problemática mundial en cuanto a la utilización de la harina de pescado anteriormente descrita, el presente trabajo también contempla evaluar el remplazo parcial de harina de pescado utilizando diferentes fuentes de origen vegetal en las formulaciones de dietas para la curvina de aleta corta, lo que se presenta como una alternativa rentable y ecológicamente amigable, ya que reducirá la dependencia actual en la harina de pescado para llevar a cabo la formulación de alimentos balanceados para esta y muchas otras especies.

III. OBJETIVOS

III.1. Objetivo general

Realizar estudios nutricionales encaminados al desarrollo de alimentos balanceados para el cultivo comercial de la curvina golfina (*C. othonopterus*), de la totoaba (*T. macdonaldi*) y de la curvina de aleta corta (*C. parvipinnis*).

III.2. Objetivos particulares

III.2.1. Determinar el requerimiento lipídico dietario de la curvina golfina, *C. othonopterus*.

III.2.2. Evaluar la sustitución de harina de pescado por harinas de origen vegetal en dietas para la curvina de aleta corta, *C. parvipinnis*.

III.2.3. Determinar el requerimiento lipídico dietario de la totoaba, *T. macdonaldi*.

IV. ARTÍCULOS PUBLICADOS O ACEPTADOS

Para cumplir con los objetivos particulares, se presentan tres artículos científicos publicados (Tabla I).

Tabla I. Relación de artículos científicos en cumplimiento a los objetivos particulares

Objetivo particular	**Título del artículo**	**Revista**	**Estatus**
III.2.1.	Influence of dietary lipid on growth performance and body composition of the Gulf corvina, *Cynoscion othonopterus*.	Aquaculture	Publicado
III.2.2.	Plant protein sources in the diets of the sciaenids red drum (*Sciaenops ocellatus)* and shortfin corvina (*Cynoscion parvipinnis*): A comparative study.	Aquaculture	Publicado
III.2.3.	Effect of dietary lipid level on growth performance, feed utilization, and body composition of totoaba, *Totoaba macdonaldi*	Aquaculture Research	Aceptado

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Influence of dietary lipid on growth performance and body composition of the Gulf corvina, *Cynoscion othonopterus*

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ABSTRACT

Based upon an iso-proteic dietary content of 40%, a 56-day experiment was conducted to evaluate the effects of incremental levels of dietary crude fat (2, 5, 8, 11, 14, 17, 20, 23 and 26%) on the performance of *Cynoscion othonopterus* juveniles, with an initial mean body weight of 32.86 ± 0.48 g. Fish were reared in a clear-water, recirculating culture system, composed of 48 circular tanks of 250 L (0.4 m^2 bottom area) filled with 200 L filtered seawater, at a density of 3 fish $tank^{-1}$ (15 fish m^{-3}), assigning each treatment to five replicate tanks. Fish were fed approximately 3% of their wet body weight daily. Overfeeding was minimized while maintaining the feeding rate close to apparent satiation, dividing the daily ration into three equal portions. A clear dose–response effect of dietary crude fat was observed on growth of the Gulf corvina, *C. othonopterus*, with the best results corresponding to fish fed 11% crude fat, while growth performance was reduced as dietary crude fat departed from this level. These results were significant for specific growth rate and thermal growth coefficient data ($P = 0.0283$ and 0.0450, respectively), and although not statistically significant, the same pattern held true numerically for the majority of the other growth response variables and feed utilization indices measured. Quadratic broken line analysis of thermal growth coefficient data estimated a requirement for dietary crude fat of 11.4% for this species, with 95% confidence interval of 9.8 to 13.0%. Significantly increased lipid deposition, concomitant with reduced moisture content in muscle and whole body were observed in response to incremental levels of dietary crude fat. Intestine pancreatic lipase content tended to decrease with increasing dietary crude fat level, although this pattern was not statistically significant.

Statement of relevance: The present manuscript provides the first documented data on lipid nutrition of the Gulf corvina, a novel candidate species for aquaculture in Northwest Mexico. Significantly increased lipid deposition, concomitant with reduced moisture content in muscle and whole body, were observed in response to incremental levels of dietary lipid. A clear dose–response effect of dietary crude fat was observed on growth, with an estimated requirement for dietary crude fat of 11.4%, when fed a diet containing 40% crude protein.

1. Introduction

The Gulf corvina (*Cysnoscion othonopterus*) is a sciaenid fish that supports the second most abundant finfish fishery, with over 3700 MT in 2010, in the Northwest Gulf of California, Mexico (Secretaría de Agricultura, Ganadería, Desarrollo Rural, Pesca y Alimentación, 2012). As other members of the family Scianidae, it is appreciated for its top quality filet. Native to the Gulf of California, the Gulf corvina performs a well-documented seasonal migration to the Colorado River Delta, where it finds lower environmental salinities and spawns (Encinas-Rivera, 2008; Rowell et al., 2005). Due to the increasing interest in the Gulf corvina as a candidate for aquaculture, research is currently underway in Northwest Mexico evaluating nutritional and environmental aspects aimed at the development of the captive rearing of this species (González-Félix et al., 2013; Perez-Velazquez et al., 2014).

Among dietary macronutrients, lipids are well recognized for playing major roles in many physiological processes, as well as for being major body constituents, and sources of energy (Tocher, 2003). For some aquacultural fish species, it has been observed that, when dietary lipid is included beyond optimal levels, body fat may accumulate excessively, affecting the quality of the product (Bromley, 1980; Hillestad and Johnsen, 1994), while for many other fish species, it limits feed ingestion rate, resulting in slow growth (Daniels and Robinson, 1986; Ellis and Reigh, 1991; McGoogan and Gatlin, 1999; Page and Andrews, 1973; Rueda-López et al., 2011; Shiau and Lan, 1996; Watanabe, 1982).

Secretion of pancreatic lipase ensures effective digestion and absorption of dietary lipids within the anterior small intestine of fish

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(duodenum in mammals), but the lipase content present in the pancreatic juice changes according to different factors including diet and age, at least in mammals (Boivin et al., 1990; Fieker et al., 2011); nevertheless, pancreatic lipase characterization or quantification has rarely been done for fish, and the effect of varying dietary lipids on the occurrence of this enzyme in fish gut has not been described for this species. Because dietary protein can also be readily used as a source of energy (Kleiber, 1975), the catabolism of dietary lipid over protein can help spare the use of this costly nutrient. For all of the reasons above, the determination of the lipid dietary requirements is an essential step for the development of feeds that produce maximum growth. However, due to its status as a possible novel species for aquaculture, to date, the dietary lipid requirement of the Gulf corvina has not been evaluated. Thus, the present study investigated the influence of dietary lipid on the growth performance and body composition of the Gulf corvina, *Cynoscion othonopterus*, as well as the influence of dietary lipid on the presence of pancreatic lipase within fish intestine.

2. Materials and methods

2.1. Experimental fish and culture system

Gulf corvina (*C. othonopterus*) juveniles from the same cohort, originating from wild spawners, were obtained from the Center for Reproduction of Marine Species of the State of Sonora (CREMES), Kino Bay, Sonora, Mexico. Fish were transported to the Kino Bay Experiment Station (KBES), University of Sonora at Kino Bay, Sonora, Mexico, placed in a 10-m^3 raceway, and fed a 46% crude protein (CP)/14% crude fat (CF) commercial fish feed (Nutripec Red Drum, Agribrands Purina®, Mexico City, Mexico) for a 15-day acclimation period.

The experimental culture system included 48 polyethylene circular tanks (0.4 m^2 bottom area, 71 cm diameter, and 250 L capacity). A 1.5-hp pump (Jacuzzi, Model 150MF-T, Little Rock, Arkansas, USA) pumped water through a 1100-L sump tank, a biofilter, a sand filter (Jacuzzi, Model L-190-7, Little Rock, Arkansas, USA), a 120-Watt UV light chamber (Rainbow Lifeguard, Model UV97, El Monte, California, USA), a 1500-Watt in-line heater (Aquatic Ecosystems, Model DE-6115, Apopka, Florida, USA), a 1-hp in-line chiller (Aquatic Ecosystems, Model AE62B, Apopka, Florida, USA), and into individual tanks at a flow rate of 1.5 L/min to achieve a full water turnover every 133 min. A daily water exchange of approximately 80% was applied using new, filtered seawater. Aeration was supplied to individual tanks with a 1.0-hp blower (Fuji, Model VFC40, Saddle Brook, New Jersey, USA) and submerged airstones.

2.2. Experimental treatments

For 56 days fish with an initial overall wet body weight of 32.86 ± 0.48 g (mean ± standard error, SE) were fed nine iso-proteic (40% CP) feeds that were formulated to contain 2, 5, 8, 11, 14, 17, 20, 23 and 26% CF, using fish oil and soybean oil as the main lipid sources (Table 1). Each experimental treatment was assigned to five replicate tanks, stocking three group-weighed fish into each tank in a completely randomized design experiment. In addition, the commercial fish feed described earlier, with 46% CP and 14% CF, was used as an external reference and assigned to three replicate tanks, but it was not included in the statistical analysis. Using a Hobart grinder (Hobart Corporation, Model A-200, Troy, Ohio, USA), feeds were prepared by cold extrusion, dried overnight at 40 °C, ground to adequate size and kept frozen at −20 °C until used. Proximate composition of experimental feeds, including moisture, crude protein, crude fat, ash, and crude fiber content, was determined according to the procedures 930.15, 976.05, 2003.05, 942.05 and 978.10 of the Association of Official Analytical Chemists (2005). Gross energy content of the experimental feeds was determined by bomb calorimetry (Model IKA C5003, IKA-Werke® GmbH, Staufen, Germany) (Table 1), while the nitrogen free extract (NFE) was calculated by difference; NFE (%) = 100 − (% CP + % CF + % moisture + % crude fiber + % ash).

2.3. Feeding and maintenance of fish

The daily feed ration, approximately 3% of their wet body weight, was administered to fish in three equal portions at 08:00, 13:00, and 18:00 h. Overfeeding was minimized while maintaining the feeding rate close to apparent satiation. Uneaten feed was collected daily out of all tanks and dried overnight to quantify, by difference, consumed feed (feed offered, g − uneaten feed, g). The concentration of dissolved oxygen, temperature, and salinity of culture water were measured daily with a multi-function oxygen meter (YSI, Model Y85, Yellow Springs, Ohio, USA). Weekly measurements of pH were performed with a hand-held pH meter (Oakton®, Model Double Junction pHTestr 1, Vernon Hills, Illinois, EUA), while the concentrations of total ammonia nitrogen and nitrite were measured following procedures adapted from those of Solórzano (1969), Spotte (1979a, 1979b) and Strickland and Parsons (1972).

2.4. Evaluation of growth performance, body and tissue composition

The growth performance of fish was evaluated in terms of final weight (g); weight gain (g) = (final weight, g − initial weight, g); percent weight gain (%) = [(final weight, g − initial weight, g) / initial weight, g] × 100; daily weight gain = weight gain, g / time (days); specific growth rate (SGR) = (ln final weight, g − ln initial weight, g) / time (days) × 100; thermal growth coefficient (TGC) = [(final weight$^{1/3}$ − initial weight$^{1/3}$) / (temperature, °C × time, days)] × 100 (as adapted by Cho, 1990); the Fulton's condition factor (K) (Ricker, 1975), an estimate of the robustness of fish = (weight, g / length^3, mm) × 100; hepatosomatic index (HSI) = (liver weight, g / final weight, g) × 100; and survival rate = (final no. of organisms × 100) / initial no. of organisms; while feed utilization was evaluated in terms of feed conversion ratio (FCR) = feed consumed, g / weight gain, g; feed efficiency (FE) = weight gain, g / feed consumed, g × 100; gross protein retention (GPR) = CP gain, g / CP consumed, g × 100; gross fat retention (GFR) = CF gain, g / CF consumed, g × 100; and protein efficiency ratio (PER) = weight gain, g / CP consumed, g.

At the beginning and end of the experiment, triplicate composite samples, each consisting of homogenates prepared with three whole fish sampled from different tanks in the same treatment, were taken to determine the contents of moisture, ash (Association of Official Analytical Chemists, 2005, methods 930.15, and 942.05, respectively), crude fat (Folch et al., 1957), and crude protein (Association of Official Analytical Chemists, 2005, method 968.06), which were analyzed via combustion by the Dumas method with a Dumas Nitrogen Analyzer (Model NDA 702, VELP® Scientifica, Usmate, Italy). In addition, following the procedures described above, triplicate composite samples, each also consisting of three fish sampled from different tanks, were taken to perform these determinations on muscle tissue and liver. Similar analyses were performed on triplicate composite samples from an initial sample of 15 fish sacrificed at the beginning of the experiment.

2.5. Evaluation of pancreatic lipase

2.5.1. Enzyme extraction

The intestines of 3 fish (*C. othonopterus*) per treatment (from different tanks) were dissected, washed with ice-cold 0.9% potassium chloride (KCl) saline solution, and then homogenized (Polytron PT10/35, Brinkmann Instruments, Westbury, NY, USA) in 35 mL of a 50 mM Tris–HCl buffer solution (pH 7.50) with 3.06 mL of a protease inhibitor solution (benzamidine HCl: 0.5 mM; E-64: 2 μM; leupeptin: 20 μM; AEBSF: 0.5 mM, and EDTA: 10 mM). Three 1.5 mL samples of the crude extract were centrifuged (Heraeus Fresco 21, ThermoFisher Scientific, Dreieich, Germany) two times at 22,000 ×*g* and 4 °C for

Table 1
Ingredients and determined values of the proximate composition (% of dry weight) of experimental feeds for *C. othonopterus*.

Ingredient	Crude fat level (%)									External reference
	2	5	8	11	14	17	20	23	26	
Fishmeal sardine[a]	42.00	42.00	42.00	42.00	42.00	42.00	42.00	42.00	42.00	N/A[m]
Squidmeal (75%)[b]	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	N/A
Casein[c]	0.00	1.10	2.20	3.20	4.30	5.50	5.90	6.30	6.70	N/A
Wheat starch[d]	22.93	18.83	14.73	10.73	6.63	2.42	2.42	2.40	2.40	N/A
Sardine fish oil[e]	0.00	1.20	2.70	6.00	9.30	12.60	15.00	17.50	20.00	N/A
Soy oil[e]	0.00	0.80	2.30	2.00	1.70	1.40	2.00	2.50	3.00	N/A
Whole wheat flour[f]	30.00	30.00	30.00	30.00	30.00	30.00	26.60	23.20	19.80	N/A
Trace mineral pre-mix[g]	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	N/A
Vitamin pre-mix w/o choline[h]	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	N/A
Choline chloride[i]	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	N/A
Vitamin stay C 35%[j]	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	N/A
Tocopherol[k]	0.02	0.02	0.02	0.02	0.02	0.03	0.03	0.05	0.05	N/A
Soy lecithin[l]	0.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	N/A
Total	100	100	100	100	100	100	100	100	100	N/A
Proximate composition[n]										
Moisture (%)	7.32	7.64	7.50	7.62	7.49	7.58	7.47	7.54	7.60	7.52
Crude protein (%)	40.68	40.33	40.44	40.63	40.52	40.41	40.56	40.51	40.57	46.15
Crude fat (%)	1.98	4.94	7.96	11.03	13.96	16.83	19.85	23.13	25.94	14.11
Ash (%)	6.71	6.71	6.75	6.82	6.70	6.78	6.70	6.77	6.81	9.76
Crude fiber (%)	1.49	1.53	1.44	1.41	1.46	1.42	1.47	1.50	1.41	2.28
NFE (%)*	41.82	38.85	35.91	32.49	29.87	26.98	23.95	20.55	17.67	20.18
Gross energy (kJ/g)	17.50	18.00	18.90	19.90	20.40	21.00	21.40	22.30	23.10	19.00
Protein (P)/energy ratio (gP/MJ)	23.25	22.41	21.40	20.42	19.86	19.24	18.95	18.17	17.56	24.29

[a] Proteínas Marinas y Agropecuarias, S.A. de C.V., Zapopan, Jalisco, Mexico.
[b] CleanFish, Guaymas, Sonora, Mexico.
[c] Fagalab, S.A. de C.V., Guamuchil, Sinaloa, Mexico.
[d] Gluten y Almidones Industriales, S.A. de C.V., Mexico City, Mexico.
[e] Aceite Nutrioli, Ragasa Industrias, S.A. de C.V., Guadalupe, Nuevo León, Mexico.
[f] Los Gallos, Molino La Fama, S.A. de C.V., Hermosillo, Sonora, Mexico.
[g] MP Biomedicals Inc., Solon, Ohio, USA, g/100 g of premix: cobalt chloride 0.004, cupric sulfate pentahydrate 0.250, ferrous sulfate heptahydrate 4.0, magnesium sulfate pentahydrate 28.398, manganese sulfate monohydrate 0.650, potassium iodide 0.067, sodium selenite 0.010, zinc sulfate heptahydrate 13.193, and α-cellulose 53.428.
[h] MP Biomedicals Inc., Solon, Ohio, USA, g/kg of premix: thiamine HCl 0.5, riboflavin 3.0, pyridoxine HCl 1.0, DL pantothenic acid 5.0, niacin 5.0, biotin 0.05, folic acid 0.18, cyanocobalamin 0.002, inositol 5.0, menadione 2.0, vitamin A acetate (20,000 IU/g) 5.0, vitamin D_3 (400,000 IU/g) 0.002, DL-α-tocopheryl acetate (250 IU/g) 8.0, and α-cellulose 865.266.
[i] Sigma Aldrich, Saint Louis, Missouri, USA.
[j] Stay C® (L-ascorbyl-2-polyphosphate 35% active C), Roche Vitamins Inc., Parsippany, NJ, USA.
[k] General Nutrition Centers, Co., Pittsburg, Pennsylvania, USA.
[l] Golden Harvest, Impulsora Golden, S.A. de C.V., Mexico City, Mexico.
[m] N/A = not applicable.
[n] Values are means ± standard deviation of triplicate samples.
* Nitrogen free extract (NFE) calculated by difference: NFE (%) = 100 − (% CP + % CF + % moisture + % crude fiber + % ash).

30 min, until the samples were clear. After centrifugation, the precipitant was discarded and the supernatant was subjected to polyethylene glycol (PEG-6000) precipitation. For this purpose, 0.75 mL of a 30% PEG-6000 solution was slowly added to the supernatant. After 1 h of stirring at 4 °C, the suspension was left for 10 min at the same temperature and subsequently centrifuged at 22,000 × *g* and 4 °C for 30 min. The supernatant was recovered and stored at −20 °C until used.

2.5.2. SDS-PAGE

In order to determine the molecular mass of native lipase and to quantify its content in the intestine of fish subjected to the different dietary lipid treatments, sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was carried out in a four gel vertical electrophoresis system (Mini-Protean Tetra Cell, Bio-Rad; CA, USA) using 10% polyacrylamide gels. The enzymes were resolved at a constant voltage 110 V applied for about 4 h. Then, the gels were fixed in a mixture of 40% ethanol and 10% acetic acid and stained overnight in gentle agitation with QC Colloidal Coomassie stain, then destained by rinsing in deionized water. An internal standard (Precision Plus Protein Standard, Bio-Rad®, Hercules, CA, USA) with proteins of 10–250 kDa, and human pancreatic lipase (BCR-693, European Community Bureau of Reference) were used as molecular markers and controls. The gels were then scanned with a GS-900 Calibrated Densitometer (Bio-Rad®, Hercules, CA, USA) for identification and quantification of protein bands.

2.6. Statistical analysis

Based on a significance level of $P \leq 0.05$, one-way analysis of variance (ANOVA) was applied to fish growth performance data: initial weight, final weight, weight gain, percent weight gain, daily weight gain, SGR, TGC, K, HSI, FCR, GPR, GFR, and PER, as well as to survival rate, and lipase quantification data. Survival rate data were arcsine-transformed, though untransformed data are presented. Tukey's honestly significant difference method was used as the mean separation procedure when significant differences among treatments were detected. Regression models for fitting the response of growth data, including broken line (linear and quadratic ascending), saturation kinetics, and two-slope, quadratic broken line model, were employed to estimate the dietary lipid requirement. The coefficient of determination (R^2) of the model herein reported was calculated according to the adjusted R^2 model proposed by Kvålseth (1985). All statistical analyses were performed using the Statistical Analysis System software (1999–2000, Software Release 8.1; SAS Institute Inc., Cary, NC, USA).

3. Results

3.1. Water quality

Temperature, salinity, pH, and the concentrations of dissolved oxygen, total ammonia nitrogen, and nitrite in the culture water were

(means ± SE) 24.8 ± 0.2 °C, 37.7 ± 0.1‰, 7.8 ± 0.02, 7.0 ± 0.1 mg/L, 0.131 ± 0.015 mg/L, and 0.019 ± 0.005 mg/L, respectively.

3.2. Evaluation of growth performance

No significant differences among treatments were observed for initial weight data (Table 2). After being fed the experimental feeds for 8 weeks, significant differences in SGR and TGC data ($P = 0.0283$ and 0.0450, respectively) were detected, with the best growth performance (the highest numerical mean value) corresponding to fish receiving 11% dietary crude fat (SGR of 0.9%/day, TGC of 0.041, and weight gain of 21.8 g), decreasing with dietary crude fat above or below this level (Table 2). Although not significant, the same pattern was also observed for the growth parameters final weight, daily weight gain, K, and the feed utilization indices FE, GPR, GFR, and PER, but not for HSI, survival, or FCR (Tables 2 and 3). Among the various regression models tested, the best fit was obtained when the two-slope, quadratic broken line analysis was applied to TGC data, estimating a dietary crude fat requirement for the Gulf corvina of 11.4%, with 95% confidence interval of 9.8 to 13.0% (Fig. 1).

3.3. Evaluation of body and tissue composition

At the beginning of the experiment, the determined values (means ± SE) of the proximate composition (% of wet weight) of whole fish, in terms of CP, CF, ash, and moisture were 16.4 ± 0.3, 6.9 ± 0.7, 3.8 ± 0.2, and 72.5 ± 0.4%, respectively. For muscle tissue, they were 18.4 ± 0.6, 3.2 ± 0.4, 1.1 ± 0.0, and 77.4 ± 0.6%, respectively; while for liver, they were 13.7 ± 2.4, 22.0 ± 1.5, 0.8 ± 0.0, and 63.3 ± 1.5%, respectively (Table 4).

At the end of the experiment, the feed with 11% crude fat elicited the highest crude protein content (treatment means) in whole fish (21.7 ± 0.8%), significantly higher than those observed in fish from all the other treatments, which decreased as dietary crude fat departed from this level (Table 4). The crude fat content of whole fish increased significantly, from 5.5 ± 0.1 to 9.5 ± 0.8%, as dietary crude fat increased from 2 to 26% (Table 4). On the contrary, the moisture content of whole fish tended to decrease with increasing dietary crude fat, with the highest moisture content recorded in fish fed 2% dietary crude fat (73.2 ± 0.7%), significantly higher than those of fish fed crude fat levels between 11 and 26% (moisture contents ranging from 68.5 to 70.4%) (Table 4). Significant differences were also detected for ash content of whole fish, with the highest value recorded for fish fed 17% dietary crude fat (4.3 ± 0.3%). However, mean separations were variable, without a clear trend being observed (Table 4).

In muscle tissue, statistical differences among treatments were observed for the proximate contents of crude fat, moisture, and ash, but not for protein. Similarly to what was observed for whole fish, crude fat in muscle tissue increased with increasing dietary crude fat level. Fish fed the highest crude fat levels of 20, 23, and 26%, had significantly greater crude fat content (5.6 ± 0.1, 6.1 ± 0.4, and 5.6 ± 0.2%, respectively) than fish fed any of the lower crude fat levels, from 2 to 17% (muscle crude fat contents ranging from 2.6 to 4.6%) (Table 4). Once more, moisture content tended to decrease as dietary crude fat increased. Moisture content recorded for muscle tissue from fish fed

Table 3
Feed utilization indices of *C. othonopterus* fed graded levels of dietary lipid.

Treatment crude fat (%)	FCR	FE	GPR	GFR	PER
2	3.7 ± 0.9	0.3 ± 0.1	14.4 ± 3.7	13.4 ± 3.5	0.8 ± 0.2
5	3.3 ± 0.4	0.4 ± 0.0	17.6 ± 2.6	16.7 ± 2.5	0.9 ± 0.1
8	3.1 ± 0.3	0.4 ± 0.1	18.8 ± 2.6	19.0 ± 2.6	1.0 ± 0.1
11	3.0 ± 0.5	0.5 ± 0.1	25.7 ± 4.9	23.1 ± 4.4	1.2 ± 0.2
14	3.5 ± 0.2	0.3 ± 0.0	15.2 ± 0.9	15.9 ± 1.0	0.8 ± 0.1
17	2.6 ± 0.2	0.4 ± 0.0	20.0 ± 1.9	19.6 ± 1.8	1.1 ± 0.1
20	3.7 ± 0.7	0.4 ± 0.1	16.4 ± 3.4	16.7 ± 3.5	0.9 ± 0.2
23	3.6 ± 0.7	0.4 ± 0.1	15.8 ± 4.9	17.2 ± 5.3	0.9 ± 0.3
26	5.5 ± 0.8	0.3 ± 0.1	11.2 ± 4.1	11.5 ± 4.3	0.7 ± 0.3
External reference[1]	2.8 ± 0.3	0.4 ± 0.1	13.8 ± 1.8	16.5 ± 2.2	0.8 ± 0.1
ANOVA $Pr > F$	0.2756	0.5612	0.2197	0.4555	0.6781

Values are means ± standard error of five replicate samples. Means with different superscripts are significantly different ($P \leq 0.05$).
Abbreviations: FCR = feed conversion ratio; FE = feed efficiency; GPR = gross protein retention; GFR = gross fat retention; PER = protein efficiency ratio.
[1] Not included in the statistical analysis.

Table 2
Growth performance of *C. othonopterus* fed graded levels of dietary lipid.

Treatment crude fat (%)	Initial weight (g)	Final weight (g)	Weight gain (g)	Percent weight gain (%)	Daily weight gain (g/day)	SGR (%/day)	TGC
2	31.3 ± 1.1	42.7 ± 3.9	11.7 ± 2.6	37.1 ± 6.9	0.20 ± 0.05	0.6^{ab} ± 0.1	0.025^{ab} ± 0.004
5	32.3 ± 1.1	49.0 ± 2.8	16.7 ± 2.5	51.8 ± 7.7	0.30 ± 0.04	0.7^{a} ± 0.1	0.034^{a} ± 0.004
8	33.9 ± 2.0	51.1 ± 2.7	17.2 ± 1.5	51.5 ± 5.7	0.30 ± 0.03	0.7^{a} ± 0.1	0.034^{a} ± 0.003
11	33.7 ± 1.6	55.4 ± 6.8	21.8 ± 6.2	64.5 ± 6.7	0.40 ± 0.11	0.9^{a} ± 0.2	0.041^{a} ± 0.009
14	33.3 ± 1.6	49.2 ± 0.5	15.9 ± 0.8	48.2 ± 6.4	0.30 ± 0.02	0.7^{a} ± 0.1	0.029^{ab} ± 0.003
17	30.7 ± 1.0	48.7 ± 0.7	18.0 ± 1.5	59.4 ± 6.9	0.30 ± 0.02	0.8^{a} ± 0.1	0.038^{a} ± 0.003
20	32.7 ± 1.1	45.8 ± 3.0	13.1 ± 2.5	40.0 ± 7.5	0.20 ± 0.04	0.6^{ab} ± 0.1	0.027^{ab} ± 0.005
23	32.8 ± 2.0	45.1 ± 4.8	12.2 ± 3.1	36.2 ± 8.0	0.20 ± 0.06	0.5^{ab} ± 0.1	0.025^{ab} ± 0.005
26	34.4 ± 2.0	42.4 ± 3.9	8.0 ± 2.4	22.7 ± 5.9	0.10 ± 0.04	0.4^{b} ± 0.1	0.016^{b} ± 0.004
External reference[1]	31.9 ± 1.7	48.4 ± 2.5	16.5 ± 2.2	52.2 ± 7.8	0.30 ± 0.04	0.7 ± 0.1	0.034 ± 0.004
ANOVA $Pr > F$	0.7563	0.3270	0.1040	0.0625	0.1040	0.0283	0.0450

Treatment crude fat (%)	K	Hepatosomatic index (%)	Survival rate (%)
2	1.2 ± 0.1	2.4 ± 0.3	91.7 ± 8.3
5	1.2 ± 0.1	2.6 ± 0.3	100.0 ± 0.0
8	1.1 ± 0.1	2.4 ± 0.1	93.3 ± 6.6
11	1.3 ± 0.1	2.6 ± 0.2	80.0 ± 8.1
14	1.2 ± 0.1	2.9 ± 0.2	100.0 ± 0.0
17	1.2 ± 0.1	2.6 ± 0.1	93.3 ± 6.6
20	1.2 ± 0.1	2.6 ± 0.2	86.7 ± 13.3
23	1.2 ± 0.0	2.8 ± 0.3	80.0 ± 13.3
26	1.2 ± 0.1	2.9 ± 0.3	93.3 ± 6.6
External reference[1]	1.2 ± 0.1	2.2 ± 0.2	100.0 ± 0.0
ANOVA $Pr > F$	0.8874	0.6556	0.6114

Values are means ± standard error of five replicate samples.
Means with different superscripts are significantly different ($P \leq 0.05$).
Abbreviations: SGR = specific growth rate; TGC = thermal growth coefficient; K = condition factor.
[1] Not included in the statistical analysis.

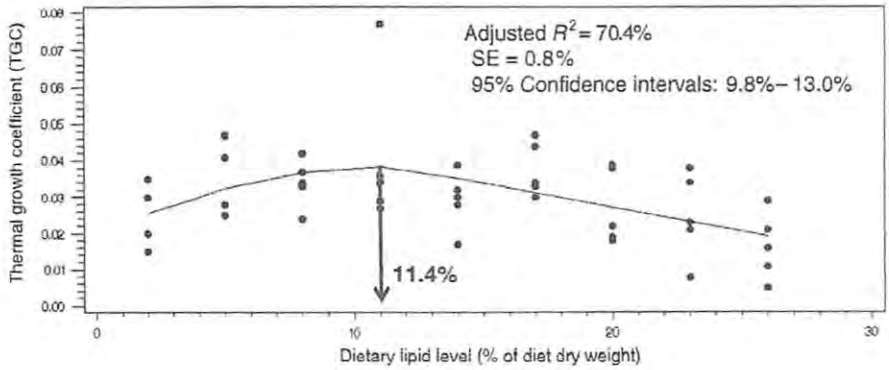

Fig. 1. Two-slope, quadratic broken line estimate of the dietary lipid requirement of *C. othonopterus* fed graded levels of dietary lipid and a constant level (40%) of crude protein.

dietary crude fat levels ranging from 2 to 17% (contents ranging from 75.9 to 76.8%), decreased significantly to 72.5–73.3% in fish fed 20–26% dietary crude fat (Table 4). Treatment means for the ash content of muscle ranged from 1.2 to 1.6%, with significant differences among treatments, but without a clear trend being observed (Table 4).

In liver, the protein content tended to decrease with dietary crude fat. Fish fed dietary crude fat levels of 2 and 8%, had statistically higher protein contents (10.2 ± 0.9 and 11.4 ± 0.8%, respectively) than fish receiving dietary crude fat levels of 23 (6.7 ± 0.7%) and 26% (6.6 ± 0.6%). The liver of fish fed the treatments 5, 11, 14, 17, and 20% crude fat also had higher protein contents, but not significantly, than those of fish receiving 23 and 26% dietary crude fat (Table 4). A trend of progressively higher crude fat content was observed in this tissue as dietary crude fat was augmented. However, this tendency was not statistically significant (Table 4). As it was observed in whole fish and muscle tissue, the moisture content of liver also decreased significantly with increasing dietary crude fat, with statistically higher contents recorded for fish fed 2, 5, and 8% crude fat (ranging from 50.1 to 51.6%), with respect to the rest of the treatments (11–26% crude fat, with moisture contents ranging from 42.2 to 47.3%) (Table 4). Finally, the ash content of liver showed

Table 4
Body and tissue proximate composition (% of wet weight) of *C. othonopterus* fed graded levels of dietary lipid.

	Treatment, crude fat (%)									ANOVA $Pr > F$
	2	5	8	11	14	17	20	23	26	
Whole body										
Crude protein	$18.2^{bc} \pm 0.1$	$19.2^{b} \pm 0.4$	$19.2^{b} \pm 0.0$	$21.7^{a} \pm 0.8$	$18.6^{bc} \pm 0.2$	$18.3^{bc} \pm 0.6$	$18.9^{b} \pm 0.2$	$17.7^{bc} \pm 0.2$	$17.1^{c} \pm 0.9$	0.0004
Crude fat	$5.5^{e} \pm 0.1$	$6.3^{de} \pm 0.2$	$6.9^{d} \pm 0.2$	$7.9^{bc} \pm 0.2$	$7.2^{cd} \pm 0.2$	$8.4^{b} \pm 0.1$	$8.8^{ab} \pm 0.4$	$9.4^{a} \pm 0.4$	$9.5^{a} \pm 0.8$	<0.0001
Moisture	$73.2^{a} \pm 0.7$	$72.3^{ab} \pm 0.1$	$72.2^{ab} \pm 0.7$	$69.9^{c} \pm 0.2$	$70.4^{bc} \pm 0.3$	$68.5^{c} \pm 1.1$	$69.4^{c} \pm 0.8$	$68.7^{c} \pm 0.4$	$69.1^{c} \pm 0.7$	0.0002
Ash	$3.8^{abc} \pm 0.1$	$3.4^{bc} \pm 0.1$	$3.2^{c} \pm 0.2$	$3.5^{bc} \pm 0.1$	$3.7^{abc} \pm 0.1$	$4.3^{a} \pm 0.3$	$4.1^{ab} \pm 0.4$	$4.1^{ab} \pm 0.1$	$3.6^{bc} \pm 0.2$	0.0224
Muscle										
Crude protein	19.3 ± 0.4	19.4 ± 0.2	19.7 ± 0.5	19.8 ± 0.2	19.8 ± 0.4	19.5 ± 0.4	18.5 ± 0.1	19.3 ± 0.2	18.6 ± 0.1	0.0747
Crude fat	$3.0^{c} \pm 0.3$	$2.6^{c} \pm 0.1$	$2.7^{c} \pm 0.1$	$3.8^{b} \pm 0.2$	$4.4^{b} \pm 0.3$	$4.6^{b} \pm 0.2$	$5.6^{a} \pm 0.1$	$6.1^{a} \pm 0.4$	$5.6^{a} \pm 0.2$	<0.0001
Moisture	$76.3^{a} \pm 0.2$	$76.8^{a} \pm 0.6$	$76.8^{a} \pm 0.4$	$76.1^{a} \pm 0.5$	$75.9^{a} \pm 0.4$	$76.1^{a} \pm 0.8$	$72.5^{b} \pm 0.7$	$73.2^{b} \pm 0.7$	$73.3^{b} \pm 0.4$	<0.0001
Ash	$1.4^{ab} \pm 0.1$	$1.3^{bc} \pm 0.1$	$1.2^{c} \pm 0.0$	$1.2^{bc} \pm 0.1$	$1.2^{bc} \pm 0.0$	$1.3^{bc} \pm 0.1$	$1.6^{a} \pm 0.1$	$1.3^{bc} \pm 0.0$	$1.3^{bc} \pm 0.0$	0.0115
Liver										
Crude protein	$10.2^{ab} \pm 0.9$	$9.8^{abc} \pm 1.8$	$11.4^{a} \pm 0.8$	$9.5^{abc} \pm 1.5$	$9.1^{abc} \pm 1.0$	$7.5^{bc} \pm 0.7$	$7.3^{bc} \pm 0.6$	$6.7^{c} \pm 0.7$	$6.6^{c} \pm 0.6$	0.0383
Crude fat	30.1 ± 1.5	29.4 ± 6.4	29.6 ± 2.2	31.8 ± 3.0	34.3 ± 5.1	35.6 ± 2.2	36.7 ± 0.6	35.6 ± 1.8	39.1 ± 2.8	0.3370
Moisture	$50.1^{a} \pm 0.6$	$51.6^{a} \pm 0.4$	$50.7^{a} \pm 0.2$	$46.8^{b} \pm 0.5$	$47.3^{b} \pm 0.8$	$46.5^{b} \pm 1.0$	$47.0^{b} \pm 0.4$	$45.5^{b} \pm 0.7$	$42.2^{c} \pm 0.5$	<0.0001
Ash	$0.7^{bc} \pm 0.1$	$0.7^{c} \pm 0.0$	$0.9^{a} \pm 0.1$	$0.9^{a} \pm 0.0$	$0.9^{ab} \pm 0.0$	$0.9^{a} \pm 0.0$	$0.9^{a} \pm 0.1$	$1.0^{a} \pm 0.1$	$1.0^{a} \pm 0.0$	0.0063

	External reference[1]	Initial fish
Whole body		
Crude protein	17.7 ± 0.2	16.4 ± 0.3
Crude fat	7.1 ± 0.6	6.9 ± 0.7
Moisture	68.8 ± 0.8	72.5 ± 0.4
Ash	3.7 ± 0.2	3.8 ± 0.2
Muscle		
Crude protein	19.8 ± 0.3	18.4 ± 0.6
Crude fat	5.3 ± 0.2	3.2 ± 0.4
Moisture	75.7 ± 0.4	77.4 ± 0.6
Ash	1.1 ± 0.0	1.1 ± 0.0
Liver		
Crude protein	9.4 ± 0.9	13.7 ± 2.4
Crude fat	31.8 ± 4.9	22.0 ± 1.5
Moisture	58.3 ± 2.0	66.3 ± 1.5
Ash	0.6 ± 0.1	0.8 ± 0.0

Values are means ± standard error of triplicate composite samples, each consisting of three fish.
Means with different superscripts within the same row are significantly different ($P \leq 0.05$).
[1] Not included in the statistical analysis.

significant differences among treatments, with significantly lower mean values recorded for fish fed the dietary crude fat levels of 2 (0.7 ± 0.1%) and 5% (0.7 ± 0.0%), in comparison with the treatments 8, 11, 17, 20, 23, and 26% crude fat (ash contents ranging from 0.9 to 1.0%), but there were no statistical differences between the 2 and 14% (0.9 ± 0.1%) treatments (Table 4).

3.4. Evaluation of pancreatic lipase

The mean content of pancreatic lipase tended to decrease with increasing dietary crude fat level, ranging from 0.85 mg/mL of homogenate in fish fed 8% crude fat, to 0.39 mg/mL of homogenate in fish receiving 15 and 26% crude fat. However, these differences were not statistically significant (ANOVA $P = 0.1682$) (Fig. 2). The overall mean molecular weight (±SE) of pancreatic lipase for the Gulf corvina was 57.4 kDa ± 0.7, while the molecular weight of the human pancreatic lipase used as molecular marker was 55.2 kDa ± 0.9, as determined by SDS-PAGE.

4. Discussion

A clear dose–response effect of dietary crude fat was observed on growth of the Gulf corvina, with the best results corresponding to fish fed 11% crude fat, while growth performance was reduced as dietary crude fat departed from this level. These results were significant for SGR and TGC data ($P = 0.0283$ and 0.0450, respectively), and although not statistically significant, the same pattern held true numerically for the majority of the other growth response variables and feed utilization indices measured (Tables 2 and 3). In addition, the fact that measurements of these variables in fish fed 11% crude fat also outperformed numerically those of fish fed a commercial diet is worth noting; this last diet had crude fat and protein contents of 14.11 and 46.15%, respectively (Table 1), and was included as an external reference only. Findings of the present study were further supported by the two-slope, quadratic broken line analysis of TGC data, which estimated a requirement for dietary crude fat of 11.4% for this species. It is known that, for many fish species, crude fat and/or the overall energy content of the diet interacts with crude protein to determine growth (Bowyer et al., 2013). In this study, dietary crude protein was maintained constant at 40% of the diet dry weight, a level reported to be nutritionally sufficient for this species (González-Félix et al., 2013). In this respect, it is worthwhile pointing out that some sciaenids and other carnivorous fish tolerate, within limits, different combinations of these macronutrients, e.g., higher protein/lower energy and vice versa, without adversely affecting growth rate (Craig et al., 2006; Hebb et al., 2003; Turano et al., 2002), an aspect that requires further investigation for the Gulf corvina. Nonetheless, the dietary crude fat requirement of 11.4% estimated for the Gulf corvina in this study, agrees with optimal levels reported for other members of the family Sciaenidae. In general, sciaenids require moderate amounts of lipid, in combination with relatively high requirements for dietary protein (usually at or above 40%). For example, optimal crude fat levels of 17.0–20.0%, in combination with 43–50%

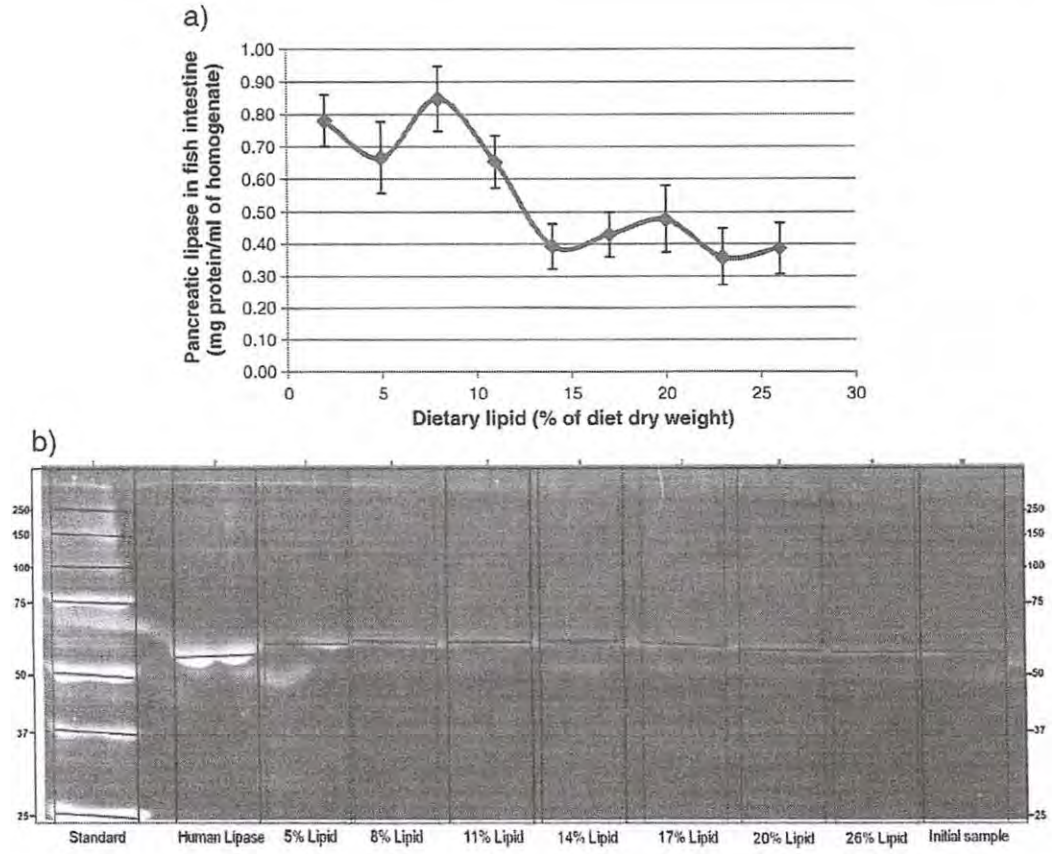

Fig. 2. a) Pancreatic lipase (mg protein/mL of homogenate ± standard error bars) in intestine of *C. othonopterus* fed graded levels of dietary lipid (ANOVA $P = 0.1682$). b) Analysis of pancreatic lipase in the intestine of *C. othonopterus* by SDS-PAGE. Electrophoresis was performed on 10% polyacrylamide gels. Lane 1: Precision Plus Protein™ Standard (Bio-Rad®, Hercules, CA, USA); lane 2: human pancreatic lipase (BCR-693, European Community Bureau of Reference); lane 3: homogenate of fish fed 5% dietary lipid; lane 4: homogenate of fish fed 8% dietary lipid; lane 5: homogenate of fish fed 11% dietary lipid; lane 6: homogenate of fish fed 14% dietary lipid; lane 7: homogenate of fish fed 17% dietary lipid; lane 8: homogenate of fish fed 20% dietary lipid; lane 9: homogenate of fish fed 26% dietary lipid; and lane 10: homogenate of initial sample of fish at the beginning of the experiment.

crude protein, were reported for *Argyrosomus regius* (Chatzifotis et al., 2010, 2012; Martínez-Llorens et al., 2011). For juvenile red drum, *Sciaenops ocellatus*, McGoogan and Gatlin (1999) observed maximum growth when fed 15% crude fat and 45% crude protein, while Turano et al. (2002) found that subadults tolerated 7.6–16.3% crude fat, combined with 36 to 44% crude protein, without showing significant differences in growth. In addition, Thoman et al. (1999) recommended 9.2% dietary crude fat and 44.0% crude protein to avoid excessive lipid carcass deposition for this species. Wang et al. (2006) observed optimal growth of the cuneate drum (*Nibea miichthioides*) fed crude fat levels between 15.0 and 17.0%. For the yellow croaker, *Pseudosciaena crocea*, Duan et al. (2001) reported optimal dietary crude fat and protein levels to be 10.5 and 47%, respectively. In turn, Chai et al. (2013) suggested dietary crude fat and protein levels of 9.0 and 48%, respectively, as optimal for the giant croaker, *N. japonica*. Finally, a diet combining 18% crude fat and 46% crude protein produced the best growth performance of juvenile *A. japonicus* (Woolley et al., 2010). In contrast to what has been described for the warm-water sciaenids, some cold-water species typically tolerate high dietary lipid levels, such as brown trout (*Salmo trutta*), which was reported to grow better when fed 29% lipid, as compared to 21% (Arzel et al., 1993), or Atlantic salmon (*Salmo salar*), which had higher growth rates when fed 38–47% lipid, compared to 31% (Hemre and Sandnes, 1999). However, excessive fat is normally stored in the body and can adversely affect flesh quality (Bowyer et al., 2013; Hillestad and Johnsen, 1994; Hillestad et al., 1998).

As expected, dietary energy increased from 17.50 to 23.10 kJ/g with increasing dietary lipid, but because the diets used in the present study were isoproteic, the dietary protein (P) to energy (E) ratio decreased, from 23.25 to 17.56 gP/MJ, as dietary lipid increased (Table 1). The experimental lipid level that elicited the best results for the Gulf corvina (11%) corresponds to a P/E ratio of 20.42 gP/MJ, which concurs with the range of P/E ratios, from 20.7 to 28.6 gP/MJ, known to produce optimal growth of other sciaenids, such as *A. japonicus*, *A. regius*, *S. ocellatus*, *N. japonica*, *N. miichthioides*, and *Totoaba macdonaldi* (Chai et al., 2013; Chatzifotis et al., 2012; Martínez-Llorens et al., 2011; McGoogan and Gatlin, 1999; Pirozzi et al., 2010; Rueda-López et al., 2011; Turano et al., 2002; Wang et al., 2006; Woolley et al., 2010) (values were either directly taken or calculated from dietary protein and energy content data reported by the authors). Comparable optimal P/E ratios also have been reported for other seawater carnivorous, non-sciaenid fish, such as *Dentex dentex* (19.5 gP/MJ for 10-g fingerlings, and 23.7 gP/MJ for 92.4-g juveniles), *Lateolabrax japonicus* (25.9 gP/MJ), *Pleuronectes americanus* (26.6 gP/MJ), and *Rachycentrum canadum* (22.4–28.8 gP/MJ) (Ai et al., 2004; Hebb et al., 2003; Skalli et al., 2004; Wang et al., 2005).

In agreement with observations of increased lipid deposition with increasing dietary lipid, a significant clear-cut trend of higher content of lipid in whole body and muscle of the Gulf corvina was observed as dietary lipid increased. This tendency has been reported in whole body or tissues from other sciaenids, such as *S. ocellatus*, *A. regius* and *N. miichthioides* (McGoogan and Gatlin, 1999; Wang et al., 2006; Woolley et al., 2010), as well as for fishes belonging to other families, such as *Paralichthys olivaceus*, *Epinephelus malabaricus*, *D. dentex*, and *S. salar* (Hemre and Sandnes, 1999; Lee et al., 2000; Shiau and Lan, 1996; Skalli et al., 2004). In this study and also in other fish species (*Liza macrolepis*, *R. canadum*, and *N. miichthioides*), increased somatic lipid occurs concomitantly with a reduction of the body moisture content (Rangaswamy et al., 1998; Wang et al., 2005, 2006). However, body moisture did not decrease with dietary lipid in *Siganus rivulatus* and *A. japonicus* (Ghanawi et al., 2011; Woolley et al., 2010). Interestingly, although the lipid content of liver of the Gulf corvina tended to increase with increasing dietary lipid, this trend was not significant, which has also been observed in viscera of *T. macdonaldi* (Rueda-López et al., 2011). In contrast, significantly increased lipid deposition has been observed in liver of cod (*Gadus morhua*) and red drum (*S. ocellatus*), in response to increasing dietary lipid (Burr et al., 2006; Grisdale-Helland et al., 2008). These results depict contrasting capacities of different fish species to use the liver as a lipid storage site. On the other hand, because the crude fat content in muscle of the Gulf corvina ranged from 2.6 to 6.1%, in fish fed 2% and 23% dietary lipid, respectively, this species would be categorized as low- to medium-fat fish, according to the classification of fishes based upon their meat lipid content (Ackman, 1990). However, it should be taken into account that the higher muscle lipid contents herein observed were experimentally elicited by high levels of dietary lipid. The muscle lipid content of 3.8% observed in fish fed the optimal lipid level ($\approx$11%), as well as the 3.2% muscle lipid content observed in the initial sample of fish, would place this species into the low-fat fish category (2–4% fat), similarly to wild *C. phoxocephalus*, another low-fat fish belonging to the family Sciaenidae (Murillo et al., 2014). For comparative purposes, it would be of interest determining the seasonal variation of the lipid content of wild Gulf corvina in future studies. Muscle crude protein content was not significantly altered by dietary lipid in the Gulf corvina, as also was reported for *E. malabaricus* and *A. regius* (Chatzifotis et al., 2010; Lin and Shiau, 2003), but in some instances, dietary lipid has elicited either increased body or tissue crude protein in some species (*E. malabaricus*, *P. olivaceus*, and *L. japonicus*), or decreased crude protein in others (*D. dentex*, *R. canadum*, and *P. olivaceus*) (Ai et al., 2004; Lee et al., 2000; Shiau and Lan, 1996; Skalli et al., 2004; Wang et al., 2005). In this study, significant differences were detected in the ash content of fish muscle, but without a clear trend observed. Similarly, no clear effect of dietary lipid on muscle ash content has been reported for *A. regius*, *R. canadum*, and *P. olivaceus* (Chatzifotis et al., 2010; Lee et al., 2000; Wang et al., 2005).

Digestion of dietary triglycerides is driven to completion in the intestine by pancreatic lipase, in conjunction with pancreatic colipase and bile in mammals (Sebban-Kreuzer et al., 2003). Triacylglycerol is a major lipid class in the diet of marine fish and is generally the predominant lipid class in the diet of freshwater fish (Tocher, 2003). In mammalian gut, they are hydrolyzed by two main lipases, the pancreatic lipase–colipase system, and the less specific bile salt-activated lipase. Although evidence points to the presence of a bile-salt activated lipase in teleost fish, there is also evidence for a pancreatic lipase–colipase system (Tocher, 2003), as postulated for trout by Leger (1985). Tocher and Sargent (1984) suggested that one enzyme predominantly hydrolyzed triacylglycerol, and the other wax and steryl esters. A pancreatic lipase has been reported in top minnow (*Tripoртheus* sp.) by Patton et al. (1978), in sardine (*Sardinella longiceps*) by Mukundan et al. (1985), in cod (*G. morhua*) by Gjellesvik (1991) and Gjellesvik et al. (1992), and in turbot (*Scophtalmus maximus*) by Koven et al. (1994a, 1994b). In some fish, such as *Dicentrarchus labrax* and *S. ocellatus*, increasing levels of dietary lipid have elicited greater pancreatic lipase activity until a plateau is reached, describing a classical enzyme–substrate interaction (Buchet et al., 2000; Zambonino Infante and Cahu, 1999, 2001). However, the correlation between dietary lipid level and the corresponding enzymes is not clear-cut for all fish. For example, in the present study, lipase content seemed to decrease with increasing dietary lipid level, although this pattern was not statistically significant. Similarly, in the euryhaline atherinid *Odontesthes bonarienis*, lipase activity decreased as dietary lipid increased from 6 to 25% (Gómez-Requeni et al., 2013). Interestingly, long-term high-fat feeding in mice elicited increased pancreatic lipase expression initially ($P < 0.05$), but at the end of the study (113 days), mice became obese and glucose-intolerant, and the lipase levels had decreased to the level of the control diet (Rippe et al., 2003). In other studies, lipase activity in *Sparus aurata* and larval *D. labrax* was not affected by high levels of dietary lipid (García-Meilán et al., 2013; Morais et al., 2004). Activity of lipase and other pancreatic enzymes is known to vary with factors such as fish species, age, lipid source, quality of dietary lipid, and prandial status, among others (Morais et al., 2007). Fish in the present study were fed for the last time approximately 15 h before being sacrificed, which may have lessened the strength of the enzyme content detected. Shorter, and possibly

variable time since last meal should be taken into account in future studies on pancreatic lipase of the Gulf corvina. With respect to the molecular weight of pancreatic lipase observed for the Gulf corvina (57.4 kDa ± 0.7) and for the human pancreatic lipase used as molecular marker and control (55.2 kDa ± 0.9), both of these results agree with values previously reported, using SDS-PAGE, for fish (e.g., 60 and 74 kDa for cod and carp pancreatic lipases, respectively) and human pancreatic lipase (50–51 kDa) (Gjellesvik et al., 1992; Görgün and Akpinar, 2012; Iizuka et al., 1991).

In summary, a clear dose–response effect of dietary crude fat was observed on growth of the Gulf corvina, *C. othonopterus*. Based upon two-slope, quadratic broken line analysis of thermal growth coefficient data, the requirement for dietary crude fat for this species was estimated to be 11.4%, when fed a diet containing 40% crude protein. Significantly increased lipid deposition, concomitant with reduced moisture content in muscle and whole body were observed in response to incremental levels of dietary lipid. Lipase content tended to decrease with increasing dietary crude fat level, although this pattern was not statistically significant.

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Plant protein sources in the diets of the sciaenids red drum (*Sciaenops ocellatus*) and shortfin corvina (*Cynoscion parvipinnis*): A comparative study

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Cynoscion parvipinnis

ABSTRACT

Two parallel feeding trials were conducted to determine the responses of red drum (*Sciaenops ocellatus*, initially weighing 4.7 ± 0.3 g) and shortfin corvina (*Cynoscion parvipinnis*, initially weighing 8.6 ± 1.0 g) to increasing dietary percentages of non-genetically modified soybean meal (SBM-3010), soybean protein concentrate (Soy-PC) and corn protein concentrate (Corn-PC) as replacements for menhaden fishmeal. A basal diet contained (dry-matter basis) 40% crude protein from menhaden fishmeal and 10% crude fat primarily from menhaden fish oil. Then, nine diets were formulated by replacing 25%, 50% and 75% of protein in the basal diet with SBM-3010, Soy-PC and Corn-PC, while adding crystalline supplements of lysine, methionine, taurine, and glycine. After 7 weeks of culture for red drum and 8 weeks for shortfin corvina, treatment mean final weights ranged from 28.8 to 40.7 g and from 35.2 to 51.6 g, respectively. Significant ($P < 0.05$) differences were detected across all growth performance and feed utilization parameters for both red drum and shortfin corvina. When supplemented with amino acids, SBM-3010 and Soy-PC can replace up to 75% of menhaden fishmeal protein, and that Corn-PC can successfully replace up to 50% of fishmeal protein without compromising the performance of red drum. Likewise, for juvenile shortfin corvina, Soy-PC and Corn-PC can replace up to 75% of fishmeal protein in the diet, while SBM-3010 successfully replaced up to 50% of fishmeal protein without compromising fish performance. The whole-body proximate composition was significantly influenced by the dietary treatments in shortfin corvina, but not in red drum.

Statement of relevance

The present manuscript provided an excellent opportunity to conduct comparative nutrition research and advance the development of more sustainable diets for the aquaculture of sciaenid species. For red drum, *Sciaenops ocellatus*, it was observed that Soybean meal (SBM) and soybean protein concentrate (Soy-PC) can replace up to 75% of fishmeal protein in the diet; while corn protein concentrate (Corn-PC) can successfully replace up to 50% of fishmeal protein, without compromising fish growth performance. For shortfin corvina, *Cynoscion parvipinnis*, Soy-PC and Corn-PC can replace up to 75% of fishmeal protein in the diet, while SBM successfully replaced up to 50% of fishmeal protein.

1. Introduction

As aquaculture production has grown, so has the demand for fishmeal to be included in cultured fish diets because it is considered a highly digestible protein component, containing high levels of most essential amino acids. However, in recent years, it has become evident that the increasing demand for fishmeal is surpassing supply, resulting in increasing prices (Tacon and Metian, 2008). Moreover, environmental pressures to use more sustainable resources and restrictions on contaminants in animal feeds demand that, for the aquaculture industry to continue to expand, sustainable and economically feasible alternatives to fishmeal must be found (NRC, 2011).

A considerable amount of effort is being devoted to identifying, developing, and evaluating alternative protein sources for aquaculture feeds. Plant based feedstuffs, including products derived from the processing of soybean, e.g., soybean meal (SBM), soy protein concentrate (Soy-PC), as well as of corn, e.g., corn gluten meal and corn protein

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concentrate (Corn-PC), are an important group of alternative ingredients currently evaluated to replace fishmeal in aquatic feedstuffs. Nonetheless, aspects such as presence of undigestible carbohydrate content, deficiency in some essential amino acids and the presence of antinutritional factors (e.g., protease inhibitors, phytic acid) must be controlled (Gatlin et al., 2007; Krogdahl et al., 2010).

Soybean protein has been recognized as one of the most feasible alternative protein sources because of its wide availability, consistent nutrient composition, balanced amino acid profile, and reasonable price (Gatlin et al., 2007; Kader et al., 2012). However, it also contains low levels of the sulfur-containing amino acids methionine and cysteine, as well as various antinutritional factors, which may restrict its level of inclusion in feeds for some aquatic species (Gatlin et al., 2007). Only recently, selective soy breeding programs have made it possible to produce novel non-genetically modified soy varieties with reduced levels of antinutritional factors, such as oligosaccharides and protease inhibitors (Buentello et al., 2015). In a similar trend, plant protein concentrates with high protein and low carbohydrate content have considerable potential as alternatives to fishmeal. Soy-PC has more than 600 g kg^{-1} of crude protein and lower levels of antinutritional factors relative to SBM (NRC, 2011). It has been utilized with significant success in diets of several marine fish species including Japanese flounder, *Paralichthys olivaceus* (Deng et al., 2006), Atlantic halibut, *Hippoglossus hippoglossus* (Berge et al., 1999), Atlantic salmon, *Salmo salar* (Storebakken et al., 2000), cobia, *Rachycentron canadum* (Salze et al., 2010), and Red Sea bream, *Pagrus major* (Takagi et al., 2001). Similarly, Corn-PC may contain up to 800 g kg^{-1} of crude protein and less than 10 g kg^{-1} of starch (AAFCO, 2007); additionally, as opposed to soybean feedstuffs, Corn-PC contains fewer antinutritional factors and is rich in sulfur-containing amino acids, but deficient in lysine (Hardy, 2010). Partial replacement of fishmeal utilizing corn-derived products has been successfully evaluated in practical diets for marine fish species such as Japanese flounder, *P. olivaceus* (Kikuchi, 1999), cobia (Luo et al., 2013), and turbot, *Psetta maxima* (Regost et al., 1999).

Two sciaenid species were the subject of the present study, red drum (*Sciaenops ocellatus*) and shortfin corvina (*Cynoscion parvipinnis*). Red drum is a euryhaline sciaenid native to the Gulf of Mexico and Atlantic coasts of the United States. This carnivorous species is extensively cultured along both coasts for stock enhancement and seafood production purposes, and has traditionally been fed fishmeal-based diets (Gatlin, 2002). However, previous studies have demonstrated the possibility of incorporating relatively high levels of SBM, Soy-PC and barley-PC in the diets of red drum (McGoogan and Gatlin, 1997; Rossi et al., 2013). On the other hand, the shortfin corvina is a related sciaenid species that inhabits inshore waters with sandy bottoms from the Gulf of California, Mexico to southern California, USA (Chao, 1995). Commercial overfishing and habitat alteration provoked a decline in their natural populations; therefore, the shortfin corvina is being evaluated as a candidate for aquaculture at several research institutions in Mexico, as has recently occurred with other commercially important sciaenids in the Gulf of California, such as totoaba, *Totoaba macdonaldi* and Gulf corvina, *Cynoscion othonopterus* (Chao, 1995; CITES, 2005; Paredes et al., 2010).

The objective of this study was to evaluate and compare the responses of red drum and shortfin corvina to the inclusion of three different dietary plant protein feedstuffs in the diet, SBM, Soy-PC, and Corn-PC, as replacements for menhaden fishmeal. This provided an excellent opportunity to conduct comparative nutrition research and advance the development of artificial diets to support the aquaculture programs for sciaenid species. Additionally, the potential substitution of fishmeal with plant protein feedstuffs will directly contribute to more environmentally and economically sustainable use of coastal and marine resources, and directly benefit public and private aquaculture operations.

2. Materials and methods

2.1. Diet formulation

Two parallel feeding trials were conducted to determine the responses of red drum and shortfin corvina to increasing dietary percentages of non-genetically modified SBM (Navita Premium Feed Ingredients, SBM-3010), Soy-PC and Corn-PC as replacements for menhaden fishmeal. With respect to the test ingredient SBM-3010, recent studies have shown that, in comparison to average contents of the antinutritional oligosaccharides stachyose (ST) and raffinose (RA) (4.0 and 1.5%, respectively), as well as of trypsin inhibitor activity (8.3 mg/g) found in conventional SBM (Ireland et al., 1986; Goda et al., 2002; Francis et al., 2001; Hart et al., 2010), the novel non-genetically modified soybean cultivars such as SBM-3010 contain significantly lower levels of these antinutritional factors. For example, 67.5% to 87.5% less stachyose, 73.3 to 93.3% less raffinose, and 1.2 to 1.7 mg/g trypsin inhibitor activity (Buentello et al., 2015). A basal, control diet was formulated and analyzed to contain, on a dry-matter basis, 40% crude protein and 10% total lipid primarily from menhaden fishmeal and menhaden fish oil, and dextrin as the soluble carbohydrate to provide approximately 3.1 kcal digestible energy g^{-1} diet, optimal nutritional requirements for red drum (Serrano et al., 1992). Then, nine experimental diets were formulated by replacing 25%, 50% and 75% of protein in the control diet with SBM-3010, Soy-PC and Corn-PC (Table 1). The experimental diets were supplemented with crystalline L-lysine HCl, DL-methionine and taurine to meet levels provided by the fishmeal control diet (NRC, 2006). Additionally, L-glycine was included in the experimental diets as a palatability enhancer (McGoogan and Gatlin, 1997). All diets were mechanically mixed and pressure pelleted as previously described by McGoogan and Gatlin (1997).

2.2. Feeding trials

2.2.1. Red drum

The feeding trial was conducted at the Texas A&M University Aquacultural Research and Teaching Facility in 110-L aquaria configured as a recirculating system (1 L min^{-1}), whereby waste water gravity flowed to a settling chamber, then to a biological filter and was pumped through an ultraviolet light chamber and sand filter before being returned to the aquaria. Water quality was maintained within acceptable levels for red drum. Salinity was maintained at 6–8 g L^{-1} by combining a synthetic seawater mixture (Fritz Industries) with sodium chloride and well water. Low-pressure electrical blowers provided aeration via air stones to maintain dissolved oxygen levels near air saturation. Water temperature was maintained at 27 ± 1 °C by controlling ambient temperature with dual air-conditioning units. A 12 h light–12 h dark photoperiod was maintained with fluorescent lights controlled by automatic timers.

At the start of the feeding trial, groups of 20 juvenile red drums were stocked into the aquaria at mean wet body weight (±standard deviation) of 4.64 ± 0.21 g per fish. Fish were subjected to a 1-week conditioning period during which the control diet was fed to apparent satiation while fish became acclimated to the culture conditions. After the conditioning period, triplicate aquaria were randomly assigned to each dietary treatment (n = 3). The fish were fed twice daily at a rate approaching apparent satiation with pre-weighed rations based on a percentage of total fish weight per aquarium (5–6% of total body weight) and visual feeding cues. Fish in each aquarium were group-weighed every week and feed rations adjusted accordingly. The feeding trial continued for a total of 7 weeks.

2.2.2. Shortfin corvina

The feeding trial was conducted at the Wet Laboratory of Aquaculture Nutrition of the Kino Bay Experiment Station (KBES), University

Table 1
Formulation and analyzed proximate composition of the experimental diets.

Ingredients	Fishmeal 100%	SBM-3010 25%	SBM-3010 50%	SBM-3010 75%	Soy-PC 25%	Soy-PC 50%	Soy-PC 75%	Corn-PC 25%	Corn-PC 50%	Corn-PC 75%
Fish meal[1]	59.3	44.5	29.6	14.8	44.5	29.6	14.8	44.5	29.6	14.8
Soybean meal 3010[2]	0.00	16.6	33.1	49.7	0.00	0.00	0.00	0.00	0.00	0.00
Soy protein concentrate[3]	0.00	0.00	0.00	0.00	14.2	28.3	42.5	0.00	0.00	0.00
Corn protein concentrate[4]	0.00	0.00	0.00	0.00	0.00	0.00	0.00	11.8	23.5	35.3
Dex. starch[5]	17.5	15.0	15.0	15.0	17.5	17.5	17.5	17.5	17.5	17.5
Menhaden oil[1]	2.55	3.77	4.99	6.20	4.14	5.73	7.32	4.18	5.80	7.43
Vitamin premix[6]	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Mineral premix[6]	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
Carboxymethylcellulose[5]	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Glycine[5]	0.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Lysine HCl[5]	0.00	0.09	0.19	0.29	0.25	0.50	0.75	0.71	1.43	2.14
DL-methionine[5]	0.00	0.19	0.37	0.56	0.06	0.12	0.18	0.00	0.00	0.00
Taurine[5]	0.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Alphacel[5]	11.7	7.95	4.72	1.49	7.44	6.20	4.95	9.40	10.1	10.8
Determined proximate composition (g/100 g DM)										
Crude protein	41.3	44.7	44.5	43.9	45.1	45.3	45.4	45.4	46.2	46.2
Crude fat	11.5	11.3	10.9	10.2	11.6	11.0	10.7	15.1	16.5	18.9
Ash	15.3	13.4	11.4	9.3	12.5	10.8	7.9	12.3	9.3	6.2
Dry matter	87.1	85.9	85.6	85.0	84.3	86.6	84.1	86.9	86.9	87.4

[1] Special Select™, Omega Protein Inc., Abbeville, LA. USA.
[2] Navita Premium Feed Ingredients, Inc., West des Moines, IA, USA.
[3] The Solae Company, St. Louis, MO, USA. As dry: crude protein = 722.3 g/kg; lipid = 21.3 g/kg.
[4] Empyreal 75, Cargill Corn Milling, Blair, NE, USA. As dry: crude protein = 826.7 g/kg; lipid = 113.6 g/kg.
[5] USB Corporation, Cleveland, OH, USA.
[6] Same as Moon and Gatlin (1991).

of Sonora at Kino Bay, Sonora, Mexico. Two identical recirculating experimental culture systems were used, each system composed of fifty 30-cm diameter circular polyethylene tanks (bottom area of 0.07 m^2 and 19-L), a 1100-L sump tank, a biofilter, a 47.5-cm sand filter, a 1.5-HP pump, a 20-μm pore size cartridge filter, a 120-W ultraviolet light chamber, a 1500-W in-line heater, and a 0.5 HP in-line chiller. Aeration for both culture systems was provided by a 1.0-HP blower. Seawater recirculation rate in each tank was 1.9 L min^{-1}, and approximately 50% daily replacement with fresh-filtered seawater was applied. The culture systems were interconnected with each other; therefore, they shared the same water quality.

Fish with an overall individual mean wet body weight of 8.6 ± 1.0 g were stocked into tanks at a rate of 4 individuals per tank, assigning each experimental treatment to 10 replicate tanks (n = 10). After a 1-week conditioning period, the fish were fed thrice daily at a rate approaching apparent satiation with pre-weighed rations based on a percentage of total fish weight per tank (3% of total body weight) and visual feeding cues at 08:00, 13:00 and 18:00 h for 8 weeks. Fish in each tank were group-weighed weekly and feed rations adjusted accordingly. Daily measurements of dissolved oxygen, temperature, and salinity of culture water were performed with a multi-function oxygen meter (YSI, Model Y85, Yellow Springs, Ohio, USA), while pH was measured with a hand-held pH meter (Oakton®, Model Double Junction pHTestr 1, Vernon Hills, Illinois, EUA). Weekly measurements of the concentrations of total ammonia nitrogen and nitrite were performed following procedures adapted from those of Solórzano (1969); Spotte (1979a, 1979b) and Strickland and Parsons (1972). Mean values (± standard deviation) for temperature, salinity, pH, dissolved oxygen, total ammonia nitrogen, and nitrite in the culture water were 30.5 ± 0.7 °C, 37.5 ± 1.1‰, 7.7 ± 0.2, 7.2 ± 0.7 mg L^{-1}, 0.134 ± 0.045 mg L^{-1}, and 0.111 ± 0.183 mg L^{-1}, respectively.

2.3. Sampling methods, performance indices and laboratory analysis

At the conclusion of both feeding trials, final fish weight and percent survival in each tank were determined. Then, three fish per tank were randomly sampled at 15 h after the last feeding (postprandial time point). Total body weight, along with filets (muscle), liver, and intraperitoneal fat weights were recorded for calculation of morphometric and body condition indices as follows: condition factor (g body weight × 100/[mm body length^3]), muscle ratio (g filet weight/100 g body weight), hepatosomatic index (HSI) (g liver weight/100 g body weight) and intraperitoneal fat (IPF) ratio (g IPF weight/100 g body weight). The IPF ratio of the shortfin corvina was not obtained because these fish did not accumulate IPF. Additionally, whole-body pooled samples of red drum (n = 3 per tank) and shortfin corvina (n = 15 per treatment) were taken and stored at −20 °C until the analysis of proximate composition using established methodology: Dumas protocol for crude protein (N factor = 6.25) (AOAC, 2005), crude fat (Folch et al., 1957), and ash (AOAC, 1990). At the start of each feeding trial, five fish were taken to obtain the initial whole-body proximate composition. All fish were euthanized (MS-222 at 300 mg/L) prior to sample collection. Growth performance and feed efficiency indicators were computed as follows: relative weight gain ([g final weight − initial weight / g initial weight] × 100), daily weight increment (g final weight gain/days), feed efficiency ratio (g weight gain/g dry feed intake), protein efficiency ratio (g weight gain/g dry protein fed) and protein retention ([final body protein − initial body protein] × 100 / total protein fed).

2.4. Non-specific immunity assays

Non-specific immunity assays were performed only on shortfin corvina. Twelve fish from each experimental treatment were randomly selected and euthanized for blood collection. Approximately 1.0 mL of blood from each fish was collected from the hemal arch in the caudal peduncle using 1-mL syringe with 26-gauge, heparinized needles. A portion of the collected blood was used to determine neutrophil oxidative radical production. Additionally, plasma was separated from the collected blood by centrifugation and stored at −80 °C for subsequent determination of lysozyme activity. Neutrophil oxidative radical production was determined spectrophotometrically by means of nitroblue tetrazolium (NBT) reduction, as described by Siwicki et al. (1994). Plasma lysozyme activity was determined as described by Engstad et al. (1992), using a suspension of *Micrococcus lysodeikticus* (Sigma-Aldrich) and hen egg white lysozyme as standard. A lysozyme activity unit was defined as the amount of enzyme producing a decrease in absorbance of 0.001 min^{-1}.

2.5. Statistical analysis

All response criteria were subjected to one-way analysis of variance (ANOVA) with significance set at $P < 0.05$. Significant one-way ANOVA were followed by Duncan's *post hoc* multiple comparison test. Prior to one-way ANOVA, data expressed as percentages were arcsine transformed. All statistical analysis was conducted using the Statistical Analysis System (SAS 9.4) software package.

3. Results

3.1. Fish performance parameters

3.1.1. Red drum

All growth performance parameters evaluated on red drum were significantly ($P < 0.01$) affected by the experimental diets (Table 2). Final weight mean values ranged from 20.0 to 40.7 g, showing daily weight increment values ranging from 0.31 to 0.73 g day^{-1} across all dietary treatments. Feed efficiency ratio presented mean values ranging from 0.62 to 0.86, showing significantly higher values in fish fed diets containing 25 and 50% SBM-3010, compared to those fed diets containing 75% Soy-PC, 25–75% Corn-PC and the control diet. With respect to protein efficiency ratio and protein retention, mean values ranged from 1.36 to 1.94 and 22.8 to 34.8, respectively. In this regard, fish fed diets containing 25–75% of SBM-3010 showed significantly higher protein efficiency ratio in comparison to those fed diets containing 75% Soy-PC and 25–75% Corn-PC, while significantly higher protein retention values were observed in fish fed diets containing 25% SBM-3010 and 50% Soy-PC, compared to those fed diets containing 75% Soy-PC, 25–75% Corn-PC and the control diet.

3.1.2. Shortfin corvina

Significant ($P < 0.05$) differences also were detected across all growth performance and feed utilization parameters measured for the shortfin corvina at the end of the feeding trial (Table 2). Final weight gain showed mean values ranging from 27.3 to 42.8 g, so that daily weight increment values ranged from 0.5 to 0.8 g day^{-1}. Fish fed diets containing plant ingredients in replacement of menhaden fishmeal showed better growth performance in comparison with those fed the control diet, with the exception of those fed the 75% SBM-3010 diet where no significant differences were observed in terms of relative weight gain when compared to the control diet. Significantly higher feed efficiency ratio was found in fish fed the 25% Soy-PC diet in comparison with those fed diets containing 75% SBM-3010, 75% Soy-PC and the control diet. Similarly, significantly higher protein efficiency ratio and protein retention values were observed in fish fed the 25% Soy-PC diet when compared with those fed the 75% SBM-3010, 75% Soy-PC and control diets.

3.2. Body condition indices and whole body composition

3.2.1. Red drum

Red drum fed the diet containing 50% SBM-3010 showed significantly higher condition factor value in comparison with the rest of the experimental treatments, except for those fish fed diets containing 25 and 75% SBM-3010. In terms of HSI, fish fed the 75% Corn-PC diet presented significantly higher value in comparison with those fed diets containing 50 and 75% SBM-3010, 25% Soy-PC and 25–50% Corn-PC. Additionally, fish fed diets containing 25% SBM-3010 and 50% Soy-PC presented higher muscle ratio values in comparison with fish fed the 75% Corn-PC. However, the IPF ratio values remained unaffected in all experimental treatments (Table 3). Finally, whole-body composition analysis

Table 2
Growth performance, feed utilization and survival of red drum, *S. ocellatus* fed experimental diets for 7 weeks and shortfin corvina, *C. parvipinnis* fed experimental diets for 8 weeks.

	Fishmeal 100%	SBM-3010 25%	SBM-3010 50%	SBM-3010 75%	Soy-PC 25%	Soy-PC 50%	Soy-PC 75%	Corn-PC 25%	Corn-PC 50%	Corn-PC 75%	P>F[3]	MSE[4]
Red drum, *S. ocellatus*[1]												
Initial mean weight, g	4.56	4.76	4.68	4.67	4.71	4.62	4.73	4.77	4.83	4.61	0.41	0.08
Final mean weight, g	28.8^{C}	40.7^{A}	37.8^{AB}	39.3^{A}	37.3^{AB}	37.4^{AB}	33.0^{BC}	32.4^{BC}	32.3^{BC}	20.0^{D}	0.00	1.16
Weight gain, g	24.2^{C}	35.9^{A}	33.1^{AB}	34.6^{A}	32.6^{AB}	32.8^{AB}	28.3^{BC}	27.6^{BC}	27.5^{BC}	15.39^{D}	0.00	0.91
Relative weight gain, %	535^{D}	758^{A}	709^{AB}	741^{A}	702^{ABC}	709^{AB}	598^{BCD}	578^{CD}	576^{CD}	334^{E}	0.00	25.68
Daily weight increment, g	0.49^{C}	0.73^{A}	0.68^{AB}	0.71^{A}	0.66^{AB}	0.67^{AB}	0.58^{BC}	0.56^{BC}	0.56^{BC}	0.31^{D}	0.00	0.01
Feed efficiency ratio	0.74^{C}	0.86^{A}	0.85^{A}	0.83^{AB}	0.83^{AB}	0.84^{AB}	0.76^{BC}	0.73^{C}	0.72^{C}	0.62^{D}	0.00	0.02
Protein efficiency ratio	1.80^{ABC}	1.93^{A}	1.94^{A}	1.94^{A}	1.85^{ABC}	1.88^{AB}	1.68^{BCD}	1.65^{CD}	1.56^{DE}	1.36^{E}	0.00	0.04
Protein retention, %	28.2^{BCDE}	34.8^{A}	32.2^{ABC}	32.5^{AB}	29.2^{ABCD}	34.0^{A}	26.5^{CDE}	27.3^{BCDE}	25.7^{DE}	22.8^{E}	0.00	1.15
Survival, %	86.7^{AB}	85.0^{AB}	88.3^{AB}	85.0^{AB}	90.0^{AB}	90.0^{AB}	85.0^{AB}	80.0^{AB}	76.7^{B}	98.3^{A}	0.04	3.69
Shortfin corvina, *C. parvipinnis*[2]												
Initial mean weight, g	7.9	8.8	8.1	9.1	8.3	9.0	8.6	8.6	8.8	8.9	0.16	0.32
Final mean weight, g	35.2^{B}	51.6^{A}	48.7^{A}	45.6^{A}	45.2^{A}	51.0^{A}	47.8^{A}	46.4^{A}	47.7^{A}	48.1^{A}	0.00	2.26
Weight gain, g	27.3^{B}	42.8^{A}	40.6^{A}	36.5^{A}	36.9^{A}	42.0^{A}	39.3^{A}	37.7^{A}	39.0^{A}	39.3^{A}	0.00	2.12
Relative weight gain, %	349^{C}	487^{A}	503^{A}	406^{BC}	448^{AB}	473^{AB}	464^{AB}	438^{AB}	447^{AB}	449^{AB}	0.00	22.72
Daily weight increment, g	0.5^{B}	0.8^{A}	0.7^{A}	0.7^{A}	0.6^{A}	0.8^{A}	0.7^{A}	0.7^{A}	0.7^{A}	0.7^{A}	0.00	0.02
Feed efficiency ratio	0.62^{CD}	0.74^{ABC}	0.74^{AB}	0.58^{D}	0.78^{A}	0.70^{ABCD}	0.65^{BCD}	0.74^{ABC}	0.73^{ABC}	0.67^{ABCD}	0.00	0.04
Protein efficiency ratio	1.7^{ABC}	1.9^{AB}	1.9^{AB}	1.5^{C}	2.0^{A}	1.8^{ABC}	1.7^{BC}	1.8^{ABC}	1.8^{ABC}	1.7^{BC}	0.04	0.10
Protein retention, %	29.9^{BC}	35.2^{AB}	35.1^{AB}	28.0^{C}	36.5^{A}	32.9^{ABC}	28.5^{C}	34.4^{AB}	33.2^{ABC}	30.8^{ABC}	0.00	1.79
Survival, %	92.5	90.0	90.0	97.5	90.0	87.5	92.5	92.5	90.0	92.5	0.09	4.03

[1] Values represent means of three replicate groups ($n = 3$).
[2] Values represent means of ten replicate groups ($n = 10$).
[3] Significance probability associated with the F-statistic. Different superscript letters within a row indicate significant ($P < 0.05$) differences as evaluated by Duncan test.
[4] Pooled mean standard error.

of red drum in terms of total protein, lipid, moisture and ash content was not influenced by the dietary treatments (Table 3).

3.2.2. Shortfin corvina

In the case of shortfin corvina, the indices K and HSI remained unaffected by the dietary treatments at the end of the experimental trial (Table 3). Nonetheless, the whole-body composition was significantly ($P < 0.05$) affected by the dietary treatments, in terms of crude protein, crude fat, moisture and ash content (Table 3). For instance, fish fed the diet containing 75% Soy-PC presented lower protein content in comparison with the rest of the experimental treatments, except for fish fed the control diet. Additionally, fish fed the 75% Corn-PC diet presented significantly higher lipid content when compared with the rest of the experimental treatments. Moreover, fish fed the 75% SBM-3010 diet presented higher moisture content in comparison to fish fed the diets containing 50 and 75% Soy-PC and 25–75% Corn-PC. Finally, the lowest ash content was presented in fish fed a diet containing 25% Soy-PC, significantly lower than those of the other experimental treatments, except for the 25% SBM-3010 treatment.

3.3. Non-specific immune response

The non-specific immune response of shortfin corvina was significantly ($P < 0.01$) affected by the dietary treatments (Table 4). Blood neutrophils (NBT mL^{-1}) and serum lysozyme activity (unit mL^{-1}) mean values ranged from 7.8 to 10.8 and from 130.0 to 237.5, respectively. In this regard, fish fed the 75% SBM-3010 diet presented significantly higher NBT when compared with the rest of the experimental treatments, with the exception of fish fed the 25% Soy-PC. Also, the lysozyme activity of fish fed the 50 and 75% SBM-3010 diet was significantly higher than those of fish receiving the other dietary treatments.

4. Discussion

The results of the present study indicate that, when supplemented with amino acids, SBM-3010, Soy-PC and Corn-PC can partially substitute menhaden fishmeal in the diet of both juvenile red drum and shortfin corvina. Although there are no preceding studies regarding the nutrition of shortfin corvina, the growth and feed efficiency responses to the different dietary treatments were similar to those found in red drum. This confirms that previous nutritional research conducted with red drum could be applied as a basis for the development of diets to support the aquaculture programs for shortfin corvina. Nonetheless, some differences between the responses of these two sciaenid species were found. For instance, the performance of red drum did not differ when fed either 25, 50, or 75% SBM-3010; in contrast, when fed the 75% SBM-3010 diet, shortfin corvina's growth was significantly reduced. On the other hand, the performance of shortfin corvina was not affected with the replacement of up to 75% of fishmeal protein with Corn-PC, whereas growth of red drum fed this diet was significantly reduced.

Previous studies have assessed the nutritional value of different plant protein ingredients in red drum. McGoogan and Gatlin (1997) found that juvenile red drum fed diets containing up to 90% protein from SBM gained as much weight as fish fed 100% protein from fishmeal. Rossi et al. (2013) concluded that Soy-PC and barley-PC can readily replace half of fishmeal protein; however, a combination of novel non-genetically modified SBM varieties and Soy-PC could not reach the 90% replacement previously reported by McGoogan and Gatlin (1997). Nonetheless, in a subsequent study, Rossi et al. (2015) found that SBM or SBM-3010, in combination with Soy-PC, can replace approximately 86% of the digestible protein provided by menhaden fishmeal without negatively affecting production of advanced juvenile red drum. These results emphasize the importance of evaluating alternative ingredients at different growth stages to optimize the use of plant-based formulations, as has been observed with other species, such as Atlantic salmon, *Salmo salar* (Burr et al., 2012). Therefore, summing to all the previous background, the results of the present study confirm that SBM-3010 and Soy-PC can replace up to 75% of fishmeal protein in the diet of juvenile red drum. In addition, Corn-PC can successfully replace up to 50% of fishmeal protein in the diet.

A wide tolerance to plant ingredients has been observed in other marine fish species. The level of inclusion of different plant feedstuffs in practical diets can go from 30% to complete replacement of fishmeal without compromising growth performance (Buentello et al., 2015; Bulut et al., 2014; Enterria et al., 2011; Jirsa et al., 2014; Kader et al., 2012; Kikuchi, 1999; Luo et al., 2013; Martínez-Llorens et al., 2007;

Table 3
Body condition indices and whole-body proximate composition of red drum, *S. ocellatus* fed experimental diets for 7 weeks and shortfin corvina, *C. parvipinnis* fed experimental diets for 8 weeks.

	Fishmeal 100%	SBM-3010 25%	SBM-3010 50%	SBM-3010 75%	Soy-PC 25%	Soy-PC 50%	Soy-PC 75%	Corn-PC 25%	Corn-PC 50%	Corn-PC 75%	P>F[3]	MSE[4]
Red drum, *S. ocellatus*[1]												
Condition factor	1.13^{BC}	1.20^{AB}	1.27^{A}	1.16^{ABC}	1.12^{BCD}	1.13^{BC}	1.12^{BCD}	1.09^{BCD}	1.07^{CD}	1.00^{D}	0.00	0.03
HSI, %	1.65^{ABCD}	1.64^{ABCD}	1.39^{CD}	1.33^{D}	1.58^{BCD}	1.96^{AB}	1.81^{ABC}	1.43^{CD}	1.57^{BCD}	2.04^{A}	0.00	0.09
IPF ratio, %	1.00	0.58	0.61	0.55	0.75	0.57	0.59	0.72	0.92	0.83	0.33	0.15
Muscle ratio, %	30.9^{AB}	34.1^{A}	33.8^{AB}	33.4^{AB}	31.2^{AB}	35.4^{A}	31.7^{AB}	32.0^{AB}	30.3^{AB}	28.5^{B}	0.01	1.07
Whole-body composition												
Moisture, %	75.43	74.01	74.59	74.40	74.96	73.14	75.37	74.04	73.98	76.74	0.09	0.71
Crude Protein, %	16.22	18.20	17.18	17.29	16.38	18.17	16.46	17.61	17.80	16.54	0.22	0.62
Crude fat, %	4.09	5.26	4.59	3.99	4.76	5.10	4.58	4.22	4.68	4.17	0.26	0.37
Ash, %	3.80	4.20	4.39	3.92	3.63	4.22	3.85	4.44	4.15	3.71	0.15	0.22
Shortfin corvina, *C. parvipinnis*[2]												
Condition factor	1.1	1.1	1.1	1.0	1.1	1.0	1.0	1.1	1.1	1.0	0.99	0.02
HSI, %	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.99	0.02
IPF ratio, %	–	–	–	–	–	–	–	–	–	–	–	–
Muscle ratio, %	44.7	47.4	47.5	47.5	47.3	47.6	47.7	47.6	47.5	47.5	0.96	1.86
Whole-body composition												
Moisture, %	73.5^{abc}	73.5^{abc}	73.8^{ab}	74.3^{a}	73.1^{abcd}	71.6^{def}	72.3^{bcde}	72.1^{cde}	71.3^{ef}	70.2^{f}	0.00	0.28
Crude protein, %	18.1^{bc}	18.8^{a}	18.4^{ab}	18.5^{ab}	18.5^{ab}	18.8^{a}	17.6^{c}	18.8^{a}	18.8^{a}	18.7^{a}	0.00	0.10
Crude fat, %	4.6^{d}	4.5^{de}	3.7^{ef}	3.3^{f}	5.3^{cd}	6.0^{bc}	6.7^{ab}	5.2^{cd}	5.9^{bc}	6.9^{a}	0.00	0.14
Ash, %	4.2^{a}	3.8^{bc}	4.2^{ab}	4.3^{a}	3.5^{c}	4.4^{a}	4.1^{ab}	4.4^{a}	4.1^{ab}	4.3^{a}	0.00	0.07

[1] Values represent means of three replicate groups ($n = 3$).
[2] Values represent means of ten replicate groups ($n = 10$).
[3] Significance probability associated with the F-statistic. Different superscript letters within a row indicate significant ($P < 0.05$) differences as evaluated by Duncan test.
[4] Pooled mean standard error.

Table 4
Blood neutrophils and serum lysozymes content in shortfin corvina, *C. parvipinnis* fed experimental diets during 8 weeks[1].

	Fishmeal 100%	SBM-3010 25%	SBM-3010 50%	SBM-3010 75%	Soy-PC 25%	Soy-PC 50%	Soy-PC 75%	Corn-PC 25%	Corn-PC 50%	Corn-PC 75%	P>F[2]	MSE[3]
Blood neutrophil NBT per mL blood	7.8^{C}	8.3^{BC}	9.1^{BC}	10.8^{A}	9.6^{AB}	9.0^{BC}	8.6^{BC}	8.4^{BC}	8.7^{BC}	8.6^{C}	0.00	0.62
Serum lysozyme, units mL^{-1}	130.0^{EF}	193.3^{BC}	234.2^{AB}	237.5^{A}	179.2^{CD}	168.3^{CDE}	135.0^{DEF}	133.3^{EF}	113.3^{F}	135.0^{EF}	0.00	16.00

[1] Values represent means of 12 fish per treatment.
[2] Significance probability associated with the F-statistic. Different superscript letters within a row indicate significant ($P < 0.05$) differences as evaluated by Duncan test.
[3] Pooled mean standard error.

Regost et al., 1999). This tolerance may be dependent on the species, stage of life and quality of the plant-based meals, in terms of digestibility and presence of antinutritional factors, which are endogenous substances in feedstuffs that could produce negative effects on health, growth performance, and nutrient balance (Gatlin et al., 2007; NRC, 2011). The SBM-3010 evaluated in this study was produced from non-genetically modified varieties of soybean that are selectively bred for relatively higher crude protein and 70–90% removal of antinutritional factors (Buentello et al., 2015). Despite the limited information about the nutritional value of novel SBM varieties, recent studies conducted with fish (Buentello et al., 2015; Suarez et al., 2013; Watson et al., 2014) and Pacific white shrimp, *Litopenaeus vannamei* (Zhou et al., 2014), have reported promising results. In the present study, SBM-3010 successfully replaced 75% of fishmeal protein in red drum's diets; however, shortfin corvina's growth was significantly reduced when fed the 75% SBM-3010 diet. It appears that red drum has a lower sensitivity to the antinutritional factors present in SBM; in fact, Rossi et al. (2013) reported that the inclusion of SBM-3010 in place of regular SBM in red drum's diet resulted in non-significant improvements.

Similar to novel SBM varieties, plant-PCs have several advantages over plant meals including a reduced oligosaccharide content and increased crude protein content. Soy-PC is an ingredient that has been previously evaluated in different fish species (Berge et al., 1999; Deng et al., 2006; Rossi et al., 2013, 2015; Salze et al., 2010; Takagi et al., 2001). On the other hand, the use of Corn-PC in fish or crustacean feeds has been studied only to a limited extent, but some studies have been conducted with corn gluten meal, a product that is relatively similar and highly digestible to juvenile cobia (Zhou et al., 2004) and rainbow trout (Morales et al., 1994). Also, it has been determined that corn gluten meal could replace up to 33% of fishmeal protein in the diet of turbot (Regost et al., 1999), up to 60% of the fishmeal in diets of juvenile gilthead sea bream (Pereira and Oliva-Teles, 2003), and up to 40% of the protein from fishmeal in the diet of Japanese flounder (Kikuchi, 1999). Additionally, a potential advantage of Corn-PC is that it can lessen the need for supplemental methionine because it has a higher content of this amino acid than what is found in other plant-based feedstuffs (NRC, 2011). In a previous study, Rossi et al. (2013) reported that replacing 50% of menhaden fishmeal protein with Corn-PC negatively affected the growth performance of red drum. Nevertheless, in the present study, Corn-PC successfully replaced 50% of fishmeal protein in red drum's diet; even more, shortfin corvina readily accepted a higher inclusion of Corn-PC (75%).

Interestingly, results of the present study showed that, in some cases, growth of fish fed the plant-based diets out-performed that of fish fed the fishmeal control diet in terms of weight gain, feed efficiency and/or protein efficiency ratio. Similarly, in another study with red drum, Rossi et al. (2013) found that 50% fishmeal protein replacement with Soy-PC resulted in fish out-performing those fed a menhaden fishmeal control diet. Taking into account that both the red drum and the shortfin corvina are carnivorous species, these results were somewhat unexpected. The use of menhaden fishmeal may partly explain the outcome, because at least for salmon, there have been indications of lower protein digestibility and lower availability of a number of amino acids in menhaden fishmeal, as compared to fishmeals derived from other fish species and from different geographical origin (Anderson et al., 1995). However, it also is important to note that the amino acid profile of all these plant-based diets was adjusted by including lysine, methionine and/or taurine. This is why different plant feedstuffs in the diet, also referred to as protein blends, may be used to produce diets with more well-matched amino acid profiles for fish (Hardy, 2010). For instance, complete replacement of fishmeal in the diet of juvenile Atlantic salmon was achieved through the utilization of such protein blends (Burr et al., 2012). Additional studies evaluating alternative protein blends may contribute to further reduce the use of fishmeal in diets for sciaenids.

Both the HSI and IPF ratio of red drum has been shown to increase in response to greater intake of digestible energy (McGoogan and Gatlin, 1998) or to SBM-based diets (Rossi et al., 2013, 2015). In the present study, the HSI and IPF ratio values found in red drum and shortfin corvina were not clearly affected by fishmeal replacement. However, other studies have reported significant reductions in HSI and/or IPF ratio in response to SBM as a substitute for fishmeal in red drum (McGoogan and Gatlin, 1997) and other carnivorous fish species (Kaushik et al., 1995; Zhang et al., 2014). Contradictions between results found in different studies may indicate that HSI and IPF ratios are not clear indicators of metabolic effects caused by fishmeal replacement with plant feedstuffs in fish diets.

Lysozyme activity and blood neutrophil oxidative radical production (NBT) are related to the non-specific immunological response before the presence of pathogens and as possible indicators of stress (Tort et al., 2003). Some antinutritional factors found in SBM have been shown to cause intestinal inflammatory processes and significantly augment lysozyme and NBT values in meager (*Argyrosomus regius*) (Ribeiro et al., 2015). Likewise, increasing activity of neutrophil and macrophages were observed in rainbow trout and Atlantic salmon in response to soybean-based diets (Rumsey et al., 1994; Krogdahl et al., 2000). In contrast, in a recent study with red drum, both the lysozyme activity and the NBT response were unaffected by dietary SBM inclusion levels (Rossi et al., 2015), in agreement with other studies evaluating plant-based diets in different fish species (Buentello et al., 2015; Güroy et al., 2013; Kokou et al., 2012; Sitjà-Bobadilla et al., 2005; Moxley et al., 2014). Hence, the relative tolerance to dietary SBM and other plant-based feedstuffs seems to be species-specific. In the present study, shortfin corvina showed significantly higher values of serum lysozyme and NBT when fed SBM-3010 and Soy-PC but not when fed Corn-PC. Interestingly, the higher lysozyme and NBT values were found with the replacement of 75% fishmeal with SBM-3010, the same treatment where shortfin corvina growth was significantly reduced. These results may support the hypothesis that nutritional imbalances and/or deficiencies caused by partial fishmeal replacement are a potential source of stress causing adverse effects on the immune response of fish species that are susceptible to specific plant feedstuffs.

In summary, based on the results of the present study, SBM-3010, Soy-PC and Corn-PC are good candidates for the partial replacement of menhaden fishmeal protein in the diets of red drum and shortfin corvina, when supplemented with amino acids. In the case of juvenile red drum, it was confirmed that SBM-3010 and Soy-PC can replace up to 75% of menhaden fishmeal protein in the diet. In addition, Corn-PC can successfully replace up to 50% of menhaden fishmeal protein without compromising fish performance. Moreover, in the case of juvenile shortfin corvina, it was observed that Soy-PC and Corn-PC can replace

up to 75% of menhaden fishmeal protein in the diet, while SBM-3010 successfully replaced up to 50% of menhaden fishmeal protein without compromising fish performance. Although there are no preceding studies regarding the nutrition of shortfin corvina, the growth and feed efficiency responses to the different dietary treatments were similar to those found in red drum. This confirms that previous nutritional research done with red drum could be applied as a basis for the development of diets to support the aquaculture programs for shortfin corvina.

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Dear Dr. González-Félix:

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Effect of dietary lipid level on growth performance, feed utilization, and body composition of totoaba, Totoaba macdonaldi (Gilbert, 1890)

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Effect of dietary lipid level on growth performance, feed utilization, and body composition of totoaba, *Totoaba macdonaldi* (Gilbert, 1890)

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Running title: Lipid utilization by *Totoaba macdonaldi*

Keywords: *Totoaba macdonaldi*; Dietary lipid; Growth response; Proximate body composition

Abstract

Using a fixed dietary crude protein (CP) level of 46%, an 8-week experiment was conducted to evaluate the influence of increasing levels of dietary crude fat (CF) (6, 8, 11, 14, 17, 20, and 22% of the diet dry weight) on growth, feed utilization and body composition of totoaba, *T. macdonaldi*, an endangered species native to the Gulf of California, Mexico, with great potential for aquaculture. Juvenile fish (overall initial mean body weight of 128.3 ± 9.9 g) were stocked into circular tanks of 250 L (0.4 m^2 bottom area) in a clear-water recirculating culture system at a density of 4 fish $tank^{-1}$, assigning each dietary treatment to five replicate tanks. Adequate and comparable growth of totoaba was observed over the 8 to 22% range of dietary CF (185.14 to 227.59 g of final weight). In contrast, growth was significantly reduced in fish fed 6% dietary CF (148.25 g of final weight), as evaluated by growth performance variables and feed utilization indices. In response to increasing dietary lipid, deposition of lipid increased significantly in whole fish (1.77 to 4.42% CF) and liver (23.23 to 41.45% CF), while moisture content was significantly reduced in these tissues. The CF content of fish muscle remained under 2% when fed up to 22% dietary lipid. Thus, to promote sustainable practices and to minimize the economic pressure caused by high fish oil prices in the manufacture of aquafeeds, a dietary lipid inclusion of 8%, provided as fish oil, is encouraged for juvenile totoaba.

48 Fish belonging to the family Sciaenidae, commonly known as drums, weakfishes,
49 croakers, or corvinas, are appreciated as food fish and cultured in different parts of the
50 world (FAO 2012, 2014). This family is well represented in Mexico, with at least 30
51 sciaenids inhabiting the Gulf of California alone (Van der Heiden 1985). Some of
52 these species, such as the Gulf corvina, *Cynoscion othonopterus* (Jordan & Gilbert
53 1882) and the totoaba, *Totoaba macdonaldi* (Gilbert 1890), both native to the Gulf of
54 California, have attracted people's attention as candidate species for aquaculture.
55 Although both species have interesting aquaculture-related features, the totoaba stands
56 out for being a legendary species that supported one of the most important fisheries in
57 the Gulf of California at the turn of the 20th century. It is the largest sciaenid known to
58 date, attaining a size of up to 2 m in length and weighing up to 135 kg (Flanagan &
59 Hendrickson 1976; CITES 2005). Unfortunately, overfishing placed this species under
60 the status of critically endangered, and a total fishing ban was imposed by the Mexican
61 Law since 1975 (DOF 1975; Bobadilla, Alvarez-Borrego, Avila-Foucat, Lara-Valencia
62 & Espejel 2011). Nevertheless, totoaba breeding programs currently underway in
63 Northwest Mexico have succeeded in the production of fry for the purpose of stock
64 enhancement, as well as for the development of aquaculture. So far, research with the
65 species has focused on its nutritional requirements and various aspects of its biology
66 and physiology in captivity (Rosas, Vázquez-Duhalt, Tinoco, Shimada, D'Abramo &
67 Viana 2008; Rueda-López, Lazo, Correa & Viana 2011; Minjarez-Osorio, González-
68 Félix & Perez-Velazquez 2012; Badillo-Zapata, Lazo, Herzka & Viana 2014; López,
69 Flores-Ibarra, Bañuelos-Vargas, Galaviz & True 2015).
70 Dietary lipids are very important constituents of aquafeeds for marine finfish,
71 both, quantitatively and qualitatively. Their importance is well recognized in science,

72 from their classical role as a source of energy and constituents of the so-called lipid
73 bilayer in cell membranes, to their role in the endocrine system played by steroid
74 hormones and terpenoids (Singer & Nicolson 1972; Tocher 2003). In practical terms,
75 insufficient or excessive dietary lipid levels are detrimental to fish growth or carcass
76 quality (Bromley 1980; Hillestad & Johnsen 1994; Shiau & Lan 1996; McGoogan &
77 Gatlin 1999; Rueda-López *et al.* 2011). Therefore, determining quantitative lipid
78 dietary requirements is essential for ensuring optimal growth of captive fish species, as
79 well as to spare dietary protein from being used as energy.

80 As a new species with aquacultural potential, nutritional studies of totoaba are
81 scarce. Rueda-López *et al.* (2011) and Minjarez-Osorio *et al.* (2012) studied the
82 influence of dietary crude protein (CP) and energy levels on growth and survival of
83 totoaba. In the first study, reduced growth rates were observed in totoaba with an
84 individual weight close to 12 g fed 18% crude fat (CF) compared to 8.5%, and 52% CP
85 was optimal. In the second study, 47% dietary CP was recommended for organisms
86 close to 75 g of individual weight when the diet included 8% CF. However, a study of
87 the effects of a wide range of dietary lipid levels on growth and survival of this species
88 is not available. Thus, the present work investigated the effects of dietary lipid level on
89 growth performance, feed utilization, and body composition of *T. macdonaldi*.

90 In the present study, totoaba juveniles belonging to the same cohort and
91 originating from captive wild broodstock were obtained from the Center for
92 Reproduction of Marine Species of the State of Sonora (CREMES), Kino Bay, Sonora,
93 Mexico. Fish were transported to the Kino Bay Experiment Station (KBES),
94 University of Sonora at Kino Bay, Sonora, Mexico, maintained in a 10 m^3 raceway,
95 and fed a commercial feed with 46% CP and 12% CF (Marine MX, Skretting ®,

96 Tooele, Utah, USA). Juveniles were then transferred into the experimental culture
97 system for initiation of the study at an overall mean wet body weight of 128.3 ± 9.9 g
98 (mean ± standard deviation, SD). The experimental recirculating culture system
99 consisted of 35 polyethylene circular tanks (71 cm diameter, 0.4 m^2 bottom area, and
100 250 L capacity) filled with 200 L filtered seawater recirculating into individual tanks
101 by means of a 1.5-HP pump (Jacuzzi, Model 150MF-T, Little Rock, Arkansas, USA)
102 at a flow rate of approximately 1.5 L min^{-1}, with a complete water turnover every 133
103 min. Water was also pumped through a 1,100-L sump tank, a sand filter (Jacuzzi,
104 Model L-190-7, Little Rock, Arkansas, USA), a biofilter, a 120-Watt UV light
105 chamber (Rainbow Lifeguard, Model UV97, El Monte, California, USA), a 1,500-
106 Watt in-line heater (Aquatic Ecosystems, Model DE-6115, Apopka, Florida, USA),
107 and a 1-HP in-line chiller (Aquatic Ecosystems, Model AE62B, Apopka, Florida,
108 USA). A water exchange of approximately 80% was performed daily adding new,
109 filtered seawater. Aeration to individual tanks was supplied with a 1.0-HP blower
110 (Fuji, Model VFC40, Saddle Brook, New Jersey, USA) and submerged airstones.
111 Using fish oil as the main lipid source, nine iso-proteic (46% CP) diets with CF
112 contents of 6, 8, 11, 14, 17, 20, and 22% (Table 1) were fed to fish for 8 weeks in a
113 completely randomized design experiment, assigning each experimental treatment to
114 five replicate tanks and stocking four group-weighed fish into each tank. Experimental
115 feeds were prepared using a Hobart grinder (Hobart Corporation, Model A-200, Troy,
116 Ohio, USA). They were dried overnight at 40°C, ground to adequate size, and kept
117 frozen at −20°C until used. The proximate composition of duplicate samples of each
118 experimental feed was determined, in terms of moisture, ash, crude fiber (AOAC 2005,
119 methods 930.15, 942.05 and 978.10, respectively), crude fat (Folch, Lees & Sloane-

120 Stanley 1957), and crude protein, which was analyzed via combustion by the Dumas
121 method (AOAC 2005, method 968.06) with a Dumas Nitrogen Analyzer (Model NDA
122 702, VELP® Scientifica, Usmate, Italy). A bomb calorimeter (Model IKA C5003, IKA-
123 Werke® GmbH, Staufen, Germany) was employed to determine gross energy content of
124 the experimental feeds (Table 1), while the nitrogen free extract (NFE) was calculated by
125 difference: NFE (%) = 100 – (% CP + % CF + % moisture + % crude fiber + % ash).
126 The initial daily feed ration was calculated based on the initial individual mean
127 weight, corresponding to 3% of the wet weight of fish, and was adjusted daily
128 depending on the feed consumed by fish to be kept close to apparent satiation. This
129 was achieved by collecting every day uneaten feed out of each tank and drying it
130 overnight to quantify, by difference, the feed consumed (feed offered, g – uneaten
131 feed, g). Additionally, fish from three randomly selected tanks per treatment were
132 group-weighed every other week and feed rations adjusted accordingly. The pre-
133 weighed daily feed ration was divided into three equal portions, administered to fish at
134 08:00, 13:00, and 18:00 hours. Daily measurements of the concentration of dissolved
135 oxygen, temperature, and salinity of the culture water were taken with a multi-function
136 oxygen meter (YSI, Model Y85, Yellow Springs, Ohio, USA). Weekly, the
137 concentrations of total ammonia nitrogen and nitrite were measured according to
138 procedures adapted from those of Solórzano (1969), Spotte (1979a,b), and Strickland
139 & Parsons (1972), while pH was measured using a hand-held pH meter (Oakton®,
140 Model Double Junction pHTestr 1, Vernon Hills, Illinois, EUA).
141 The growth performance of fish was evaluated by final weight (g); weight gain
142 (g) = (final weight, g – initial weight, g); percent weight gain (%) = [(final weight, g –
143 initial weight, g) /initial weight, g] × 100; daily weight gain (g d^{-1}) = weight gain,

g/time (days); thermal growth coefficient (TGC) = [(final weight$^{1/3}$ – initial weight$^{1/3}$)/(Temperature, °C × time, days)] × 100 (as adapted by Cho 1990), and specific growth rate (SGR, % d^{-1}) = (ln final weight, g – ln initial weight, g)/time (days) × 100. Additional measurements included the Fulton's condition factor (K) (Ricker 1975), an indirect estimate of the robustness/healthiness of fish = (weight, g/length^3, mm) × 100; hepatosomatic index (HSI, %) = (liver weight, g/final weight, g) × 100; viscerosomatic index (VSI, %) = (viscera weight, g/final weight, g) × 100; and survival (%) = (final No. organisms × 100)/initial No. organisms). Feed utilization was evaluated in terms of feed conversion ratio (FCR) = feed consumed, g/weight gain, g; feed efficiency (FE) = weight gain, g/feed consumed, g; gross protein retention (GPR, %) = CP gain, g/CP consumed, g × 100; gross fat retention (GFR, %) = CF gain, g/CF consumed, g × 100; and protein efficiency ratio (PER) = weight gain, g/CP consumed, g.

Upon termination of the experiment, fish in all tanks were group-weighed and percent survival in each tank was determined. Fish in each tank were euthanized by immersion in iced water (≈ 4°C), stored in a ziploc bag per tank, and transported in an ice-filled cooler to the Nutrition Laboratory of the Department of Scientific and Technological Research of the University of Sonora, where they were stored at −20°C until the analysis of proximate composition. Subsequently, fish were thawed and individually weighed and measured for total length; then, they were dissected and visceral organs and liver were weighed. Triplicate composite whole fish samples, each consisting of homogenates prepared with three fish sampled from different tanks in the same treatment, were used to determine the proximate contents of CF, CP, moisture, and ash, as described earlier. In addition, triplicate composite samples, each consisting

168 of muscle or liver homogenates of three fish sampled from different tanks, were used
169 to perform the same determinations. These analyses and procedures also were
170 performed on triplicate composite samples of whole fish, muscle tissue and liver from
171 18 fish sacrificed at initiation of the experiment.

172 Using a significance level of $P \leq 0.05$, one-way analysis of variance (ANOVA)
173 was applied to growth performance, feed utilization, and fish proximate composition
174 data: initial weight, final weight, weight gain, percent weight gain, daily weight gain,
175 TGC, SGR, K, HSI, VSI, FCR, GPR, GFR, PER, and survival. Prior to one-way
176 ANOVA, data expressed as percentages were arcsine transformed; however,
177 untransformed data are reported. When significant differences among treatments were
178 detected, Duncan's multiple range test was used as the mean separation procedure.
179 Statistical analyses were conducted using SAS statistical software version 9.1 (SAS
180 Institute Inc., Cary, NC, USA).

181 The concentrations (means ± SD) of dissolved oxygen, total ammonia nitrogen,
182 and nitrite in the culture water were 6.0 ± 0.8 mg L^{-1}, 0.146 ± 0.042 mg L^{-1}, and 0.074
183 ± 0.035 mg L^{-1}, respectively; while temperature, salinity, and pH were 28.3 ± 1.1°C,
184 36.9 ± 0.6 g L^{-1}, and 7.8 ± 0.1, respectively.

185 No differences in weight were observed among treatments at the beginning of
186 the study (Table 2). At the end of the growth trial, fish fed dietary CF levels from 8 to
187 22% had, in general, a similar growth response. The highest growth performance
188 across all parameters evaluated, *i.e.*, final weight, weight gain, percent weight gain,
189 daily weight gain, TGC, and SGR corresponded to fish fed 20% dietary CF,
190 significantly higher than that of fish fed 14% CF, but not statistically different from
191 fish receiving 8, 11, 17, and 22% CF. Furthermore, no significant differences were

192 observed among fish fed the dietary treatments 8, 11, 12, 14, 17, and 22%. Conversely,
193 fish fed 6% CF consistently had significantly lower growth performance than fish from
194 all the other dietary treatments across all parameters evaluated. Mean survival rates
195 ranged from 95.0 to 100.0%, without significant differences among treatments (Table
196 2).

197 The growth response observed was reflected in the measurements of the feed
198 utilization indices; fish fed 6% dietary CF had the significantly highest FCR, as well as
199 the lowest FE, GPR, and PER values, compared to fish fed the other dietary treatments
200 (Table 3). In addition, significant differences among treatments were observed for
201 GFR, HSI, and VSI values; however, treatment means did not consistently increase or
202 decrease in relation to dietary lipid, leading to an unclear pattern of *post hoc* means
203 separation. The mean Fulton's condition factor at the beginning of the study was 1.35;
204 at the end of the trial it ranged from 1.06 to 1.24, with no statistically significant
205 differences detected among treatments (Table 3).

206 The determined values of the proximate composition of whole fish at the
207 beginning of the growth trial were similar to those of animals fed 22% CF at the end of
208 the trial, and muscle tissue showed more CF but lower moisture content than fish fed
209 the different experimental diets (Table 4). The CF content of whole fish progressively
210 increased with increasing dietary CF, being significantly higher (4.42%) in fish fed
211 22% dietary CF, and significantly lower (1.77%) in fish fed 6%. In contrast, the
212 moisture content decreased significantly with increasing dietary CF. No statistically
213 significant differences among treatments were detected for CP content of whole fish,
214 but they were detected for ash content, though no clear trend was observed after *post*
215 *hoc* means separation. In muscle tissue, similarly to what was observed in whole fish,

significant differences among treatments were detected for the content of CF, moisture, and ash, but not for CP. The means separation procedure for CF provided variable results without a clear trend being observed, but moisture content in this tissue also decreased with increasing dietary CF (Table 4). In liver, the CF content increased significantly and progressively from 23.23% at the lowest dietary CF level to 41.45 and 40.55% at 20 and 22%, respectively, and the moisture content also tended to decrease with increasing dietary CF, as previously observed (Table 4).

In the present study and under these experimental conditions, the lowest dietary lipid level tested, 6%, clearly did not support acceptable growth of *T macdonaldi*, as indicated by the statistically significant decreased growth observed across all the growth response variables measured in fish receiving this diet. It is worthwhile pointing out that the experimental diets of the present study had a fixed dietary CP level of 46%, intentionally chosen to be in close proximity to what was previously reported as nutritionally sufficient for adequate growth of this species (47% dietary CP) according to Minjarez-Osorio *et al.* (2012), hence, CP was not in excess in order to promote the preferential use of the dietary lipid for energy, considering that not only the limited provision of non-protein energy sources, but also the excess of protein may lead to the inefficient use of protein as energy and limit the allocation of this nutrient for growth in aquatic organisms (Gómez-Montes, García-Esquivel, D'Abramo, Shimada, Vásquez-Peláez & Viana 2003). In contrast to the response observed to the dietary lipid level of 6%, totoaba seemed to tolerate a relatively wide range of dietary lipid, from 8 to 22%, without significantly affecting growth, except only for the dietary lipid level of 14%, which elicited an odd and significantly lower growth response than

239 fish fed 20% CF, but not significantly different from fish fed 8, 11, 17 or 22%, due to
240 some unidentified reason, but probably unrelated to the dietary lipid level.

241 Lipid levels known to satisfy dietary lipid requirements of other scieaenids,
242 which, depending on factors such as dietary CP, species, and body size, may range
243 from 7.6 to 20%. For example, 17.0-20.0% CF for meagre *Argyrosomus regius* A.
244 (Chatzifotis, Panagiotidou, Papaioannou, Pavlidis, Nengas & Mylonas 2010;
245 Chatzifotis, Panagiotidou & Divanach 2012; Martínez-Llorens, Espert, Moya, Jover &
246 Tomás-Vidal 2011); 15% for juvenile and 7.6-16.3% for subadult red drum *Sciaenops*
247 *ocellatus* L. (McGoogan & Gatlin 1999; Thoman, Davis & Arnold 1999; Turano,
248 Davis & Arnold 2002); 15.0 to 17.0% for cuneate drum *Nibea miichthioides* C.L.W.
249 (Wang, Guo & Bureau 2006); 10.5% for yellow croaker, *Pseudosciaena crocea* R.
250 (Duan, Mai, Zhong, Si & Wang 2001); 9.0% for giant croaker *N. japonica* T.S. (Chai,
251 Ji, Han, Dai & Wang 2013), 18% for dusky kob *A. japonicus* T.S. (Woolley, Jones &
252 Britz 2010), and 11.4% for Gulf corvina *C. othonopterus* (González-Félix, Minjarez-
253 Osorio, Perez-Velazquez & Urquidez-Bejarano 2015). It has been observed that when
254 dietary lipids are provided in excess to marine fish, feed intake is typically reduced and
255 growth rate diminishes (Wang, Liu, Tian, Mai, Du, Wang & Yang 2005; Rueda-López
256 *et al.* 2011), although this was not evident for totoaba in this study, similarly to what
257 has been reported for Atlantic cod, *Gadus morhua* L. (Grisdale-Helland, Shearer,
258 Gatlin & Helland 2008) and *S. ocellatus* (Turano *et al.* 2002), so totaba, just as cod and
259 red drum, also is able to consume a high quantity of dietary lipids without a severe
260 effect on growth.

261 Dietary lipid and the overall dietary energy interact with crude protein to
262 determine fish growth (Bowyer, Qin & Stone 2013). Different dietary CP/CF

263 combinations, within certain limits, may be manipulated to achieve similar growth
264 responses, as has been shown for grouper, *Epinephelus malabaricus* B.S. (Shiau & Lan
265 1996), *S. ocellatus* (Turano *et al.* 2002), winter flounder *Pleuronectes americanus* W.
266 (Hebb, Castell, Anderson & Batt 2003), and cobia *Rachycentron canadum* L. (Craig,
267 Schwarz & McLean 2006), among other species. In a factorial study testing CP levels
268 of 43, 48, and 52%, and CF levels of 8.5 and 18% in diets for totoaba, Rueda-López *et*
269 *al.* (2011) observed reduced growth when fish were fed 18% CF at all three protein
270 levels tested, as well as when fed 8.5% CF in combination with 43 or 48% CP, while
271 the best growth performance was reported for animals fed 8.5% CF and 52% CP. In
272 our study growth was practically homogeneous over the range of 8 to 22% CF at a
273 fixed 46% CP level. The differences observed in both studies may reflect a size effect,
274 since Rueda-López *et al.* (2011) employed much smaller fish, 12.12 g *vs.* 128.3 g in
275 this study, which also may explain the reduced weight gain observed here, since fish
276 exhibit a decrease in growth rate as body weight increases (Cook, McNiven,
277 Richardson & Sutterlin 2000). In addition, they reported a relatively higher dietary
278 energy content, ranging from 20.1 to 22.5 kJ g^{-1}, and possibly fish at that size were
279 more responsive to concomitantly higher dietary CP and energy levels. In the present
280 study dietary energy increased progressively from 17.8 to 21.7 kJ g^{-1} as dietary lipid
281 increased from 6 to 22%, while, as expected, the dietary protein/energy (P/E) ratio
282 decreased from 26.2 to 21.3 mg P kJ^{-1} (Table 1). The dietary P/E ratios of the 8 to 22%
283 lipid levels (21.3-25.7 mg P kJ^{-1}) that elicited comparable growth and are within the
284 range of values, from 20.4 to 28.6 mg P kJ^{-1} (available values, or calculated from
285 dietary protein and energy contents provided by the authors), reported for adequate
286 growth of various sciaenids (McGoogan & Gatlin 1999; Turano *et al.* 2002; Wang *et*

287 *al.* 2006; Pirozzi, Booth & Allan 2010; Woolley *et al.* 2010; Martínez-Llorens *et al.*
288 2011; Rueda-López *et al.* 2011; Chatzifotis *et al.* 2012; Chai *et al.* 2013; González-
289 Félix *et al.* 2015; Perez-Velazquez, González-Félix, Viana, Lazo-Corvera &
290 Maldonado-Othón 2015).

291 The ranges of the values recorded for the various indices of fish fed 8 to 22%
292 dietary lipid, such as FE (0.30-0.45), PER (0.71-1.00), HSI (0.88-1.63%), and K (1.06-
293 1.22) are comparable to values previously reported for this species (FE, 0.5-0.7; PER,
294 0.7-1.2; HSI, 1.1-1.3; and K, 1.1) (Rueda-López *et al.* 2011; Minjarez-Osorio *et al.*
295 2012). The FCR attained by fish fed 8% and 20% CF, 2.42 and 2.48, respectively, are
296 comparable to the reported range of 2.2-2.3 by Minjarez-Osorio *et al.* (2012), but they
297 were higher for the rest of the dietary lipid levels (2.82-3.43). This may be related to
298 the feed preparation process, since the experimental feeds were prepared using a meat
299 grinder (Hobart, Troy, OH, USA) at room temperature. Better results may be expected
300 by preparing feeds through extrusion cooking, the best method available to date for
301 manufacturing finfish aquafeeds.

302 Increased lipid deposition with increasing dietary lipid was observed in whole
303 fish and liver of *T. macdonaldi* in the present study, concurring with a number of
304 reports of either sciaenids, such as *S. ocellatus*, *A. regius*, *N. miichthioides*, *A.*
305 *japonicus*, and *C. othonopterus* (McGoogan & Gatlin 1999; Chatzifotis *et al.* 2010,
306 2012; Wang *et al.* 2006; Woolley *et al.* 2010; González-Félix *et al.* 2015, Perez-
307 Velazquez *et al.* 2015), or non-sciaenid fish, such as Atlantic salmon *Salmo salar* L.,
308 Japanese flounder *Paralichthys olivaceus* T.S., grouper *Epinephelus malabaricus* B.S.,
309 and common dentex *Dentex dentex* L. (Hemre & Sandnes 1999; Lee, Cho & Kim
310 2000; Shiau & Lan 1996; Skalli, Hidalgo, Abellán, Arizcun & Cardenete 2004). This

311 phenomenon is often accompanied by a significant reduction of the moisture content,
312 which was observed in this study for whole fish and liver, and also in studies of other
313 fish species, such as *R. canadum*, *N. miichthioides*, and mullet *Liza macrolepis* S.
314 (Wang *et al.* 2005, 2006; Rangaswamy, Gopal & Swamy 1998); although this has not
315 always been observed for all fish, *e.g.*, in *A. japonicus* and *Siganus rivulatus* F.N.,
316 (Woolley *et al.* 2010; Ghanawi, Roy, Davis & Saoud 2011).

317 The liver of fish fed diets with 8 and 11% CF showed similar lipid content as
318 initial fish, but higher dietary lipid increased the accumulation of liver fat significantly,
319 which may be detrimental to fish health since this may lead to fatty liver syndrome, a
320 condition associated to increased lipid peroxidation and impaired liver function,
321 leading in turn to inefficient nutrient utilization, as reported for *S. ocellatus* (Tucker,
322 Lellis, Vermeer, Roberts & Woodward 1997; Craig, Washburn & Gatlin 1999). This
323 observation also supports the report made by Rueda-López *et al.* (2011), stating that
324 totoaba possibly has a limited capacity to metabolize lipid, consequently, it
325 accumulates in liver when excess dietary lipid is fed to this species. On the other hand,
326 the muscle lipid content at the beginning of this study was 1.71%., but in spite of being
327 fed relatively high lipid levels, the lipid content of fish muscle ranged from 1.22 to
328 1.57% at the end of the trial, placing this species under the category of lean fish (< 2%
329 fat) according to Ackman's (1990) classification of fish based upon meat lipid content.
330 A similar report has been made for another lean fish like *G. morhua* with a high lipid
331 requirement (20%) but low lipid muscle content (Grisdale-Helland *et al.* 2008). In
332 contrast, using dietary lipid levels of 8.5 and 18%, and higher dietary energy contents,
333 Rueda-López *et al.* (2011) reported values ranging from 2.3 to 3.7%, indicating that

334 the muscle lipid content of totoaba can be altered through dietary manipulation,
335 particularly in younger fish.

336 In summary, at a fixed crude protein level of 46%, *Totoaba macdonaldi*
337 juveniles showed similar growth performance when fed dietary lipid levels ranging
338 from 8 to 22% for 8 weeks, but it was significantly reduced at 6%, with fish displaying
339 the lowest FE, GPR, and PER values. In response to increasing dietary lipid, the
340 deposition of lipid in whole fish and liver also increased significantly. In spite of this,
341 the lipid content of fish muscle remained under 2% when fed up to 22% dietary lipid,
342 and it is therefore considered a lean-fish. Nevertheless, to promote sustainable
343 practices and to minimize the economic pressure caused by high fish oil prices in the
344 manufacture of aquafeeds, a dietary lipid inclusion of 8%, provided as fish oil, is
345 encouraged for juvenile totoaba.

346

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355

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Table 1. Ingredient and proximate composition (% of dry weight) of experimental diets fed to *Totoaba macdonaldi* L. for 8 weeks.

Ingredient	Crude fat level (%)						
	6	8	11	14	17	20	22
Sardine fishmeal[a]	50.00	50.00	50.00	50.00	50.00	51.00	51.70
Sardine fish oil[a]	0.00	1.75	4.80	7.86	10.91	13.93	16.96
Soybean meal[b]	6.00	6.00	6.00	6.00	6.00	6.00	6.00
Corn gluten meal[c]	6.00	6.00	6.00	6.00	6.00	6.00	6.00
Casein[d]	3.25	3.67	4.15	4.62	5.05	5.45	6.06
Whole wheat flour[e]	25.98	22.91	19.48	16.05	12.77	8.35	4.01
Wheat starch[f]	5.50	5.40	5.30	5.20	5.00	5.00	5.00
Soy lecithin[g]	0.00	1.00	1.00	1.00	1.00	1.00	1.00
Vit-Min Pre-Mix[h]	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Choline chloride[i]	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Vitamin Stay C 35%[j]	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Tocopherol[k]	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Total	100	100	100	100	100	100	100
Proximate composition[l]							
Crude fat (%)	6.00 ± 0.19	8.14 ± 0.07	10.50 ± 0.27	13.57 ± 0.06	16.89 ± 0.25	19.68 ± 0.40	22.21 ± 0.02
Crude protein (%)	46.66 ± 0.62	46.54 ± 0.74	46.35 ± 0.99	45.58 ± 0.27	45.42 ± 0.39	45.15 ± 1.13	46.24 ± 1.34
Moisture (%)	7.78 ± 0.01	8.44 ± 0.10	7.91± 0.06	8.34 ± 0.08	8.15 ± 0.11	7.33 ± 0.08	7.51 ± 0.11
Ash (%)	12.49 ± 0.04	12.44 ± 0.15	12.15 ± 0.11	12.36 ± 0.02	12.27 ± 0.06	12.09 ± 0.09	12.19 ± 0.35
Crude fiber (%)	2.71 ± 0.08	2.26 ± 0.07	2.14 ± 0.10	2.05 ± 0.00	2.07 ± 0.08	1.60 ± 0.03	1.47 ± 0.07
NFE (%)[m]	24.36	22.18	20.95	18.10	15.20	14.15	10.38
Gross energy (E) (kJ g^{-1})	17.8	18.1	18.8	19.3	19.7	20.7	21.7
P/E ratio (mg P kJ^{-1})	26.2	25.7	24.7	23.6	23.0	21.8	21.3

[a]Proteínas Marinas y Agropecuarias, S.A. de C.V., Zapopan, Jalisco, Mexico.

[b]Consorcio Super, S.A. de C.V. Guadalajara, Jalisco, Mexico.

[c]CPIngredientes, S.A. de C.V. Guadalajara, Jalisco, Mexico.

[d]FagaLab S.A. de C.V., Guamuchil, Sinaloa, Mexico.

[e]Los Gallos, Molino La Fama S.A. de C.V., Hermosillo, Sonora, Mexico.

[f]Gluten y Almidones Industriales, S.A. de C.V., Mexico City, Mexico

[g]Golden Harvest, Impulsora Golden, S.A. de C.V., Mexico City, Mexico.

[h]Rovimix for Carnivorous Marine Fish, Insumos Nubiot, Obregon City, Sonora, Mexico.

[i]Sigma Aldrich, Saint Louis, Missouri, USA.

[j]Stay C® (L-ascorbyl-2-polyphosphate 35% active C), Roche Vitamins Inc., Parsippany, NJ, USA.

[k]General Nutrition Centers, Co., Pittsburg, Pennsylvania, USA.

[l]Values are means of triplicate samples ± standard deviation.

[m]Nitrogen free extract (NFE) calculated by difference: NFE (%) = 100 – (% CP + % CF + % moisture + % crude fiber + % ash).

Table 2. Growth performance and survival of *T. macdonaldi* fed different levels of dietary lipid for 8 weeks.

Treatment Crude fat (%)	Initial weight (g)	Final weight (g)	Weight gain (g)	Weight gain (%)	Daily weight gain (g d^{-1})	TGC	SGR (% d^{-1})	Survival (%)
6	125.20 ± 7.16	148.25^{c} ± 16.79	23.05^{c} ± 12.83	18.28^{c} ± 9.85	0.42^{c} ± 0.24	0.018^{c} ± 0.009	0.30^{c} ± 0.15	100.0 ± 0.0
8	131.88 ± 14.80	211.15^{ab} ± 23.23	79.29^{ab} ± 11.77	60.27^{ab} ± 7.42	1.44^{ab} ± 0.22	0.055^{ab} ± 0.006	0.84^{ab} ± 0.08	95.0 ± 11.2
11	127.08 ± 6.21	207.53^{ab} ± 26.49	80.46^{ab} ± 21.64	62.97^{ab} ± 14.97	1.47^{ab} ± 0.39	0.056^{ab} ± 0.012	0.87^{ab} ± 0.16	95.0 ± 11.2
14	127.35 ± 13.26	185.14^{b} ± 5.72	57.80^{b} ± 10.17	46.28^{b} ± 11.89	1.05^{b} ± 0.18	0.042^{b} ± 0.009	0.68^{b} ± 0.15	95.0 ± 11.2
17	130.20 ± 10.25	201.17^{ab} ± 7.22	76.07^{ab} ± 7.10	60.81^{ab} ± 5.67	1.38^{ab} ± 0.13	0.054^{ab} ± 0.005	0.85^{ab} ± 0.07	100.0 ± 0.0
20	120.08 ± 8.95	227.59^{a} ± 36.64	104.78^{a} ± 40.75	86.45^{a} ± 37.89	1.91^{a} ± 0.74	0.071^{a} ± 0.024	1.09^{a} ± 0.35	100.0 ± 0.0
22	134.95 ± 6.90	219.19^{ab} ± 15.23	84.24^{ab} ± 9.60	62.36^{ab} ± 5.25	1.53^{ab} ± 0.18	0.057^{ab} ± 0.005	0.87^{ab} ± 0.06	100.0 ± 0.0
ANOVA $Pr > F$	0.5062	0.0009	0.0003	0.0008	0.0003	0.0001	0.0001	0.6771

Values are means of five replicate tanks ± standard deviation. Means within columns with different superscripts are significantly different ($P \leq 0.05$).

TGC = thermal growth coefficient; SGR = specific growth rate.

Table 3. Body and feed utilization indices of *T. macdonaldi* fed different levels of dietary lipid for 8 weeks.

Treatment Crude fat (%)	K	HSI (%)	VSI (%)	FCR	FE	GPR (%)	GFR (%)	PER
Initial fish	1.35 ± 0.32	0.90 ± 0.29	2.85 ± 0.39	-	-	-	-	-
6	1.24 ± 0.32	$0.72^{d} \pm 0.27$	$2.66^{d} \pm 0.52$	$6.84^{a} \pm 1.26$	$0.15^{c} \pm 0.03$	$5.77^{b} \pm 1.25$	$4.45^{c} \pm 0.97$	$0.33^{c} \pm 0.07$
8	1.19 ± 0.20	$1.63^{a} \pm 0.42$	$3.76^{a} \pm 0.45$	$2.42^{b} \pm 0.43$	$0.43^{ab} \pm 0.09$	$16.16^{a} \pm 3.51$	$11.04^{a} \pm 0.33$	$0.92^{ab} \pm 0.20$
11	1.22 ± 0.13	$0.88^{cd} \pm 0.21$	$2.92^{bcd} \pm 0.36$	$2.82^{b} \pm 0.24$	$0.36^{ab} \pm 0.03$	$13.85^{a} \pm 1.12$	$8.53^{ab} \pm 0.69$	$0.77^{ab} \pm 0.06$
14	1.13 ± 0.27	$1.07^{c} \pm 0.22$	$2.83^{cd} \pm 0.31$	$3.43^{b} \pm 0.73$	$0.30^{b} \pm 0.06$	$12.24^{a} \pm 2.34$	$5.75^{c} \pm 1.10$	$0.66^{b} \pm 0.13$
17	1.15 ± 0.21	$1.00^{c} \pm 0.24$	$3.27^{b} \pm 0.33$	$2.89^{b} \pm 0.17$	$0.35^{ab} \pm 0.02$	$13.44^{a} \pm 0.81$	$6.86^{bc} \pm 0.42$	$0.77^{ab} \pm 0.05$
20	1.06 ± 0.16	$1.33^{b} \pm 0.26$	$3.15^{bc} \pm 0.54$	$2.48^{b} \pm 0.86$	$0.45^{a} \pm 0.20$	$17.46^{a} \pm 7.63$	$8.99^{ab} \pm 3.93$	$1.00^{a} \pm 0.44$
22	1.10 ± 0.32	$1.31^{b} \pm 0.30$	$2.97^{cd} \pm 0.38$	$3.09^{b} \pm 0.26$	$0.33^{ab} \pm 0.03$	$12.68^{a} \pm 1.07$	$6.47^{bc} \pm 0.55$	$0.71^{ab} \pm 0.06$
ANOVA $Pr > F$	0.5137	< 0.0001	< 0.0001	< 0.0001	0.0021	0.0026	0.0003	0.0023

Values are means of five replicate tanks ± standard deviation. Means within columns with different superscripts are significantly different ($P \leq 0.05$).

K = Fulton's condition factor; HSI = hepatosomatic index; VSI = viscerosomatic index; FCR = feed conversion ratio; FE = feed efficiency; GPR = gross protein retention; GFR = gross fat retention; PER = protein efficiency ratio.

1 Table 4. Determined proximate composition (% of wet weight) of whole body and tissues of *T. macdonaldi* fed different levels of dietary
2 lipid for 8 weeks.

		Treatment, Crude fat (%)							
	Initial fish	6	8	11	14	17	20	22	ANOVA $Pr > F$
Whole fish									
Crude fat	4.73 ± 0.46	$1.77^{e} \pm 0.17$	$2.37^{d} \pm 0.05$	$2.51^{d} \pm 0.15$	$2.60^{d} \pm 0.16$	$3.34^{c} \pm 0.22$	$3.93^{b} \pm 0.11$	$4.42^{a} \pm 0.08$	<0.0001
Crude protein	18.46 ± 0.92	17.84 ± 1.15	17.69 ± 0.77	17.98 ± 0.18	18.60 ± 0.24	17.59 ± 0.37	17.51 ± 0.35	18.04 ± 0.53	0.6552
Moisture	72.13 ± 0.35	$75.53^{ab} \pm 0.23$	$75.86^{a} \pm 0.16$	$75.64^{ab} \pm 0.52$	$74.92^{b} \pm 0.46$	$75.06^{b} \pm 0.49$	$74.21^{c} \pm 0.17$	$72.73^{d} \pm 0.38$	<0.0001
Ash	4.36 ± 0.12	$5.28^{a} \pm 0.20$	$4.22^{c} \pm 0.07$	$4.09^{c} \pm 0.15$	$4.85^{b} \pm 0.02$	$4.08^{c} \pm 0.09$	$4.23^{c} \pm 0.06$	$5.13^{a} \pm 0.14$	<0.0001
Muscle									
Crude fat	1.71 ± 0.03	$1.28^{c} \pm 0.06$	$1.45^{ab} \pm 0.10$	$1.22^{c} \pm 0.04$	$1.34^{bc} \pm 0.04$	$1.57^{a} \pm 0.06$	$1.29^{c} \pm 0.01$	$1.31^{c} \pm 0.10$	0.0014
Crude protein	21.35 ± 0.33	19.59 ± 0.44	20.68 ± 0.47	21.20 ± 1.94	20.67 ± 1.45	19.82 ± 0.56	20.61 ± 1.57	20.38 ± 1.14	0.7121
Moisture	75.14 ± 0.75	$79.55^{a} \pm 0.47$	$78.83^{ab} \pm 0.55$	$78.04^{bc} \pm 0.85$	$78.43^{bc} \pm 0.70$	$78.31^{bc} \pm 0.36$	$77.53^{c} \pm 0.64$	$77.45^{c} \pm 0.46$	0.0092
Ash	1.45 ± 0.06	$1.33^{b} \pm 0.06$	$1.36^{b} \pm 0.13$	$1.34^{b} \pm 0.02$	$1.29^{b} \pm 0.07$	$1.25^{b} \pm 0.03$	$1.30^{b} \pm 0.04$	$1.47^{a} \pm 0.06$	0.0243
Liver									
Crude fat	25.03 ± 0.81	$23.23^{c} \pm 1.89$	$24.49^{c} \pm 0.70$	$25.03^{c} \pm 1.64$	$30.75^{b} \pm 2.66$	$31.38^{b} \pm 2.40$	$41.45^{a} \pm 0.46$	$40.55^{a} \pm 0.50$	<0.0001
Crude protein	8.72 ± 1.50	$16.00^{a} \pm 1.48$	$12.47^{bc} \pm 1.22$	$12.33^{bc} \pm 1.68$	$14.52^{ab} \pm 1.82$	$12.84^{bc} \pm 0.28$	$9.86^{c} \pm 1.49$	$10.91^{c} \pm 1.63$	0.0057
Moisture	53.98 ± 1.78	$60.64^{a} \pm 1.74$	$61.88^{a} \pm 2.67$	$55.57^{b} \pm 2.54$	$57.13^{ab} \pm 4.64$	$52.42^{bc} \pm 2.18$	$46.50^{d} \pm 2.14$	$48.61^{cd} \pm 1.50$	<0.0001
Ash	1.41 ± 0.02	$1.12^{b} \pm 0.05$	$0.97^{bcd} \pm 0.03$	$1.59^{a} \pm 0.18$	$1.03^{bc} \pm 0.08$	$1.03^{bc} \pm 0.12$	$0.84^{d} \pm 0.03$	$0.89^{cd} \pm 0.04$	<0.0001

3 Values are means of triplicate composite samples, each consisting of three fish ± standard deviation.

4 Means with different superscripts within the same row are significantly different ($P \leq 0.05$).

V. DISCUSIÓN

El porcentaje de lípido en la dieta presentó una clara influencia sobre el crecimiento de la curvina golfina *C. othonopterus*, donde los peces que fueron alimentados con la dieta que contenía 11% de grasa cruda mostraron los valores más altos de crecimiento, el cual disminuyó conforme el porcentaje de lípido en la dieta se alejaba de este nivel. Este comportamiento se presentó en la mayoría de los índices de crecimiento y utilización del alimento evaluados, sin detectarse diferencias significativas entre los tratamientos experimentales, con excepción de la tasa de crecimiento específica (SGR) y la tasa de crecimiento térmico (TGC) (P = 0.0283 y 0.0450, respectivamente). Los resultados obtenidos en el presente estudio se corroboraron mediante la aplicación de un análisis cuadrático de línea quebrada de dos pendientes utilizando los datos de la tasa crecimiento térmico, con lo que se logró establecer el requerimiento de grasa cruda de la especie en 11.4%, el cual resulta similar a los valores reportados para otros miembros de la familia Sciaenidae.

Para la totoaba, *T. macdonaldi*, se determinó que la dieta con un nivel de inclusión de lípido de 6% resultó ser inadecuada, ya que en los peces alimentados con esta dieta se observó una disminución significativa en su crecimiento y utilización del alimento, en comparación con los peces alimentados con el resto de las dietas experimentales. Por otra parte, se determinó que la especie puede tolerar un porcentaje de inclusión de lípido que puede ir desde 8 hasta 22%, sin mostrar efectos negativos sobre el crecimiento, con excepción de nivel de 14%, ya que los peces alimentados con esta dieta presentaron un rendimiento significativamente menor en términos de crecimiento, en comparación con los que fueron alimentados con la dieta con un nivel de 20% de lípido, lo cual parecería ser un comportamiento extraño para este grupo de peces. Sin embargo, los peces alimentados con la dieta que contenía 14% de lípido no mostraron diferencias significativas con respecto a aquellos alimentados con las dietas que contenían 8, 11, 17 y 22% de lípido. Es probable que, por razones fortuitas, los organismos alimentados con la dieta que contenía 14% de lípido mostraran una disminución en el crecimiento, sin estar este comportamiento relacionado con el porcentaje de inclusión de lípido en las dietas experimentales.

De acuerdo a la literatura, los niveles de lípido y/o la cantidad de energía contenida

en las dietas pueden interactuar con el contenido de proteína en muchas especies de peces, actuando como factores determinantes en el crecimiento (Bowyer *et al.*, 2013). En el presente estudio, el contenido de proteína se mantuvo estable en 40% para la curvina golfina y en 46% para la totoaba, valores reportados como nutricionalmente adecuados para ambas especies (Minjarez-Osorio *et al.*, 2012; Perez-Velaquez *et al.*, 2015). Es importante señalar que algunos esciénidos y otros grupos de peces carnívoros pueden tolerar, dentro de los ciertos límites, diferentes combinaciones de estos macronutrientes, es decir, niveles más altos de proteína en combinación con un nivel menor de lípido en la dieta y viceversa, sin mostrar efectos negativos en las tasas de crecimiento (Craig *et al.*, 2006; Hebb *et al.*, 2003; Turano *et al.*, 2002). Sin embargo, se requiere llevar a cabo más estudios en estas especies para lograr comprender completamente la interacción de estos macronutrientes.

Por lo general, los esciénidos requieren de moderadas cantidades de lípido, en combinación con un alto requerimiento de proteína en la dieta (40% ≤). Por ejemplo, se ha reportado que el nivel óptimo de grasa cruda en la especie *Argyrosomus regius* puede ir desde 17.0 hasta 20%, en combinación con niveles de proteína de 43 a 50% (Chatzifotis *et al.*, 2010, 2012; Martínez-Llorens *et al.*, 2011). McGoogan y Gatlin (1999) reportaron un máximo crecimiento para la especie *S. ocellatus* utilizando niveles de grasa cruda y proteína de 15 y 45%, respectivamente, mientras que Turano *et al.* (2002) reportaron que los subadultos pueden tolerar un nivel de lípido desde 7.6 hasta 16.3%, en combinación con niveles de proteína de 36 a 44%, sin mostrar diferencias significativas en términos de crecimiento. Sin embargo, Thoman *et al.* (1999) recomendaron la inclusión de un nivel de lípido de 9.2% en combinación con 44% de proteína para evitar el exceso de acumulación de lípido en la piel de esta especie. Wang *et al.* (2006) observaron un crecimiento óptimo de la especie *Nibea miichthioides* con niveles de grasa entre 15 y 17%. Para el roncador amarillo, *Pseudosciaena crocea*, Duan *et al.* (2001) reportaron un nivel óptimo de grasa y proteína de 10.5 y 47%, respectivamente. A su vez, Chai *et al.* (2013) reportaron niveles óptimos de grasa cruda y proteína cruda de 9.0 y 48%, respectivamente, en dietas para el roncador gigante, *N. japonica*. Finalmente, una dieta con un nivel de lípido de 18%, en combinación con un nivel de proteína de 46%, resultó en un mejor desempeño en términos de crecimiento para la especie *A. japonicus* (Woolley *et al.*, 2010).

Diversos estudios han reportado que algunas especies de peces de agua fría, por lo general, requieren un aporte mayor de lípido en la dieta, contrario a lo que se ha descrito para especies de esciénidos de aguas cálidas. Arzel *et al.* (1993) reportaron un mejor crecimiento de la trucha café, *Salmo trutta* cuando se alimentó con una dieta que contenía 29% de lípido, en comparación con los que fueron alimentados con la dieta que contenía 21%. A su vez, el salmón del atlántico, *S. salar*, presentó un mejor crecimiento cuando se alimentó con dietas con 38–47% de lípido, en comparación con aquellos que fueron alimentados con una dieta que contenía 31% (Hemre y Sandnes, 1999). Sin embargo, se encuentra bien documentado en la literatura que el excedente de lípido en la dieta tiende a depositarse en los diferentes tejidos, lo cual puede afectar en forma negativa la calidad del filete (Bowyer *et al.*, 2013; Hillestad y Johnsen, 1994; Hillestad *et al.*, 1998). De forma similar a los estudios que sugieren un incremento en el depósito de lípido en los tejidos conforme se aumenta el porcentaje del lípido en la dieta, en el presente estudio también se observó de manera clara esta tendencia, tanto en la curvina golfina como en la totoaba, ya sea en organismos completos, hígado o músculo. Este mismo patrón ha sido reportado para otros esciénidos como la curvina roja, *S. ocellatus*, *A. regius* y *N. miichthioides* (McGoogan y Gatlin, 1999; Wang *et al.*, 2006; Woolley *et al.*, 2010), así como también para otras especies de peces marinos no esciénidos como *P. olivaceus*, *E. malabaricus*, *D. dentex*, y *S. salar* (Hemre y Sandnes, 1999; Lee *et al.*, 2000; Shiau y Lan, 1996; Skalli *et al.*, 2004).

Los análisis proximales llevados a cabo en la curvina golfina y en la totoaba mostraron una reducción en el porcentaje de humedad de los tejidos analizados conforme la cantidad de lípido depositado en los tejidos se incrementaba. Este comportamiento también ha sido reportado para otras especies de peces como *Liza macrolepis*, *R. canadum*, y *N. miichthioides* (Rangaswamy *et al.*, 1998; Wang *et al.*, 2005, 2006). En cuanto al almacenamiento de lípido en el hígado de la curvina golfina, se observó una tendencia a incrementarse conforme el porcentaje de lípido en la dieta aumentaba; sin embargo, esta tendencia no resultó ser significativa, en tanto que sí lo fue en el caso de la totoaba. Estos resultados son similares a los reportados para el bacalao del Atlántico, *Gadus morhua* y para la curvina roja *S. ocellatus*, en los que se observó que conforme el porcentaje de lípido en la dieta se incrementaba, también lo hacia el porcentaje de acumulación de lípido en el hígado

(Burr *et al.*, 2006; Grisdale-Helland *et al.*, 2008). Resulta interesante que el contenido de lípido en el músculo de totoaba al inicio de experimento fue de 1.71%, y que a pesar de ser alimentados con dietas con un contenido de lípido de hasta 22%, el porcentaje de acumulación al final del experimento presentó valores bajos, de 1.22 hasta 1.57%, por lo que esta especie puede clasificarse como una especie magra (< 2% grasa), de acuerdo con la clasificación de Ackman (1990). Por el contrario, Rueda-López *et al.* (2011), utilizando dietas con niveles de proteína de 43 a 52% y niveles de lípido 8 y 18%, reportaron valores de acumulación de lípido en el músculo de totoaba desde 2.3 hasta 3.7%, con lo que se podría catalogar como una especie con bajo contenido de grasa (contenido de lípido de 2-4%). Por consiguiente, los resultados observados en estos experimentos sugieren que el porcentaje de acumulación de lípido en el músculo de esta especie puede ser manipulado por medio de la dieta.

En cuanto a los valores del contenido de lipasa en la curvina golfina, éstos mostraron una disminución conforme se incrementó el porcentaje de lípido en la dieta, aunque este patrón no fue estadísticamente significativo. De forma similar, en la especie *Odontesthes bonarienis*, se observó una disminución de la actividad de la lipasa conforme se aumentó el porcentaje de lípido de 6 a 25% en la dieta (Gómez-Requeni *et al.*, 2013). Sin embargo, en otros estudios llevados a cabo en la dorada, *S. aurata* y en larvas de *D. labrax,* se observó que la actividad de la lipasa se mantuvo estable aún con la inclusión de altos niveles de lípido en la dieta (García- Meilán *et al.*, 2013; Morais *et al.*, 2004). De acuerdo con la literatura, la actividad de la lipasa, al igual que otras enzimas pancreáticas, puede variar en los peces de acuerdo a factores tales como la edad, fuente, calidad del lípido y estado prandial de los organismos, entre otros (Morais *et al.*, 2007). Con respecto al peso molecular de la lipasa pancreática de la curvina golfina (57.4 kDa ± 0.7) reportado en el presente estudio, así como para la lipasa pancreática de humano utilizada como marcador molecular control (55.2 kDa ± 0.9), los valores obtenidos fueron similares a los reportados utilizando la técnica SDS-PAGE en algunos peces (60 y 74 kDa para la lipasa pancreática de bacalao y carpa, respectivamente) y para la lipasa pancreática humana (Gjellesvik *et al.*, 1992; Görgün y Akpinar, 2012; Iizuka *et al.*, 1991).

Por otra parte, la utilización de dietas con un nivel de inclusión de 25, 50 y 75% de

harina de soya no transgénica, concentrado de proteína de soya y concentrado de proteína de maíz, resultaron en un mejor rendimiento de la curvina de aleta corta, *C. parvipinnis*, en términos de peso final (PF), peso ganado (%) (PG%) e incremento de peso diario (IPD), en comparación con los peces alimentados con la dieta control (100% harina de pescado). Estos resultados son similares a los reportados para otras especies de peces marinos como el pargo, *P. major* (Kader *et al.*, 2012), el jurel de California, *S. lalandi* (Buentello *et al.*, 2015), la dorada, *S. aurata* (Martínez-Llorens *et al.*, 2007), la curvina roja (Moxley *et al.*, 2014; Rossi Jr. *et al.*, 2015 y Rossi Jr. *et al.*, 2013), la curvina blanca, *Atractoscion nobilis* (Jirsa *et al.*, 2014), la cobia (Luo *et al.*, 2013), el lenguado Japonés (Kikuchi, 1999), el turbot, *P. maxima* (Regost *et al.*, 1999) y el lenguado de verano, *P. dentatus* (Enterria *et al.*, 2011). Se ha observado que los peces presentan, por lo general, una amplia tolerancia a los ingredientes de origen vegetal, ya que los niveles de inclusión pueden ir desde el 30% hasta el reemplazo total de la harina de pescado en la formulación de dietas, sin mostrar efectos negativos sobre el crecimiento de los peces cultivados. Adicionalmente, el nivel de tolerancia por estos ingredientes alternos puede depender de la especie, estadio de vida y calidad de los ingredientes, en términos de digestibilidad y presencia de compuestos antinutricionales.

Los compuestos antinutricionales presentes en la harina de soya pueden incluir proteínas antigénicas, inhibidores de las proteasas, lectinas y ácido fítico, los cuales, en combinación con la deficiencia de algunos aminoácidos, han causado severas consecuencias en el crecimiento de los peces cuando el porcentaje de inclusión es mayor al 40% (Gatlin *et al.*, 2007). Sin embargo, la implementación de nuevas técnicas en el refinamiento de estos ingredientes han producido los llamados concentrados de proteína, los cuales presentan menor cantidad de anti nutrientes y mayor cantidad de proteína que las harinas convencionales (NRFS, 2011), permitiendo un mayor nivel de inclusión de estos ingredientes sin mostrar efectos adversos sobre el crecimiento de los organismos. De acuerdo con lo anterior, en el presente estudio fue posible reemplazar hasta el 75% de harina de pescado en dietas para la curvina de aleta corta utilizando harina de soya no transgénica, concentrado de proteína de soya y concentrado de proteína de maíz. Sin embargo, los peces alimentados con la dieta que contenía 75% de harina de soya no transgénica presentaron valores más bajos en términos del porcentaje de peso ganado y tasa de crecimiento específica, en comparación con aquellos

alimentados con dietas que contenían 25 y 50% de reemplazo. El factor de conversión alimenticia fue claramente afectado por el nivel de reemplazo de harina de pescado, mostrando los valores más altos en los peces que fueron alimentados con la dieta que contenía el 75% de inclusión de harina de soya no transgénica, en comparación con los peces alimentados con dietas con un nivel menor de inclusión de harina de soya, concentrado de proteína de soya y concentrado de proteína de maíz. Estos resultados podrían ser atribuidos posiblemente a la mayor presencia de compuestos antinutricionales en la harina de soya, en comparación con los concentrados de proteína de soya y de maíz.

Las lisozimas en los peces se encuentran relacionadas con la respuesta inmunológica no especifica ante la presencia de patógenos y como claro indicador de los niveles de estrés (Tort *et al.*, 2003). En el presente estudio, la mayor concentración de lisozimas y neutrófilos sanguíneos presentes en la curvina de aleta corta se encontraron en los peces alimentados con las dietas que contenían 50 y 75% de harina de soya. Estos resultados son similares a los reportados previamente por Ribeiro *et al.* (2015), quienes observaron que una mezcla de harinas y aceites de origen vegetal tienden a incrementar significativamente los valores de lisozimas en la especie *A. regius*, en comparación con la dieta referencia (100% harina de pescado). Sin embargo, estudios realizados por Buentello *et al.* (2015), Kokou *et al.* (2012) y Sitjà-Bobadilla *et al.* (2005) indican que los valores de lisozimas se mantienen estables en la dorada al ser alimentada con dietas con niveles de inclusión de harina de soya bioprocesada, concentrado de proteína de maíz y harina de soya no transgénica, respectivamente. Adicionalmente, Dawood *et al.* (2014) reportaron que los niveles más bajos de lisozimas para el jurel, *S. dumerili,* se obtuvieron en peces alimentados con una dieta que contenía 45% de harina de soya, relacionando este resultado con un pobre estado de salud de los peces. A su vez, Rossi Jr. *et al.* (2015) no observaron algún efecto en la concentración de lisozimas y neutrófilos sanguíneos en la curvina roja después evaluar la inclusión de harina de soya y harina de soya no transgénica en dietas experimentales para esta especie. Sin embargo, es bien sabido que algunos compuestos antinutricionales encontrados en la harina de soya como las saponinas pueden ser responsables de procesos inflamatorios en el tracto digestivo de los peces (Hedrera *et al.*, 2013; Knudsen *et al.*, 2008; Ribeiro *et al.*, 2015). Con respecto a lo anterior, Krogdahl *et al.* (2000) reportaron un aumento en la concentración de lisozimas y una

menor resistencia a enfermedades en el salmón del Atlántico alimentado con harina de soya.

Los resultados obtenidos en el presente estudio podrían apoyar la hipótesis de que los desbalances nutricionales y/o las deficiencias causadas por el parcial o total reemplazo de harina de pescado, representan una fuente potencial de estrés causando efectos adversos sobre la respuesta del sistema inmune, tal y como ha sido descrito por algunos autores (Merrifield *et al.*, 2011; Sitjà-Bobadilla *et al.*, 2005). Sin embargo, se requiere de mayor información para determinar acertadamente la respuesta del sistema inmune no específico en los peces marinos, ya que los criterios de interpretación en términos de la obtención de altas o bajas concentraciones de lisozimas, permanece aún inconsistente para algunas especies.

VI. CONCLUSIONES

- Con base en el análisis cuadrático de línea quebrada de dos pendientes, se estableció el requerimiento de lípido de la curvina golfina, *C. othonopterus*, en 11.4%, en combinación con un nivel de proteína de 40%, información de vital importancia para la formulación de alimentos balanceados para esta especie.
- La acumulación de lípido en los diversos tejidos analizados en la curvina golfina aumentó de manera proporcional con el nivel de lípido dietario, acompañado de una reducción del porcentaje de humedad en músculo y organismos completos.
- El contenido de lipasa pancreática en la curvina golfina tendió a disminuir conforme el porcentaje de lípido en la dieta se incrementaba, aunque esta tendencia no resultó ser estadísticamente significativa.
- La totoaba, *T. macdonaldi*, puede tolerar un porcentaje de inclusión de lípido desde 8 hasta 22%, en combinación con un nivel de proteína del 46%, sin mostrar efectos negativos en el crecimiento de los organismos. Por otra parte, la dieta que contenía 6% de lípido resultó ser inadecuada para la totoaba, al afectar significativamente el rendimiento de la especie en términos de crecimiento y utilización del alimento. Sin embargo, para promover prácticas sustentables en la formulación de alimento balanceado para juveniles de esta especie, se recomienda la inclusión de 8% de lípido dietario cuando se provee como aceite de pescado.
- El porcentaje de lípido dietario influyó claramente en la composición proximal de los diversos tejidos de totoaba analizados. Adicionalmente, se observó una reducción del porcentaje de humedad en músculo y organismo completo conforme los niveles de lípido en la dieta se incrementaban.
- De acuerdo con el porcentaje de acumulación de lípido en el músculo de la totoaba, la especie se cataloga como magra. Sin embargo, con base en los resultados en el presente experimento, así como en otras investigaciones, resulta evidente que el porcentaje de lípido en el músculo puede ser manipulado a través de la dieta.
- La curvina de aleta corta, *C. parvipinnis*, puede tolerar exitosamente la inclusión de hasta el 75% de concentrado de proteína de soya, 50% de harina de soya no

transgénica y 75% de concentrado de proteína de maíz, sin comprometer su rendimiento en términos de crecimiento y utilización del alimento.

VI. RECOMENDACIONES

- En el presente estudio se logró determinar el requerimiento de lípido dietario de la curvina golfina, *C. othonotperus*, y con ello, se logró elucidar el requerimiento de los dos macronutrientes de mayor importancia, proteína (determinado previamente) y lípido. Por lo anterior, se recomienda utilizar esta información para la formulación y elaboración de alimentos balanceados para juveniles de esta especie, con el fin de facilitar su cultivo a escala comercial.
- Así mismo, en el presente estudio se logró determinar que la totoaba, *T. macdonaldi*, puede tolerar dietas con un nivel de inclusión de lípido desde 8 hasta 22%, sin mostrar efectos negativos en su crecimiento. Con base en la problemática mundial que gira en torno a la restricción de la utilización de insumos marinos en la formulación de dietas para organismos marinos, del intervalo de niveles de lípido tolerados por la totoaba, se recomienda utilizar el menor (8%), por ser congruente con un uso racional y más amigable con el ambiente de este recurso.
- En este mismo sentido, se recomienda llevar a cabo la evaluación del reemplazo de harina y aceite de pescado, utilizando ingredientes alternos en la elaboración de dietas para la curvina golfina y la totoaba.
- Se recomienda determinar los requerimientos nutricionales, en términos de lípido y proteína cruda, de la curvina de aleta corta, *C. parvipinnis*, con lo que se podrían mejorar los índices de crecimiento y utilización del alimento para la especie.
- De la misma forma, se recomienda evaluar el rendimiento biológico de la curvina golfina, la totoaba y la curvina de aleta corta en tanques de concreto y estanques de tierra, con lo que se podrían aprovechar las instalaciones destinadas para el cultivo de camarón, muchas de las cuales, han sido abandonadas debido su problemática actual.
- En el presente estudio, los valores de las variables fisicoquímicas del agua de cultivo en las que se llevaron a cabo los experimentos fueron similares a los valores óptimos reportados para el cultivo de otras especies de peces marinos, por lo que se considera que éstas no afectaron el desempeño de los organismos. Sin embargo, se recomienda

determinar los valores óptimos de oxígeno disuelto, salinidad, temperatura, pH y amonio, con el fin de maximizar el crecimiento de la curvina golfina, totoaba y curvina de aleta corta, lo que permitirá un mejor manejo de estos organismos en cautiverio.

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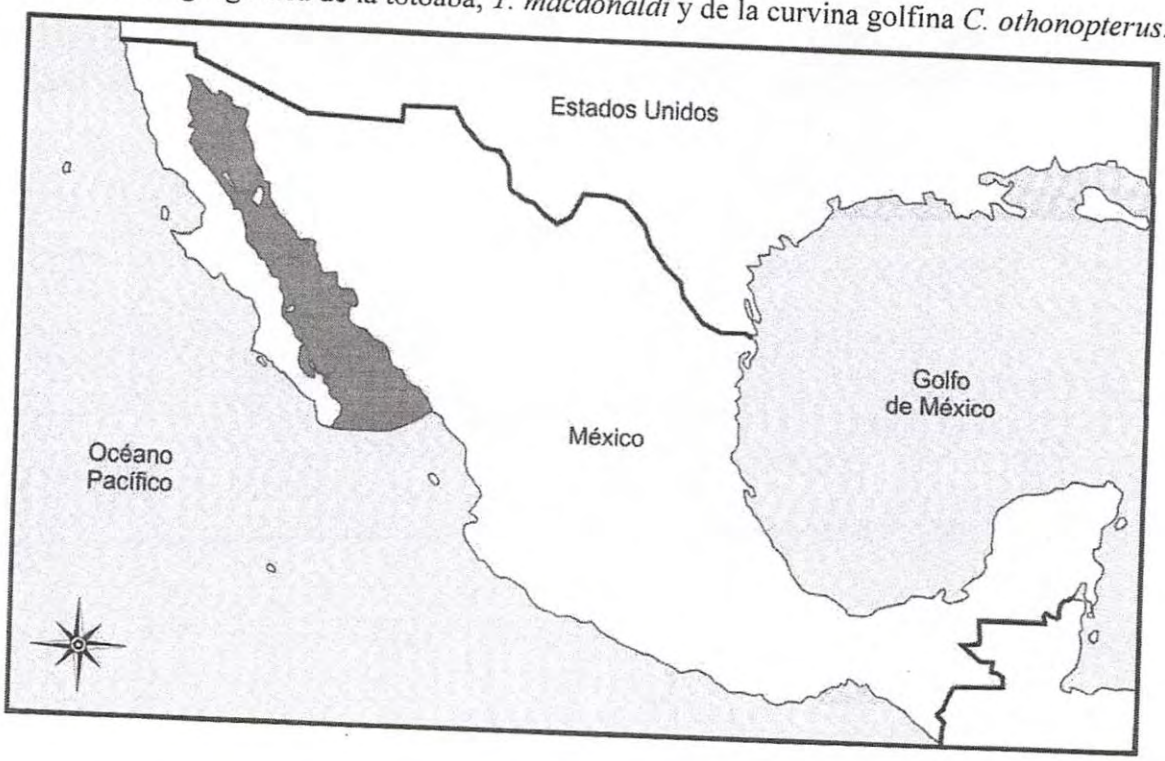
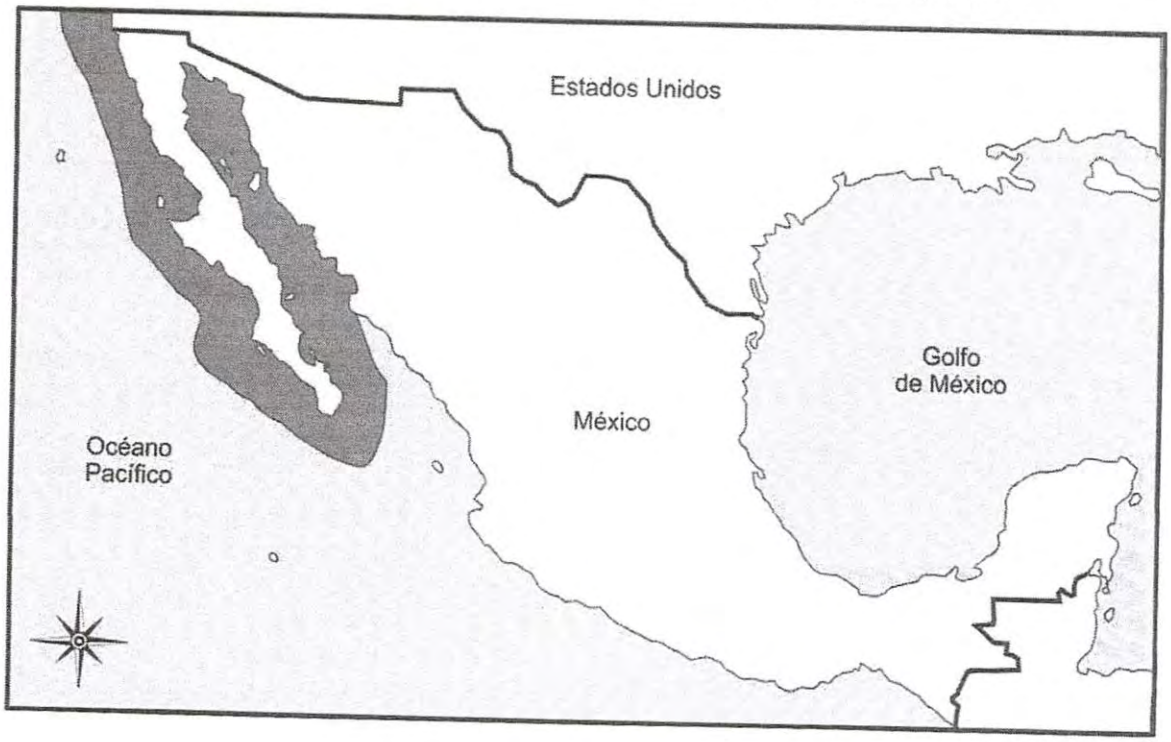
APÉNDICE I.

Distribución geográfica de la totoaba, *T. macdonaldi* y de la curvina golfina *C. othonopterus*.



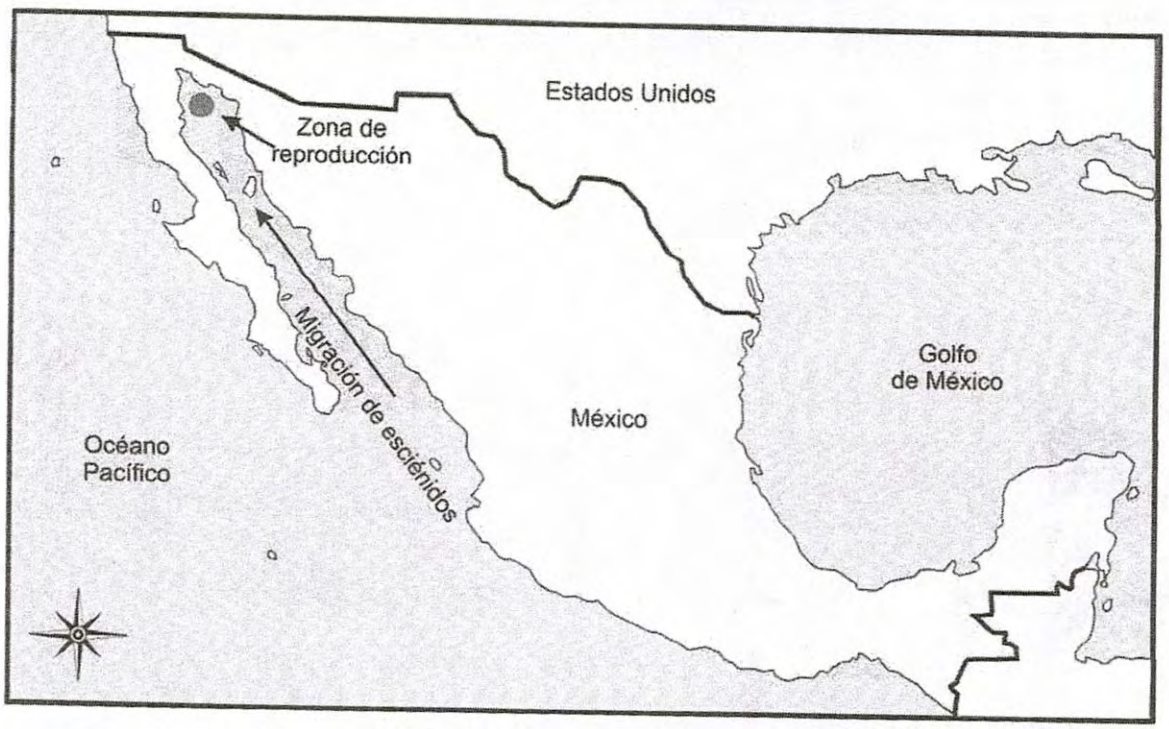
APÉNDICE II.

Distribución geográfica de la curvina de aleta corta, *C. parvipinnis.*



APÉNDICE III.

Migración y zona de reproducción de la curvina golfina, *C. othonopterus*, de la curvina de aleta *corta C. parvipinnis* y de la totoaba, *T. macdonaldi*.



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