UNIVERSIDAD NACIONAL AUTÓNOMA DE MÉXICO
POSGRADO EN CIENCIAS DEL MAR Y LIMNOLOGÍA

Valor nutricional del biofloc sobre la historia nutricional y desempeño reproductivo del camarón blanco del Pacífico (*Litopenaeus vannamei*) y del camarón rojo del Caribe (*Farfantepenaeus brasiliensis*)

TESIS
POR ARTÍCULOS CIENTÍFICOS

QUE PARA OBTENER EL GRADO ACADÉMICO DE:
DOCTOR EN CIENCIAS

PRESENTA:
EDÉN MAGAÑA GALLEGOS

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DR. GERARD CUZON
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MÉXICO, CD. MX., OCTUBRE, 2018

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Si te caes te levantas y si alguien te dice que no puedes, lo haces incluso mejor. Nunca pierdas la humildad. Transmite la información…que: ¡para eso es!

DEDICATORIA

A mi esposa…

A mis padres biológicos…

A mis padres académicos…

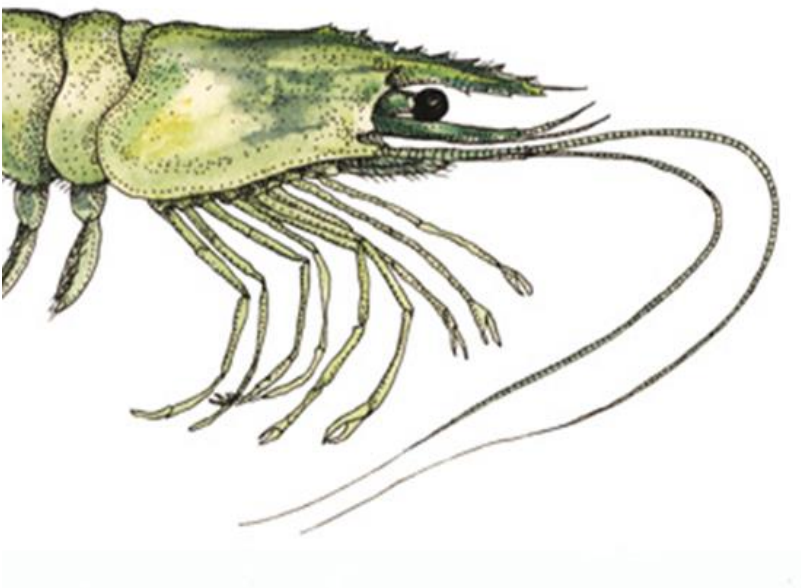
A todos mis hermanos…

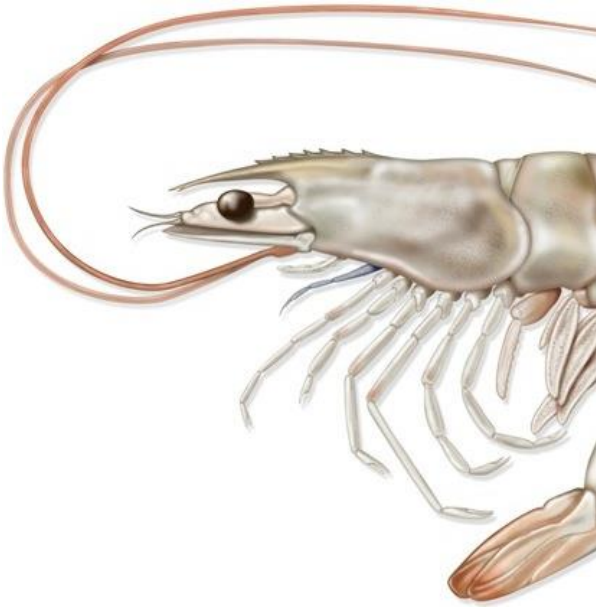
INDICE GENERAL

CAPÍTULO I

Estructura general de la tesis

Introducción general, hipótesis y objetivos





CAPÍTULO 1

Estructura general de la tesis:
Introducción general, hipótesis, objetivos y preguntas de investigación

Resumen

Este capítulo tiene como finalidad mostrar la estructuración de la presente tesis así como proveer una breve introducción del uso del biofloc en la camaronicultura. Esta tesis contiene siete capítulos (incluyendo este **primer capítulo** titulado: *Estructura general de la tesis*). El **segundo capítulo** tuvo la finalidad de adentrarnos en el tema "*¿Cuál es la participación real del biofloc para camarones peneidos*?", y abarca temas relacionados a la evolución de los sistemas de cultivo y su arribo a los sistemas biofloc, el uso de técnicas analíticas que direccionan la contribución de los nutrientes a los organismos de engorda y el efecto del biofloc en la reproducción. El **tercer capítulo** tuvo el objetivo de determinar cuál es la contribución de diferentes fuentes alimenticias (biofloc, alimentos frescos y artificiales) en la engorda y generación de huevos de *Farfantepenaeus brasiliensis* y se tituló: "*Contribución del biofloc y alimento artificial en la engorda y reproducción de F. brasiliensis (Latreille, 1817) determinado por ácidos grasos e isótopos estables*". El **cuarto capítulo** se tituló: "*Contribución nutricional del biofloc durante la engorda y reproducción de Litopenaeus vannamei, determinado por isótopos estables y ácidos grasos"*. Tanto el tercer y cuarto capítulo utilizan herramientas metodológicas tales como: i) isótopos estables, ii) ácidos grasos, iii) análisis bromatológicos de varios alimentos, iv) modelos de mezcla lineales basados en balance de masas para calcular la contribución de diversas fuentes alimenticias en la dieta de los camarones y producción de huevos. El **quinto capítulo** fue titulado: "*¿La ablación unilateral del pedúnculo ocular afecta la calidad de las larvas del camarón rosado F. brasiliensis (Latreille, 1817) (Decapoda: Dendrobranchiata: Penaeidae)?*". El **sexto capítulo** se tituló: "*¿El biofloc mejora la producción y calidad de huevos de L. vannamei (Boone, 1931) (Decapoda: Dendrobranchiata: Penaeidae) sin ablación unilateral del pedúnculo ocular?*". Tanto el quinto y sexto capítulo, utilizaron varios indicadores reproductivos así como metabólicos (p.ej. proteína total soluble, acilglicéridos, colesterol, glucosa). Y finalmente, el **séptimo capítulo** comprende una discusión general y conclusiones.

Abstract

The aim of this chapter is to show the structure of the thesis as well as a short introduction about the use of biofloc in shrimp aquaculture. This thesis has six chapters (including this one entitled "*general structure of the thesis"*). **Second chapter** has the aim to show "*¿What is the real contribution of biofloc for peneid shrimps?*", and related topics as aquaculture system evolution and the use of biofloc, the use of techniques that help to know what is the contribution of some food sources to the shrimp diet during grow-out and reproduction. **Third chapter** had the aim to determine the contribution of different food sources (biofloc, fresh and artificial foods) during the grow-out and egg production of *F. brasiliensis* and was entitled: "*Biofloc and food contribution to grow-out and broodstock of Farfantepenaeus brasiliensis (Latreille, 1817) determined by stable isotopes and fatty acids*". **Fourth chapter** was entitled: "*Nutritional contribution of biofloc within the diet of growout and broodstock of Litopenaeus vannamei, determined by stable isotopes and fatty acids*". Both third and fourth chapter used methodological tools as: i) stable isotopes, ii) fatty acid, iii) bromatological analysis, iv) mass balance lineal models to calculate the food source contribution for shrimp and eggs. **Fifth chapter** was entitled: "*Does unilateral eyestalk ablation affect the quality of the larvae of the pink shrimp Farfantepenaeus brasiliensis (Latreille, 1817) (Decapoda: Dendrobranchiata: Penaeidae)?*". **Sixth chapter** was entitled: "*Does biofloc improve the production of quality eggs in Litopenaeus vannamei (Boone, 1931) (Decapoda: Dendrobranchiata: Penaeidae) without unilateral eyestalk ablation?*" Both fourth and fifth chapters used reproductive, metabolic and morphological indicators. Finally, the **seventh chapter** addressed topics related to general discussion and conclusions.

Introducción general

La aplicación de la tecnología biofloc (BFT) en el cultivo de camarón ha evolucionado de sistemas intensivos hasta hiperintesivos. Además, este sistema ya no es solamente aplicado a la etapa de engorda de camarón si no que actualmente se están llevando a cabo estudios en la etapa de pre-maduración de reproductores de camarones como *L. vannamei* y *L. stylirostris* (Cardona *et al.*, 2016; Chim *et al.*, 2014; Magaña-Gallegos *et al.*, 2018b). No obstante, no ha sido determinado cual es la contribución del biofloc a la progenie. A pesar de esto, es bien sabido que esta tecnología provee muchos beneficios a la camaronicultura (Braga *et al.*, 2015; Suita *et al.*, 2016) tales como mejorar la respuesta inmune (Xu & Pan, 2013), incrementar la producción (Chan-Vivas *et al.*, 2018; Martínez-Córdova *et al.*, 2014), efecto probiótico y bioseguridad (Aguilera-Rivera *et al.*, 2014; Aguilera-Rivera *et al.*, 2018), mejor calidad del producto final y disminución del factor de conversión alimenticia (Chan-Vivas *et al.*, 2018), y nutrición (los flóculos contienen entre un 25-30% de proteína cruda y pueden ser ingeridos por los organismos de cultivo) (Ekasari *et al.*, 2014b; Magaña-Gallegos *et al.*, 2018b). Los principios básicos de este sistema han sido especificados como: i) bajo recambio de agua, ii) la adición de una fuente de carbono orgánico externa para mantener una relación C:N deseada, con el fin de promover el crecimiento microbiano heterotrófico, iii) una aireación constante para mantener los flóculos en suspensión y iv) una alta densidad de siembra (Avnimelech, 1999; Crab *et al.*, 2012; Emerenciano *et al.*, 2013b).

En el sistema biofloc, las comunidades planctónicas surgen a través del periodo de cultivo, y los microorganismos que crecen en el sistema pueden representar una fuente de alimento continuo rico en proteínas, lípidos y carbohidratos (Becerra-Dórame *et al.*, 2012; Ekasari *et al.*, 2010; Magaña-Gallegos *et al.*, 2018a). Es por esto, que la productividad natural más el alimento comercial pueden mejorar las prácticas de alimentación y consecuentemente reducir los desechos de las granjas acuícolas (Abreu *et al.*, 2007; Avnimelech, 1999; Gamboa-Delgado, 2014). De hecho, la contribución relativa de los flóculos puede alcanzar hasta un 60% en el crecimiento de los camarones (Chim *et al.*, 2014; Magaña-Gallegos *et al.*, 2018b). A pesar de este entendimiento, es necesario investigar cual es el valor nutricional de los flóculos de diferentes tamaños de partícula durante la engorda de *L. vannamei* y *F. brasiliensis* y a su vez, es necesario direccionar, cual es la participación del

biofloc en la producción de huevos de camarones peneidos con y sin ablación. La ablación es una técnica que ha sido utilizada con el fin de acelerar la maduración gonádica (Vaca & Alfaro, 2000). No obstante, ha sido muy criticada en años recientes debido a que provoca un desbalance hormonal a los camarones, provocando una pérdida de su homeostasis (Munkongwongsiri *et al.*, 2015). El biofloc ha demostrado ser útil en mejorar la producción y calidad de huevos de camarones peneidos, no obstante, no hay reportes de si la ablación puede ser suspendida de camarones que han sido previamente madurados en biofloc.

Por ello es de primordial importancia generar conocimiento básico que relacione los nuevos sistemas alternativos de producción, tales como el de la BFT (Avnimelech, 1999) con las ventajas de manejo, especialmente la nutrición y desempeño reproductivo. Debiendo considerar las diferencias del ciclo de vida que presentan *L. vannamei* y *F. brasiliensis* tales como las conductuales y reproductivas. Por ello, es importante generar estudios comparativos con el fin de establecer las particularidades de manejo que se requieren y así proponer modelos que puedan aplicarse a la producción en otras regiones del país.

Generalidades de *L. vannamei*

El camarón blanco del Pacífico *L. vannamei* es la especie de camarón de más alta importancia de cultivo y ha presentado los más altos valores en el mercado de crustáceos (Emerenciano *et al.*, 2012b; FAO, 2017; 2018). Estudios sobre los hábitos alimenticios han demostrado que es una especie omnívora-herbívora (McTigue & Zimmerman, 1991) y tiene la habilidad de utilizar diferentes proporciones de proteínas como sustrato metabólico (Rosas *et al.*, 1995), siendo esta plasticidad del organismo una ventaja potencial para utilizarla en sistemas con BFT. Debido a que la formulación de los alimentos y tipo de cultivo es la base del éxito para los productores (Velasco *et al.*, 2000), la nutrición y alimentación especialmente de *L. vannamei* ha recibido gran atención en los últimos años (Cuzon *et al.*, 2004; NRC, 2011). Por esta razón, la tendencia ha sido buscar fuentes alternativas de proteínas y técnicas de cultivo que influyan en la velocidad de crecimiento, desempeño reproductivo o para alcanzar las tallas máximas de la especie (Carrillo *et al.*, 2000; Emerenciano *et al.*, 2013a; Galindo *et al.*, 2001). En cuanto a sus requerimientos de calidad del agua, *L. vannamei* ha sido la especie más estudiada y los niveles de seguridad para amonio, nitrito y nitrato han sido establecidos en diferentes salinidades (Lin & Chen,

2001; 2003). Como factor de seguridad en biofloc se ha tomado como regla general 1 mg/L de nitrógeno amoniacal total para esta especie a 33-35 de salinidad (Serra *et al.*, 2015), aunque desafortunadamente muchos trabajos no tomen en cuenta ni la salinidad ni el pH como factores que afectan la concentración del amonio no ionizado en el agua. En cuanto a los valores de alcalinidad, dureza por calcio/magnesio y potasio estos valores han sido definidos previamente (Furtado *et al.*, 2011; Van Wyk & Scarpa, 1999).

Generalidades de *F. brasiliensis*

En México, *F. brasiliensis* tiene gran importancia pesquera junto con otras seis especies (*F. aztecus, F. duorarum, L. setiferus, Sicyonia brevirostris* y *Xiphopenaeus kroyeri* (SAGARPA, 2002). Con respecto a la acuacultura *F. brasiliensis* tiene tasas de crecimiento relativamente buenas, y el tamaño máximo de los adultos es grande comparado a otras especies de peneidos (Gaxiola *et al*., 2010); los adultos pueden llegar a alcanzar pesos de entre 50-60 g. En cuanto a su alimentación, en un estudio realizado en la laguna de Imbossica sobre el contenido estomacal, se le describe como una especie omnívora y de carácter oportunista, lo que la haría una especie objetivo para la acuacultura debido a que tiene la capacidad de aprovechar diferentes sustratos alimenticios (Albertoni *et al.*, 2003). Esta especie ha sido previamente cultivada en sistemas con BFT, sin embargo, información clave en cuanto a desempeño reproductivo y nutrición no son del todo claros (Braga *et al.*, 2011; Magaña-Gallegos *et al.*, 2018a). A pesar de esto, ha habido un gran esfuerzo por establecer los valores de tolerancia de los compuestos nitrogenados más importantes como son el amonio en su forma no ionizada, el nitrito y el nitrato (Campos *et al.*, 2014; Hostins *et al.*, 2015). No obstante, es difícil encontrar información relacionada a otros parámetros fisicoquímicos como lo son alcalinidad, dureza del agua por calcio y por magnesio, potasio y fósforo para el manejo de esta especie. No obstante, es posible utilizar los valores recomendados para otros camarones peneidos (Van Wyk & Scarpa, 1999), aunque es necesario llevar a cabo estudios para determinar las concentraciones óptimas y su efecto en el crecimiento de este organismo.

Mortalidades masivas tempranas de camarón

La enfermedad de la necrosis hepatopancreática aguda en camarones comúnmente referida como Síndrome de Mortalidad Temprana (SMT) fue reportada por primera vez en algunas

ciudades de China en 2009 y es vinculada a la bacteria patógena *Vibrio paraemoliticus* (Nunan *et al.*, 2014). Posteriormente, según Lightner, (2012) menciona que en 2011 el SMT se expandió hasta Vietnam y Malasia. Asimismo, el SMT fue reportado en 2011 en México (Nunan *et al.*, 2014). Los principales síntomas son letargia y anorexia; al parecer el hepatopáncreas es el órgano objetivo por lo que se le encuentra atrofiado y blancuzco con rayas negras. En los estados terminales, ocurren severas infecciones causadas por bacterias oportunistas del género *Vibrio* (Lightner, 2012).

Los principales problemas con esta nueva enfermedad es que ataca a camarones entre postlarva 20 y 30 de las especies *Penaeus monodon* y *L. vannamei* provocando mortalidades muy cercanas al 100%. Además, ha tenido un impacto directo en la producción de camarón; por ejemplo, para México la producción cayó casi un 65% de 2011 a 2013 y la enfermedad se encuentra en expansión por el territorio nacional (Nunan *et al.*, 2014). En términos de mercado, varios países han suspendido o prohibido la importación de camarón vivo y/o todas las formas de productos de camarón, encontrándose México entre estos países (Ecuador, República Dominicana, Nicaragua, las Filipinas, y los Estados Unidos de América son otros ejemplos de países que han detenido la exportación de camarón; FAO, 2013). Dado lo anterior es necesario desarrollar nuevos esquemas de cultivo que sean capaces de aumentar la bioseguridad en el cultivo de camarón y que ayuden a mejorar las buenas prácticas de manejo (Aguilera-Rivera *et al.*, 2018).

Cultivo de camarón con biofloc

Los sistemas modernos de cultivo son desarrollados con la premisa de disminuir la cantidad de agua utilizada, tal es el caso de los sistemas de recirculación de agua clara, pero nuevas alternativas han surgido como es el caso de la BFT. Este último sistema tiene dos principios: i) cero o mínimo intercambio de agua y ii) el desarrollo de una comunidad microbiana heterotrófica o actualmente llamada mixotrófica, la cual forma flóculos que contienen bacterias, fitoplancton y zooplancton (Emerenciano *et al.*, 2013c; Samocha *et al.*, 2017; De Schryver *et al.*, 2008). Asimismo, se han vinculado a este sistema más altas producciones (kg/ha), reciclamiento de la proteína del alimento, mantenimiento de la calidad del agua, retención de nutrientes por parte de los organismos cultivados así como un mejor desempeño reproductivo (Avnimelech, 2007; Azim *et al.*, 2008; Emerenciano *et*

al., 2012; Xu and Pan, 2012; Aguilera-Rivera *et al*., 2014; Luo *et al*., 2014). Estos flóculos compuestos de la microbiota nativa, pueden incrementar la eficiencia de la utilización del alimento comercial, debido a que son una fuente de alimentación disponible 24 horas al día (Avnimelech, 2007; De Schryver *et al*., 2008; Ray *et al*., 2010; Emerenciano *et al*., 2011).

Para evaluar el uso de la BFT en peneidos como una fuente alimenticia (Avnimelech, 2007; Azim *et al.*, 2008; Luo *et al.*, 2014), varios criterios tales como tamaño de partícula (Ekasari *et al.*, 2014a; Magaña-Gallegos, 2014), desarrollo de la comunidad microbiana (Avnimelech, 2009; Azim *et al.*, 2008; Emerenciano *et al.*, 2011; De Schryver *et al.*, 2008), crecimiento y supervivencia (Emerenciano *et al.*, 2012a), digestibilidad (Becerra-Dórame *et al.*, 2012; Magaña-Gallegos, 2014), contenido nutricional, aminoácidos, ácidos grasos y micronutrientes (Ekasari *et al.*, 2014a; Magaña-Gallegos, 2014) e isótopos estables (Cardona *et al.*, 2015; Gamboa-Delgado, 2014) han sido utilizados. Sin embargo, es necesario desarrollar investigaciones que incluyan un carácter integral de todos estos factores, así como el factor tiempo ya que los camarones presentan cambios fisiológicos a través de su ciclo de vida.

El biofloc es un sistema que requiere un manejo adecuado de las concentraciones de carbono y nitrógeno, ya que un desbalance en ciertos iones, por ejemplo el bicarbonato puede afectar seriamente el funcionamiento del sistema. Con el fin de montar un sistema biofloc, varios aspectos en la calidad del agua deben ser tomados en cuenta previamente y van desde la cantidad de nitrógeno proveniente de compuestos nitrogenados como el amonio y nitrito, la cantidad de carbono orgánico generalmente abastecida mediante melaza, azúcar u otra fuente rica en carbono orgánico, la cantidad de carbono inorgánico relacionado a la alcalinidad, dureza por calcio y concentraciones de fósforo en el agua. Una vez se hayan establecido estos parámetros, es posible empezar una pre-fertilización (fertilización previa a la siembra de los animales) que puede ir desde los siete a 30 días previos de la siembra de los animales (Avnimelech, 2009; Effendy *et al.*, 2016). Esto dependerá de los objetivos del trabajo y de la disponibilidad de tiempo; aunque es muy recomendable, ya que se evitan aumentos de amonio y nitritos posteriores a la siembra de los animales (Avnimelech, 2009; Emerenciano *et al.*, 2017; Otoshi *et al.*, 2011). Generalmente, 15 días previos de fertilización inicial en una relación C:N 20:1 son

suficientes para establecer una comunidad heterotrófica y nitrificante. La cantidad de nitrógeno a suministrar, debe emular la cantidad máxima de alimento que ingresará al día al sistema cuando los animales sean sembrados, con esto, aseguramos que el sistema tenga la capacidad de absorber todo el nitrógeno que puede llegar a ser tóxico para los animales. La recomendación de Emerenciano *et al*. (2009), es agregar la cantidad de nitrógeno equivalente a la entrada de alimento de 300 post-larvas por metro cuadrado; aunque es posible utilizar la densidad de siembra y peso del camarón para el experimento, con el fin de adecuar el arranque del sistema para la entrada específica de alimento que ingresará al sistema (Avnimelech, 2009). Esta fertilización puede ser suministrada en el día uno y posteriormente cada cinco días hasta alcanzar los sólidos deseados o hasta que empiece la formación de sólidos suspendidos. Para esto, se puede utilizar alimento comercial sobrante de las granjas (el cual tiene nitrógeno y carbono orgánico), o fertilizantes ricos en nitrógeno como el cloruro de amonio (~26% es nitrógeno) o la urea (~45% es nitrógeno). Junto con algún fertilizante rico en nitrógeno, se utiliza algún material rico en carbono orgánico como la melaza (~30-50% de carbono dependiendo de la calidad) o azúcar (100% de carbohidratos con ~42% de carbono) en la relación C:N 20:1 o a la establecida en el experimento. Es muy importante, monitorear dos o tres días previos a la siembra de organismos la concentración del amonio y nitrito con el fin de asegurarnos que no estén en niveles tóxicos y preferentemente deben estar por debajo de los niveles de seguridad establecidos para la especie. Una vez sembrados los animales en el día 16, es posible seguir fertilizando con relación al alimento suministrado una fuente de carbono en una relación C:N 20:1 con el fin de incrementar los sólidos suspendidos hasta su concentración deseada, que para camarones es mayor a 5 ml/l pero menor a 15 ml/l (específicamente para *L. vannamei*, en la UMDI-SISAL estas concentraciones han funcionado de manera similar para *F. brasiliensis*). Una vez alcanzada la cantidad de sólidos suspendidos, se suspende la adición de la fuente de carbono y se empieza una fase de mantenimiento, donde se utiliza la regla de que por cada 1 g de nitrógeno amoniacal total se agregan 6 g de carbono orgánico al agua (Ebeling et al. 2006). Además de este esquema, será necesario tener en cuenta otros parámetros de la calidad del agua en el estanque, como son el pH, el cual direcciona la toxicidad del amonio, ya que a mayor pH mayor será la cantidad de amonio no ionizado, el cual es tóxico para los animales. Con el fin de mantener un adecuado pH, sin variaciones

tan drásticas durante el día, debido a la actividad fitoplanctónica, heterotrófica y nitrificante, es necesario mantener la alcalinidad entre 120-180 mg/L de CaCO3, por lo que se puede llevar un monitoreo rutinario, con el fin de determinar su concentración en el agua y hacer las correcciones necesarias (Furtado *et al.*, 2011). Los estaques deben tener acoplados sedimentadores con el fin de controlar la cantidad de sólidos suspendidos si se requiriera. En términos prácticos, un sistema biofloc, consumirá por cada gramo de amonio, una cierta cantidad de oxígeno, carbono inorgánico (alcalinidad) y carbono orgánico y producirá dióxido de carbono, iones hidrógeno y biomasa bacteriana (Ebeling *et al.*, 2006). Actualmente, existen otros métodos para hacer biofloc, con otra premisa, la cual es no depender directamente de las bacterias heterotróficas, si no balancear el sistema de tal manera que las bacterias nitrificantes jueguen un papel en la remoción de compuestos nitrogenados (Samocha *et al.*, 2017). Asimismo, otros métodos de fertilización inicial pueden ser seguidos, tal es el caso del inicio del sistema con nitrito de sodio (Otoshi *et al.*, 2011).

Variación de los microorganismos y valor nutricional del biofloc

El esquema tradicional de la dinámica trófica en los sistemas con flóculos microbianos es muy similar a la de ambientes naturales (Tabla 1). No obstante, resulta ser bastante compleja al momento de caracterizarla (Emerenciano *et al.*, 2011; 2012a; 2013c). Por ejemplo, la BFT ha sido implementada en varios países y el análisis de los microorganismos y potencial nutricional (análisis proximal) varía ampliamente por lo que no conservan las mismas características espacio-temporales (Hargreaves, 2013; Martínez-Cordava et al. 2018).

En un estudio realizado por Emerenciano *et al*., (2013) se describen los principales taxa encontrados en los sistemas con BFT. Como resultados, se reporta que los principales organismos son cianobacterias filamentosas, protozoarios, nematodos y copépodos los cuales van cambiando sus densidades a través del tiempo en un periodo experimental de siete meses. En su estudio, Emerenciano *et al*., (2013b) determinan contenidos de proteína cruda y lípidos de 24.7 y 0.6% respectivamente. Los valores anteriores son el promedio de los siete meses de su experimento y estos variaron respecto al mes. Los menores valores de proteína y lípidos concuerdan con una baja concentración de copépodos, nematodos y

cianobacterias filamentosas. Asimismo, Emerenciano *et al*., (2013a, 2012b) han reportado que la concentración de ácidos grasos altamente insaturados (HUFA) en el biofloc más el aporte de alimento fresco puede provocar un mejor desempeño reproductivo en *L. vannamei* y *L. stylirostris.*

En otro estudio, desarrollado por Becerra-Dórame *et al*., (2012) se encontraron niveles de proteína más altos en sistemas con biofloc (17.5%) que en aquellos con sistemas autotróficos (11.5%). De manera inversa, el contenido de lípidos en BFT fue 6.5% y en el sistema autotrófico fue de 13.3%. El alto porcentaje de proteína en los flóculos está relacionado a la composición química de las bacterias heterotróficas y otros microorganismos asociados. Mientras que el alto porcentaje de lípidos en el sistema autotrófico está relacionado a la alta concentración de microalgas.

Los dos casos de estudio mencionados anteriormente (Becerra-Dórame *et al.*, 2012; Emerenciano *et al.*, 2013c), demuestran como el cambio en la estructura de los microorganismos del biofloc tiene una influencia en su calidad y por lo tanto en el aporte nutricional hacia los organismos de cultivo. Asimismo, Ekasari *et al*., (2014) menciona que el tamaño de los flóculos microbianos puede influir en su calidad nutricional por lo que la evaluación del aporte de diferentes tamaños del biofloc en la síntesis del músculo de los camarones debe ser evaluada.

De manera similar, en un estudio realizado por Martínez-Córdova et al. (2018) se evaluaron dos tipos de agentes nucleantes (salvado de trigo y amaranto), con el fin de evaluar la dinámica bacteriana de los sistemas biofloc y su impacto en la calidad nutricional. Sus resultados demuestran que el agente nucleante tiene un gran efecto en la composición de bacterias al inicio y al final del experimento. Por ejemplo, se determinó que las bacterias del género Bacteroidetes fueron mayormente representadas al inicio del cultivo con salvado de trigo, representando entre un 75-85% de la abundancia de bacterias (baja diversidad bacteriana); no obstante, al final del experimento la diversidad bacteriana aumento. Por el contrario, el biofloc iniciado con amaranto, tuvo una mayor diversidad inicial, estando mayormente representado por las bacterias del género Planctomycetes, Proteobacteria y Bacteroidetes, y no sufrió cambios sustanciales en la composición bacteriana entre inicio y final del experimento. Lo interesante, es que el valor nutricional del biofloc cambio entre

tratamientos, presentado la mayores concentración de proteína cruda al final el tratamiento con salvado de trigo al final del experimento y por lo tanto los parámetros productivos favorecieron el cultivo de *L. vannamei* con salvado de trigo.

Dado lo anterior, el componente bacteriano es un compartimiento nutricional clave en el biofloc, y no solamente está relacionado al mantenimiento de la calidad del agua, sino también al valor nutricional de los flóculos (Martínez-Córdova *et al*., 2018; Ortiz-Estrada *et al*., 2018). Además, a corto o mediano plazo, la dinámica bacteriana puede tener importantes implicaciones en el tipo de microorganismos que posteriormente colonizan el sistema como el fitoplancton y zooplancton. De hecho, se ha demostrado que existen relaciones entre los ascensos y descensos en las concentraciones de bacterias con las del fitoplancton en sistemas biofloc (Bianchi y Martin, 1978). Otro factor importante, es que la calidad de los flóculos puede estar relacionada también con la concentración de microrganismos pertenecientes al zooplancton y estos a su vez pueden estar direccionados a la diversidad bacteriana de los sistemas biofloc (Martínez-Córdova *et al.*, 2018). De hecho, las interrelaciones bacteria-fitoplancton-zooplancton deberían ser estudiadas a mayor profundidad, ya que por ejemplo, hay hipótesis que sugieren que el valor nutricional del zooplancton está directamente relacionado con la composición nutricional del fitoplancton (principalmente relacionado a la concentración de ácidos grasos altamente insaturados) (Brett y Muller-Navarra, 1997). De hecho, Ortiz-Estrada et al. (2018), mencionan que las comunidades bacterianas en los sistemas de acuacultura han sido pobremente descritas, debido a la falta de herramientas que permitan caracterizar las comunidades bacterianas. Por ejemplo, se menciona que alrededor del 80% de las bacterias de una muestra cualquiera, no son cultivables y por lo tanto la caracterización de la comunidad bacteriana se ha visto limitada y por lo tanto su dinámica y función. No obstante, con el desarrollo de herramientas pertenecientes a la biología molecular tales como el uso del ARNr 16S (parte del ribosoma bacteriano), reacción en cadena de la polimerasa (PCR) y secuenciación del ADN es posible prescindir de los métodos basados en el cultivo bacteriano y pasar a la caracterización a partir de la información genética de una muestra y su función en un sistema (metagenómica).

Por lo tanto, la productividad natural tiene un importante papel en la suplementación nutricional de los camarones (Burford *et al.*, 2004; Emerenciano *et al.*, 2012b; 2013b; Martínez-Córdova & Peña-Messina, 2005; Samocha *et al.*, 2004) ya que contribuye con proteína nativa, carbohidratos y lípidos, así como aminoácidos, ácidos grasos, vitaminas y minerales (Crab *et al.*, 2012; Xu *et al.*, 2012).

En un estudio previo (Magaña-Gallegos, 2014) se reporta que los flóculos pueden variar en tamaño y con esta variación el valor nutricional puede cambiar. Asimismo, este estudio determinó que el biofloc puede ser visto como una dieta multifásica, tomando en cuenta los conceptos previos de una dieta difásica de Provasoli, (1971), y podrían explicar las mejoras en cuanto a parámetros de producción (Emerenciano *et al.*, 2012a), desempeño reproductivo (Emerenciano *et al.*, 2012a), calidad del producto final (Chan-Vivas, 2014) y estado de salud de los organismos (Aguilera-Rivera *et al.*, 2014). Sin embargo, es necesario entender la dinámica de los microorganismos, la manera en la que los camarones aprovechan el biofloc y la capacidad que tienen para utilizarlo ya que son temas muy importantes para un mejoramiento en cuanto a la producción de camarones en cultivo (Magaña-Gallegos, 2014).

Tabla 1 Estudios sobre contenido estomacal de camarones peneidos en ambientes naturales y controlados.

Especie	Alimento	Tipo de ambiente	Referencia
F. brasiliensis	Poliquetos, larvas de insecto y pequeños crustáceos.	Natural	Albertoni *et al*., 2003
F. paulensis	Larvas de insecto, pequeños crustáceos y poliquetos.	Natural	Albertoni *et al*., 2003
F. duorarum	Pequeños crustáceos, bivalvos, algas calcáreas, detritus vegetal, copépodos, fragmentos de pastos marinos, nematodos y diatomeas	Natural	Schwamborn y Criales, 2000
P. merguiensis	Restos no identificados, protozoarios, chelicerata, insectos, moluscos, anélidos, nematodos, equinodermos, peces, materia vegetal, algas y diatomeas.	Natural	Chong y Sasekumar, 1981
L. vannamei* y *L. stylirostris	Exuvia de camarón, granos de arena, zooplancton, presas del bentos, materia orgánica no identificable, macroalgas y microalgas.	Controlado (monocultivo y policultivo)	Martínez-Córdova y Peña-Messina, 2005

Nutrición en camarones y su relación con su desempeño reproductivo

Wouters *et al*., (2001) mencionan que la limitada e inconsistente disponibilidad de post-larvas junto con la urgente necesidad de establecer programas de reproducción, incrementa el interés de la reproducción en cautiverio de peneidos alrededor del mundo. Por lo tanto, esquemas de alimentación y manejo de pre-reproductores y reproductores están evolucionando (Cardona *et al.*, 2016; Emerenciano *et al.*, 2013a). El papel que juega la nutrición en la reproducción de los camarones aún se encuentra en estudio y es un tópico de gran debate (Wouters *et al.*, 2001). Sin embargo la disponibilidad de una dieta óptima es identificada como un factor crucial para la maduración sexual y la reproducción de los camarones. Por ejemplo, se ha reportado que dietas no balanceadas o incompletas causan desempeños reproductivos pobres o pueden hacer que los animales detengan su reproducción (Wouters *et al.*, 2001).

El papel de diferentes alimentos frescos y prácticas alimenticias no son completamente claras. Sin embargo Emerenciano *et al*. (2012b, 2013a) determinan que los alimentos marinos frescos tales como calamar y mejillón son ricos en fuentes de HUFA y por lo tanto juegan un papel particular en la reproducción de los camarones. Asimismo, se ha demostrado que los HUFA son esenciales en el proceso pre-reproductivo ya que gran parte de estos ácidos grasos son transferidos a los huevos de los camarones, influyendo en su calidad (Emerenciano *et al.*, 2012b).

Como se ha mencionado, el biofloc puede constituir una dieta multifásica así como puede proveer a los camarones de cultivo proteínas, lípidos y carbohidratos complementarios a su dieta basal. De hecho, Gamboa-Delgado, (2014) sugiere que la productividad natural puede proveer una fuente complementaria de nutrientes respecto al alimento balanceado en la nutrición de los camarones. A pesar de que se cuenta con el conocimiento de que el biofloc contribuye en gran medida con la nutrición de los camarones, es necesario realizar análisis proximales así como de determinación de aminoácidos y ácidos grasos con el fin de detectar cual es el valor nutricional del biofloc en cuanto a parámetros de producción y reproducción se refiere.

Asimismo, la mayoría de los estudios integran la ablación unilateral del pedúnculo ocular con el fin de acelerar la maduración gonádica de las hembras, no obstante actualmente está surgiendo el término BIO-etiqueta el cual considera que las granjas con esta práctica no lo puedan adquirir (Munkongwongsiri *et al.*, 2015). Por lo tanto surge la iniciativa de identificar si el biofloc puede activar la maduración de la gónada sin llevar a cabo la ablación. Experiencias positivas han sido observadas en camarones cultivados en sistemas con biofloc durante la segunda etapa del experimento de Magaña-Gallegos, (2014) ya que se identificaron hembras impregnadas con espermatóforos al final del experimento, lo que indicaría que las hembras están madurando antes de iniciar incluso los periodos establecidos de maduración. Por lo que surge la pregunta ¿el biofloc puede presentar un factor-biofloc como ha sido demostrado con el calamar cuando es provisto como alimento fresco a los reproductores en etapas pre-reproductivas? (Emerenciano *et al.*, 2012c).

Capacidad de los camarones peneidos para aprovechar el biofloc

Una de las principales preguntas hechas por los productores acuícolas es si cualquier especie elegida se puede cultivar en sistemas con biofloc. La respuesta a esta pregunta tiene que tomar en cuenta ciertos factores tales como i) capacidad de tolerar los sólidos suspendidos en el agua (i.e. para camarones se recomienda mantener los SST <15 mL/L), ii) digestión de los flóculos producidos en el sistema (*L. vannamei* y *F. brasiliensis* han demostrado tener una plasticidad enzimática bastante amplia, por lo que su cultivo es viable en sistemas biofloc), iii) apéndices especializados para cosechar los flóculos y poder ingerirlo (Hargreaves 2013). *L. vannamei* ha demostrado ser una especie apta para su cultivo en sistemas con biofloc, no obstante no ha sido evaluado como es que capturan los microorganismos y los ingieren. Al respecto, se ha demostrado que los camarones peneidos presentan en su tercer par de maxilípedos setas en forma de red que capturan microorganismos >10 μm de tamaño (Kent *et al*., 2011).

Contribución relativa de la productividad natural en la generación de músculo de camarones

Varios estudios han intentado determinar la contribución relativa de la productividad natural en la síntesis del músculo de varias especies de camarón utilizando la herramienta de isótopos estables ($\delta^{15}N$ y $\delta^{13}C$). Al respecto, Nunes *et al*. (1997), Zhang *et al*. (2000) y

Anderson *et al*. (1987) han determinado que la contribución relativa de la productividad natural en la generación del músculo son de 75.09%, 61.67% y 53-77% respectivamente. No obstante, resultados contrastantes han sido obtenidos por Cam *et al*. (1991) y más recientemente por Yuepeng *et al*. (2008) los cuales reportan contribuciones por parte del alimento balanceado de 86.5% y 93.5% y la contribución relativa remanente es aportada por la productividad natural (Tabla 3). Sin embargo estos resultados mantienen como observación general que, dado que la productividad natural no es constante, la principal fuente alimenticia para la generación de músculo es el alimento comercial.

No obstante, se ha reportado que el biofloc, puede contribuir alrededor de un 35% a la nutrición de los camarones. Esto depende del estadio ontogénico, calidad nutricional del biofloc, tamaño de partícula e incluso la especie de estudio (Cardona et al. 2015; Suita et al. 2016). En camarones como *L. vannamei* y *F. brasiliensis* se ha determinado que las partículas de mayor tamaño son las más importantes para su nutrición cuando los camarones son juveniles o pre-adultos (Emerenciano *et al*., 2012c). Incluso, se ha demostrado que el biofloc puede tener importantes beneficios durante la reproducción producto de una mejora en la historia nutricional de los camarones (Magaña-Gallegos, 2014).

Tabla 2 Condiciones de cultivo, contribución del alimento balanceado y la productividad natural. Día (d); SI (semi-intensivo).

Especies	Esquema de cultivo	Fertilización	Alimento balanceado (%)	Productividad natural (%)	Método de determinación	Referencia
L. vannamei	SI	-	23-47	53-77	^{13}C	Anderson *et al*., 1987
P. japonicus	SI	No	d26: 15 d88: 87	d26: 85 d88: 14	^{13}C	Cam *et al*., 1991
F. subtilis	Cerrado, SI	Sí	25	75	^{13}C	Nunes *et al*., 1997
F. chinensis	SI	Sí	38.33	62	^{13}C	Zhang *et al*., 2001
F. chinensis	SI	No	94	7	^{13}C	Yuepeng *et al*., 2008
F. brasiliensis	Cerrado, intensivo, BFT	Sí	0-7	93-100	^{13}C, ^{15}N	Magaña-Gallegos, 2014
L. vannamei	Cerrado, intensivo, BFT	Sí	0-41	59-100	^{13}C, ^{15}N	Magaña-Gallegos, 2014

Justificación e importancia

La acuacultura está creciendo de manera exponencial a nivel mundial, lo cual ha conllevado al cultivo comercial de muchas especies de organismos acuáticos, entre ellos los camarones. Entre los camarones, el género más explotado es el Penaeus (Alvarez *et al*., 1996; Ezquerra *et al*., 2004), sin embargo la limitada disponibilidad de post-larvas, el poco entendimiento nutricional de las especies de cultivo y los impactos de la camaronicultura están conllevando a que se estanque (Wouters *et al*., 2001).

El interés en la camaronicultura a nivel mundial ha incrementado los estudios en la fisiología de muchos camarones. En la literatura, varios estudios se enfocan en los aspectos biológicos de varias especies de camarón, lo que ha permitido desarrollar condiciones de cultivo específicas para cada especie. Sin embargo, *F. brasiliensis* a pesar de ser de alta importancia comercial en México, no cuenta con información clave de su biología y acuacultura, aunque su distribución está bien definida (Brito *et al*., 2000). En cuanto a *L. vannamei*, las bases de su cultivo son bien entendidas. No obstante, hay un déficit de información en cuanto al desempeño reproductivo de ambos camarones cultivados en sistemas biofloc (solamente existen dos trabajos de investigación desarrollados por Emerenciano *et al*., 2013a, 2012b) y por lo tanto hay un pobre entendimiento de cuál es el verdadero aporte del biofloc tanto en los camarones que se cultivan como en su progenie. Por lo tanto, es necesario asentar las bases sobre el papel que juega el biofloc como nueva alternativa de cultivo de estas dos especies de camarón, y la manera en la que aprovechan el alimento los camarones proveerá las bases para desarrollar más esfuerzos en la comprensión de mejores sistemas de cultivo y ahorros en el uso de alimento fresco y comercial.

Debido a la reciente problemática política y social, se han buscado alternativas de cultivo que causen menos impacto ambiental y que incrementen la bioseguridad y la productividad en los cultivos (Emerenciano *et al*., 2007; Emerenciano *et al*., 2011). Por lo tanto es necesario llevar a cabo más investigación con la BFT que nos permita entender de manera clara cuales son las interrelaciones de los organismos cultivados con el sistema mismo, permitiéndonos utilizar tecnologías amigables con el medio ambiente y que a su vez sean técnicamente apropiadas, económicamente viables y socialmente aceptables.

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Hipótesis

1. Si el biofloc es un complejo que aporta biomoléculas de alta calidad tales como aminoácidos, ácidos grasos, vitaminas y minerales en la nutrición de camarones pre-reproductores, entonces las hembras cultivadas en tanques de liner con biofloc tanto con o sin ablación del pedúnculo ocular presentarán un mejor desempeño reproductivo en comparación con las hembras cultivadas en agua clara.

2. Dado que el biofloc puede proveer una contribución relativa de nutrientes mayor a la del alimento comercial, entonces el biofloc contribuirá de manera más importante en la generación de la progenie en términos de reservas nutricionales tales como triglicéridos, colesterol, proteína total soluble y glucosa en comparación con otro tipo de alimentos tales como mejillón, calamar, poliqueto, biomasa de *Artemia spp*. y alimento semi-húmedo balanceado.

Objetivo general

Determinar el efecto del biofloc en la historia nutricional y el desempeño reproductivo de hembras con y sin ablación unilateral del pedúnculo ocular del camarón blanco del Pacífico (*Litopenaeus vannamei*) y camarón rojo del Caribe (*Farfantepenaeus brasiliensis*) cultivado en sistemas con biofloc y de agua clara en estanques con liner.

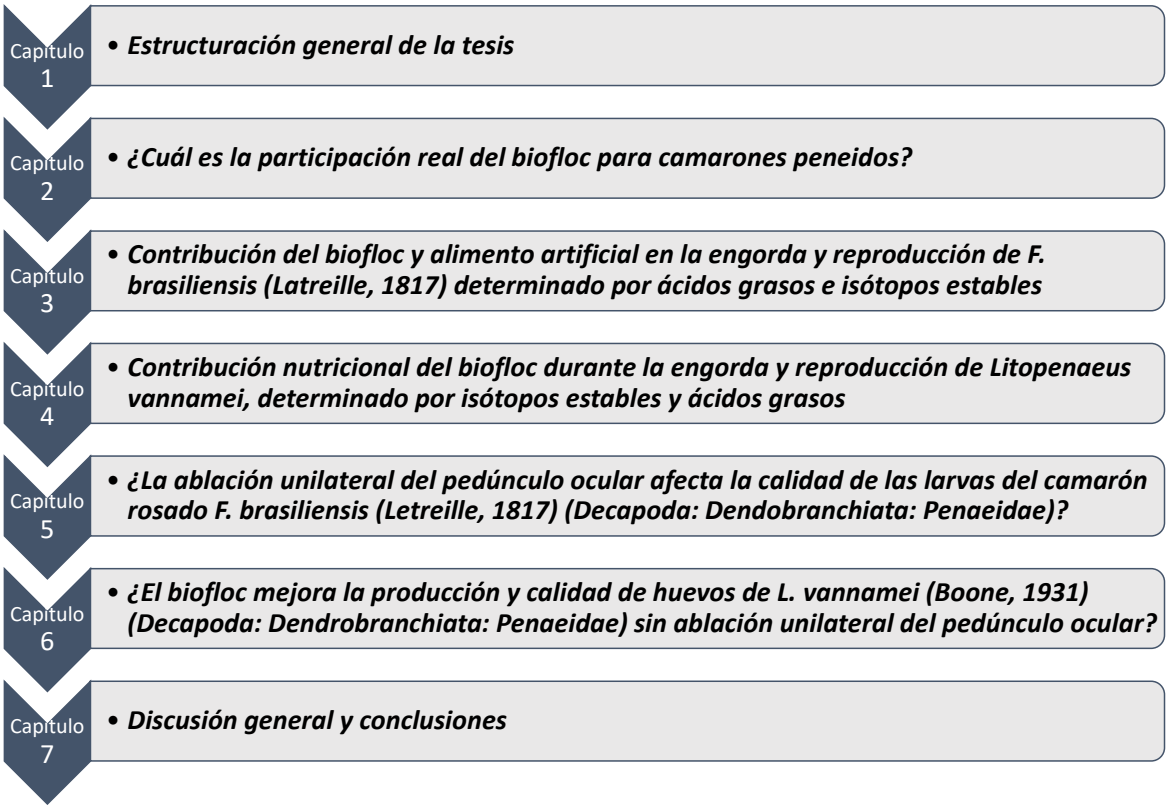
Objetivos específicos

- Evaluar el efecto del biofloc en la historia nutricional de *F. brasiliensis* en cuanto a engorda y reproducción, a partir de indicadores como isótopos estables, ácidos grasos y análisis químico-proximales de las fuentes alimenticias.
- Evaluar el efecto del biofloc en la historia nutricional de *L. vannamei* en cuanto a engorda y reproducción, a partir de indicadores productivos, de isótopos estables, ácidos grasos y análisis químico-proximales de las fuentes alimenticias.
- Evaluar el efecto de la ablación unilateral del pedúnculo ocular aplicado a *F. brasiliensis* y su relación con la calidad de la progenie, mediante el uso de indicadores productivos, morfométricos y bioquímicos.

- Evaluar el efecto de la ablación unilateral del pedúnculo ocular aplicada a *L. vannamei* proveniente de sistemas de agua clara y biofloc en relación a la calidad de huevos, mediante el uso de indicadores productivos, morfométricos y bioquímicos.

Esquema general de la tesis

A continuación (Figura 1) se presenta la estructuración general de la tesis. Se define el número de capítulos así como su respectivo título.

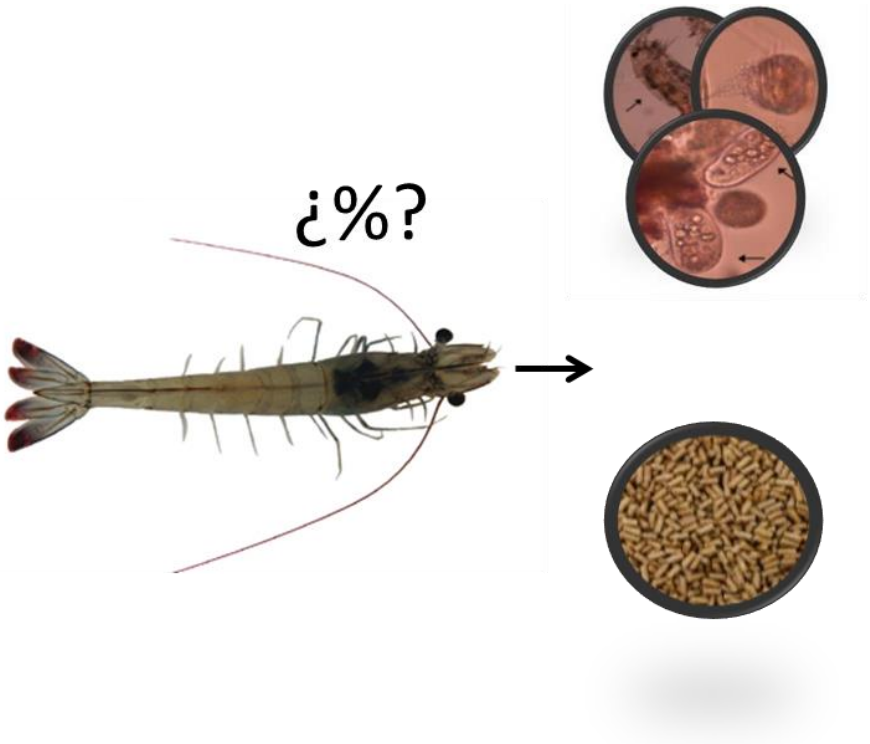


CAPÍTULO II

Artículo I

¿Cuál es la contribución real del biofloc para camarones peneidos?

Será sometido a: Reviews in Aquaculture



Será sometido a: Reviews in aquaculture

¿Cuál es la participación real del biofloc para camarones peneidos?

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Resumen

Los microorganismos en la acuacultura son parte de la dieta que los peneidos ingieren en cualquier situación de cultivo (biofloc, estanques de tierra, raceways, jaulas flotantes, etc.). En sí, en cada situación, tanto el alimento artificial y los microorganismos son provistos para sostener el crecimiento durante los pocos meses de cultivo hasta alcanzar el tamaño comercial. Sin embargo, en muchos casos ha sido poco direccionada la contribución de cada fuente alimenticia (alimento artificial vs productividad natural) y si se ha calculado, los reportes presentan muchas variaciones conllevando a cierto grado de incertidumbre. A pesar de esto, el biofloc ha demostrado ser una excelente fuente alimenticia para los camarones, llegando en algunos casos a superar en importancia a los alimentos artificiales. Además, el biofloc ha demostrado tener un impacto positivo durante la pre-maduración, reproducción y producción de la progenie.

Introducción

Los microorganismos juegan un papel crucial en el buen funcionamiento de la acuacultura (Nevejan *et al*., 2018). Dentro de los beneficios, se encuentra el mantenimiento de la calidad del agua (rutas heterotróficas, autotróficas y fotoautotróficas), nutrición de los organismos, mejora de la producción (hg/ha) y supervivencia (Avnimelech 1999; Ebeling *et al*., 2006; Serra *et al*., 2015). De manera contraria, también están relacionados con patógenos (Rurangwa and Verdegem 2015). El biofloc, es un sistema que fue desarrollado para el mantenimiento de la calidad del agua con altas densidades de siembra, sin embargo, como subproducto, se forman flóculos microbianos colonizados por otros microorganismos como fitoplancton y zooplancton (p. ej. proteínas, lípidos, vitaminas, etc.) los cuales son ricos en nutrientes, por lo que pueden mejorar la historia nutricional de los camarones (Martínez-Córdova *et al*., 2014; Samocha *et al*., 2017).

Ha sido demostrado que los camarones tienen la capacidad de ingerir, digerir y asimilar los nutrientes de los flóculos microbianos (Kent *et al*., 2011; Ekasari *et al*., 2014). Como consecuencia, mejoras en crecimiento, producción, supervivencia y mejor respuesta inmune han sido detectadas (Aguilera-Rivera *et al*., 2014; Aguilera-Rivera *et al*., 2018; Magaña-Gallegos *et al*., 2018b). Los camarones cuentan con apéndices especializados para ramonear las partículas del ambiente llamados maxilípedos, los cuales tienen setas en forma

de red que les permiten capturar los flóculos (Kent *et al*., 2011). A pesar de esto, el biofloc es un sistema en constante cambio y múltiples sucesiones a lo largo del periodo de cultivo pueden ser detectadas, por lo que el manejo de los microorganismos del biofloc puede ser algo complejo (Figura 1). No obstante, una buena comprensión del biofloc ha sido direccionado por Avnimelech, (1999) y Ebeling *et al*. (2006).

A pesar del buen entendimiento y manejo de los microorganismos en los sistemas biofloc, la contribución de los flóculos microbianos ha sido poco direccionada (Cardona *et al*., 2015). A pesar de que se ha propuesto que los alimentos inertes más los microorganismos del sistema pueden tener un efecto sinérgico que resulta en mejores cosechas y mejor desempeño reproductivo debido a una mejora en su historia nutricional (Magaña-Gallegos *et al*., 2018c). Por esto, el presente trabajo, se enfoca en aspectos de: contribución del biofloc durante la engorda y reproducción de camarones peneidos.

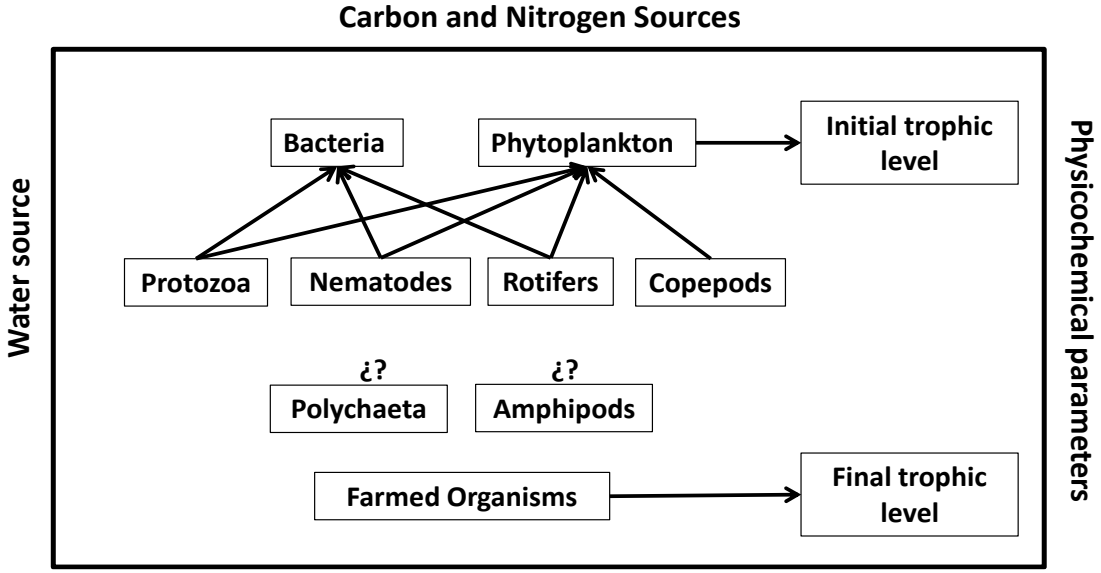

Figura 1. El nivel trófico inicial corresponde al conjunto de bacterias nitrificantes, heterotróficas y al fitoplancton, los cuales están interrelacionados (Bianchi and Martin 1978; Martínez-C). El zooplancton, integrado por varios grupos, entre los principales se encuentran los protozoarios, nematodos, rotíferos, copépodos y en algunos casos poliquetos y anfípodos (Ray *et al*., 2010). Los flóculos microbianos están representados por bacterias, fitoplancton y zooplancton. Los nutrientes contenidos en los flóculos, pueden ser ingeridos, digeridos y asimilados por los camarones, los cuales son el nivel trófico final en un tanque. Fuente de agua: impacto directo en la colonización inicial de microorganismos y directamente relacionado con la calidad del agua (p. ej. agua de pozo y agua marina). Parámetros fisicoquímicos: directamente relacionados con los microorganismos, ya que algunos requieren luz (fotosintéticos), carbono orgánico (bacterias heterotróficas), carbono inorgánico (bacterias heterotróficas y autotróficas), fósforo (fitoplancton, principal factor limitante), nitrógeno (en forma de NH_4/NH_3, NO_2, NO_3), rango de pH, alcalinidad y dureza entre otros. Relación C/N: relacionado principalmente al tipo de metabolismo bacteriano dominante (heterotrófico vs autotrófico) y con algunas especies de microalgas.

Direccionando la contribución real de las fuentes alimenticias durante la engorda y reproducción de camarones peneidos

Los microorganismos en la acuacultura han sido utilizados desde sus inicios. Pero los beneficios reales en cuanto a su impacto en la nutrición de los camarones, han sido poco direccionados con el uso de herramientas que permitan evaluar realmente lo que los camarones peneidos están asimilando (Gamboa-Delgado 2014). Generalmente, se ha utilizado el análisis de contenido estomacal con el fin de analizar las preferencias alimenticias de los camarones peneidos en la acuacultura (Hyslop 1980; Albertoni *et al*., 2003; Martínez-Córdova and Peña-Messina 2005). No obstante, debido a que los camarones presentan particularidades de manejo en su alimento como son: la fragmentación del alimento previo a su ingestión, y posteriormente el paso del alimento a través del molino gástrico**,** el reconocimiento de las entidades alimenticias ha resultado relativamente impráctico, aunque su uso ha provisto de luz en las preferencias alimenticias de los camarones. Como nueva herramienta está el uso de isótopos estables (ver Gamboa-Delgado, 2014), la cual nos brinda información de las entidades alimenticias principalmente asimiladas por los camarones y que por lo tanto realmente están siendo utilizadas para la generación de su biomasa. Actualmente, los isótopos estables, principalmente los de carbono (p.ej. ^{12}C y ^{13}C) y nitrógeno (p. ej. ^{14}N y ^{15}N) en combinación con modelos de mezcla permiten medir la contribución relativa de diferentes fuentes alimenticias a la generación de músculo camarones peneidos (Phillips 2001; Gamboa-Delgado and Le Vay 2009; Nevejan *et al*., 2018).

Los métodos tradicionales de acuacultura, se basan en la fertilización periódica de los estanques con el fin de generar la proliferación de una comunidad microbiana que ayude no solamente al mantenimiento de la calidad del agua si no que sirva como fuente de alimento para los camarones peneidos (Avnimelech 2009; Gamboa-Delgado 2014). De hecho, la contribución de la productividad natural en la generación de músculo ha sido direccionada con isótopos estables previamente en varias especies de camarones peneidos como se muestra en la Tabla 1. De manera interesante, *L. vannamei* cultivado en tanques con presencia de productividad natural, ha demostrado que principalmente asimila alimento natural y en menor proporción alimento artificial (Tabla 1). En estos estudios, se evaluó en paralelo el análisis de contenido estomacal y los resultados fueron que alrededor del 50%

del alimento era proporcionado por el alimento artificial y 50% por el alimento natural. Lo anterior, demuestra que si bien, los organismos están ingiriendo en iguales proporciones ambas fuentes alimenticias, la asimilación se produce de manera disímil. Esto se puede deber a: i) baja digestibilidad de los ingredientes en los alimentos artificiales en comparación con los alimentos naturales y ii) los factores antinutricionales que algunas harinas pueden provocar baja digestión y asimilación de los nutrientes utilizados en los alimentos artificiales.

Otro factor importante relacionado al bajo aporte de la productividad natural respecto al alimento artificial está relacionado al incorrecto establecimiento de la comunidad microbiana en el tanque, lo que provoca una disminución en las densidades de microorganismos a través del tiempo. Por ejemplo, en *Penaeus japonicus* se ha demostrado que cuando la productividad natural es alta (día 30) en el sistema, esta puede representar hasta un 87% de la generación del músculo, con solamente un 13% de contribución del alimento artificial (Cam *et al*., 1991). Sin embargo, cuando la productividad natural decayó hacia el día 120 de cultivo, esta solo represento un 34% en la generación de músculo y el resto (66%) lo proveyó el alimento artificial (Cam *et al*., 1991). Esto indica que el manejo de la densidad de microorganismos en el sistema tiene un impacto directo en la capacidad de utilizar estas entidades alimenticias como principal fuente para su crecimiento. Además, la densidad de siembra puede jugar un papel muy importante en la estabilidad de las poblaciones de microorganismos, haciendo que disminuyan a lo largo del periodo de cultivo. De hecho, Su *et al*. (2008), reportan que *F. chinensis* solamente debe la construcción de músculo en un 7% a la productividad natural y un 93% al alimento artificial. Lo interesante de este estudio, es que los tanques no fueron fertilizados para generar un aumento en la productividad natural del sistema, como señalan si hizo *(Zhang et al*., 2000) con la misma especie y donde la productividad natural represento un 63% de la contribución al crecimiento con el remanente 17% por parte del alimento artificial (Tabla 1).

Tabla 1. Contribución real del alimento natural al crecimiento somático de camarones Peneidos indicado por isótopos estables.

Especie/ambiente	Contribución real al crecimiento del tejido (%)		Referencia
	Alimento formulado	Alimento natural	
[1] *L. vannamei*	23-47	53-77	Anderson *et al*. 1987
[1] *L. vannamei*	27	73	Gamboa-Delgado and Le Vay, 2009
[1] *L. vannamei*	20	80	Gamboa-Delgado et al., 2011
P. japonicus	13-65	35-87	Cam *et al*. 1991
F. subtilis	25	75	Nunes *et al*. 1997
[2]*F. chinensis*	93	7	Su *et al*. 2008
[1]*F. chinesis*	17	63	Zhang *et al*. 2000

[1]semi-intensivo, fertilización. [2] sin fertilización.

En los sistemas biofloc, una comunidad heterotrófica es favorecida mediante la adición de materiales ricos en carbono orgánico (p. ej. melaza, salvado de trigo, harina de trigo, etc.) generando que se consuman los compuestos nitrogenados presentes en el agua (principalmente amonio y nitrito provenientes del catabolismo de las proteínas) que podrían llegar a ser tóxicos para los animales de cultivo (ver Ebeling *et al*., 2006). Como un subproducto, se forman flóculos microbianos, los cuales posteriormente son colonizados por otros microorganismos pertenecientes al fitoplancton y zooplancton (De Schryver *et al*., 2008). Debido a esto, los flóculos microbianos pueden ser vistos como partículas súper ricas en nutrientes que ayudan a nutrir al camarón (Cardona *et al*., 2016; Magaña-Gallegos *et al*., 2018b). Además, esta comunidad heterotrófica ayuda a reciclar la proteína de los alimentos ya que utilizan los principales desechos nitrogenados producidos por el camarón como medio estructural en combinación con carbono orgánico e inorgánico (ver *Ebeling et al*., 2006 para más especificaciones).

En varias condiciones de biofloc y trabajando con varias especies de camarones peneidos (*F. brasiliensis, L. vannamei, P. monodon* y *L. stylirostris*) hay una concordancia general de que más del 45-50% de la contribución al tejido proviene de los flóculos microbianos, siendo lo remanente el alimento artificial (Tabla 2). A pesar de esto, Burford *et al*., (2004) y Cardona *et al*., (2015) han reportado contribuciones del biofloc menores (18-36.9%). Esto podría ser un efecto de la densidad de cultivo, cantidad de sólidos suspendidos totales, edad del biofloc, calidad nutricional del biofloc, fuente de carbono orgánico, el estado ontogénico de los organismos y el grado de domesticación de los camarones (Suita *et al*.,

2016; Magaña-Gallegos *et al*., 2018b, c). No obstante, en todos los casos, el cultivo de camarones con biofloc ayuda a explicar las velocidades de crecimiento, supervivencia y estado de salud en comparación con tratamientos control donde no hay generación de flóculos. Lo anterior puede estar relacionado con la alta digestibilidad y buena asimilación del biofloc más una sustancial cantidad de aminoácidos libres, junto con la presencia de sustancias solubles en el medio como glucosa, aminoácidos libres y vitaminas que contribuyen a la síntesis de proteínas. De hecho, se ha detectado que cuando la productividad natural es favorecida, el alimento artificial de los camarones puede disminuir significativamente su concentración de vitaminas (Moss *et al*., 2006). Esto indica que la productividad natural de los estanques, puede producir vitaminas de manera endógena y estas ser trasferidas a través de la cadena trófica. Faltaría llevar a cabo estudios para demostrar que esto prevalece en sistemas biofloc.

En apoyo a lo anterior, en un estudio llevado a cabo por Chan-Vivas *et al*. (2018) se caracterizó el balance bioenergético de camarones de 7 y 12 gramos de peso final cultivados en agua clara y biofloc respectivamente; dado que el biofloc contribuyó de manera significativa en la nutrición de los camarones, estos obtuvieron un mayor peso final al día 45. Los resultados muestran que hay un más alto consumo de oxígeno por parte de los camarones de agua clara (1.3±0.3 mg·O^2·camarón) que los de biofloc (0.5±0.2 mg·O^2·camarón). Por calorimetría fue determinado que los camarones de agua clara presentan un más alto incremento de calor aparente atribuido a la ingesta exclusiva de alimento inerte. Al final, los camarones cultivados en biofloc mostraron más alta energía retenida (~448 Joules camarón^{-1} día^{-1}) que los de agua clara (~246 Joules camarón^{-1} día^{-1}; Fig. 3) y por lo tanto los camarones de biofloc presentaron un mayor crecimiento.

Tabla 2. Contribución real del biofloc y alimento formulado al crecimiento somático de camarones Peneidos indicado por isótopos estables.

Especie/ambiente	Contribución real al crecimiento del tejido (%)		Referencia
	Alimento formulado	Biofloc	
L. stylirostris	40	60	Chim *et al*. 2014
[1] *L. stylirostris*	~60	~40	Cardona *et al*. 2015
[2] *L. vannamei*	71-82	18-29	Burford *et al*. 2004
[3] *L. vannamei*	41.5-72.3	27.7-58.5	Ray and Lotz, 2017
[4] *L. vannamei PL*	13-68	0-50	Suita *et al*. 2016
[5] *L. vannamei*	<50%	>50	Magaña-Gallegos *et al*. 2018c
[6] *F. brasiliensis*	<5%	>95	Magaña-Gallegos *et al*. 2018b

PL = postlarva. [1] Alrededor de 40% de la contribución de carbono y 40% de nitrógeno para el biofloc. [2] Mayor cantidad de flóculos, mayor la contribución al camarón. [3] Problemas de manejo relacionados a amonio, probablemente afectaron la contribución, aun así es bastante alta. [4] Postlarvas, hábitos alimenticios filtrador, traslape señal isotópica del fitoplancton y biofloc puede enmascarar la determinación más certera. [5] Organismos provenientes de padres cultivados en biofloc, y todo el cultivo fue en biofloc. [6] Organismos provenientes de padres silvestres, pobre domesticación.

Fig. 1 Partición de energía de *L. vannamei* cultivado en sistema de agua clara y biofloc (Chan-Vivas *et al*., 2018). HiE = incremento de calor aparente; HeE = metabolismo de rutina; RE = energía retenida.

Como un efecto de la historia nutricional y selección natural, los reproductores silvestres de camarones peneidos tienen mejor desempeño reproductivo que camarones domesticados (Emerenciano *et al*., 2012b). En los esquemas tradicionales de cultivo de pre-reproductores, el uso de alimentos inertes o semi-húmedos más el uso de alimentos frescos como mejillón y calamar han sido ampliamente utilizados. Un factor clave en la reproducción de los camarones es el uso de una dieta óptima que permita a los reproductores contar con los nutrientes esenciales para la formación de la gónada y posterior transferir a la progenie

(Wouters *et al*., 2001). Se ha demostrado previamente, que los camarones peneidos explotan de manera eficiente los microorganismos y partículas floculadas de los sistemas de cultivo, confiriéndoles grandes ventajas. De hecho, actualmente el biofloc es visto como un sistema de pre-maduración de camarones peneidos (Cardona *et al*., 2016) que puede mejorar: i) la nutrición de los organismos, ii) la bioseguridad de los pre-reproductores, iii) el desempeño reproductivo de las hembras y iv) la calidad de las larvas. Por esto se han llevado a cabo estudios donde se compara el desempeño reproductivo de camarones peneidos que provienen de sistemas biofloc contra otro tipo de sistemas (Tabla 3). No obstante, es necesario llevar a cabo más estudios comparativos con otras especies de camarones peneidos (p. ej. *P. monodon*) con el fin de determinar el potencial del uso del biofloc en etapas de pre-maduración.

Además de la mejora en el desempeño reproductivo de las hembras, los camarones peneidos cultivados en sistemas biofloc tienden a mejorar la calidad de la progenie; evaluado en términos de biomoléculas de alta importancia para el desarrollo del embrión (acilglicéridos, colesterol, glucosa y proteína total soluble) hasta nauplio, así como en el perfil de ácidos grasos presentes en los huevos (Emerenciano *et al*., 2012a, 2013). Los acilglicéridos son una fuente de energía muy importante durante el desarrollo del embrión e incluso son importantes durante los primeros estadios larvarios lecitotróficos de las larvas de camarones peneidos. El colesterol es precursor de hormonas y constituyente esencial de la pared celular, por lo que juega un papel clave en el crecimiento de los organismos, además de que no puede ser sintetizado *de novo* mediante el metabolismo de camarones peneidos. La glucosa sirve como una molécula de energía rápida. La proteína total soluble es importante ya que puede brindar energía y a su vez los sillares para la estructuración de tejidos.

Tabla 3. Desempeño reproductivo de varias especies de camarones peneidos en sistemas de pre-maduración biofloc y otro tipo de sistemas. PS = peso corporal.

Especie	Otros métodos de pre-maduración					Biofloc					Referencias
	# huevos ($\times 10^3$)/ g PC	Maduraciones consecutivas	Periodo de latencia (días)	Desovaron al menos una vez (%)	Tasa de fertilización (%)	# huevos ($\times 10^3$)/ g PC	Maduraciones consecutivas	Periodo de latencia (días)	Desovaron al menos una vez (%)	Tasa de fertilización (%)	
[1] *L. stylirostris*	3.3	2.5	10.7	53.8	-	3.3	2.5	10.7	53.8	-	Emerenciano *et al*. 2011
[2] *L. vannamei*	-	-	-	-	-	2.7-3.1	5-7	22-23	88.9-94.4	73-75	Emerenciano *et al*. 2013
[3] *L. vannamei*	-	-	-	-	-	3.2	-	20	-	95.7	Magaña et al. 2018b
[4] *F. duorarum*	1.1	7	25	25	-	1.5-2.1	7-8	23-29	80-81	-	Emerenciano *et al*. 2014
[5] *F. brasiliensis*	4.4	-	11	-	86.1	5.6	-	16	-	79.6	Magaña *et al*. 2018a

[1] reproductores de estanques de tierra vs biofloc. [2] Ambos tratamientos fueron biofloc (con y sin adición de alimentos frescos), por eso se muestran los rangos. [3] solamente reproductores de biofloc. [4] Dos tratamientos biofloc y un tratamiento agua clara. [5] Reproductores silvestres vs biofloc.

En términos generales, la concentración de estas biomoléculas en huevos y nauplios de camarones peneidos se ve incrementada cuando los padres han sido pre-madurados en sistemas biofloc (Emerenciano *et al*., 2012a, 2014; Magaña-Gallegos *et al*., 2018b). Por lo que el uso de la tecnología biofloc va más allá de su implementación para mejorar el desempeño reproductivo, si no que mejora la historia nutricional de los reproductores, aumenta el número de huevos desovados y mejora la calidad de la progenie. Este último punto es importante, porque una mala calidad de la larva puede provocar transmisión de enfermedades verticales.

En la reproducción tradicional de camarones peneidos, se ha utilizado la ablación unilateral del pedúnculo ocular con el fin de acelerar la maduración de las hembras y poder predecir picos de desove (Vaca and Alfaro 2000). No obstante, se ha demostrado que la ablación puede provocar que el bienestar de los reproductores se vea afectado debido a que la mayor parte de su energía es canalizada a la reproducción, dejando a un lado la inversión de energía para su mantenimiento (Rosas *et al*., 1993; Munkongwongsiri *et al*., 2015). Además de esto, la ablación genera que la progenie sea de menor calidad como se ha demostrado en *F. brasiliensis* (Magaña-Gallegos *et al*., 2018a). Debido a esto, es necesario buscar esquemas de reproducción que no atenten contra el bienestar de los animales pero que a su vez mantengan la reproducción constante. De hecho, una de las causas por las cuales surgió la ablación, fue que muchas especies de camarones peneidos no se reproducían de manera natural en los estanques, si no que tenían que ser ablacionadas para inducir el desarrollo de la gónada y finalmente la copula. Esto ocurría en los años 70´s, cuando se empezaron a hacer las pruebas de reproducción y se desconocía mucho sobre el comportamiento alimenticio, nutrición y esquemas de pre-maduración de camarones peneidos. Hoy en día, ya hay esquemas de pre-maduración de varias especies de camarones peneidos descritas por (Emerenciano *et al*., 2012c). Por esto, es indispensable llevar a cabo estudios de organismos provenientes de sistemas biofloc sin ser ablacionados en la fase de reproducción y determinar si la copula natural se lleva a cabo y si hay remaduración y desoves fértiles. De hecho, hay varios lugares donde se lleva a cabo la reproducción sin ablación como son Cuba (a nivel producción; Com. Pers. Dra. Laida Ramos), México (Magaña-gallegos *et al*., 2018c) y Tailandia (Munkongwongsiri *et al*., 2015).

Conclusión

El uso de microorganismos en el cultivo de camarones peneidos trae consigo grandes beneficios a nivel de engorda, ya que una mayor proporción del alimento natural es asimilado en comparación con los alimentos artificiales. El biofloc, que es el manejo de una comunidad de microorganismos fotoautotróficos, heterotróficos y autotróficos nitrificantes ha demostrado ser de gran relevancia durante la engorda e incluso la reproducción de camarones peneidos. Es por esto, que es necesario seguir llevando a cabo investigaciones que esclarezcan cuales son las ventajas de usar esta tecnología, su manejo y aplicación a la acuacultura moderna.

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Alan Michel quien levanto Aquacop en Tahití; dio muchos avances sobre el Floc y compartimos aquí los puntos de vista de su equipo de trabajo conducido en los 70´s hasta los 80´s. Al Equipo Camarón de la UAS-Sisal, FC-UNAM, por su apoyo en los múltiples experimentos realizados.

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CAPÍTULO III

Artículo II

Biofloc and food contribution to grow-out and broodstock of *Farfantepenaeus brasiliensis* (Latreille, 1817) determined by stable isotopes and fatty acids

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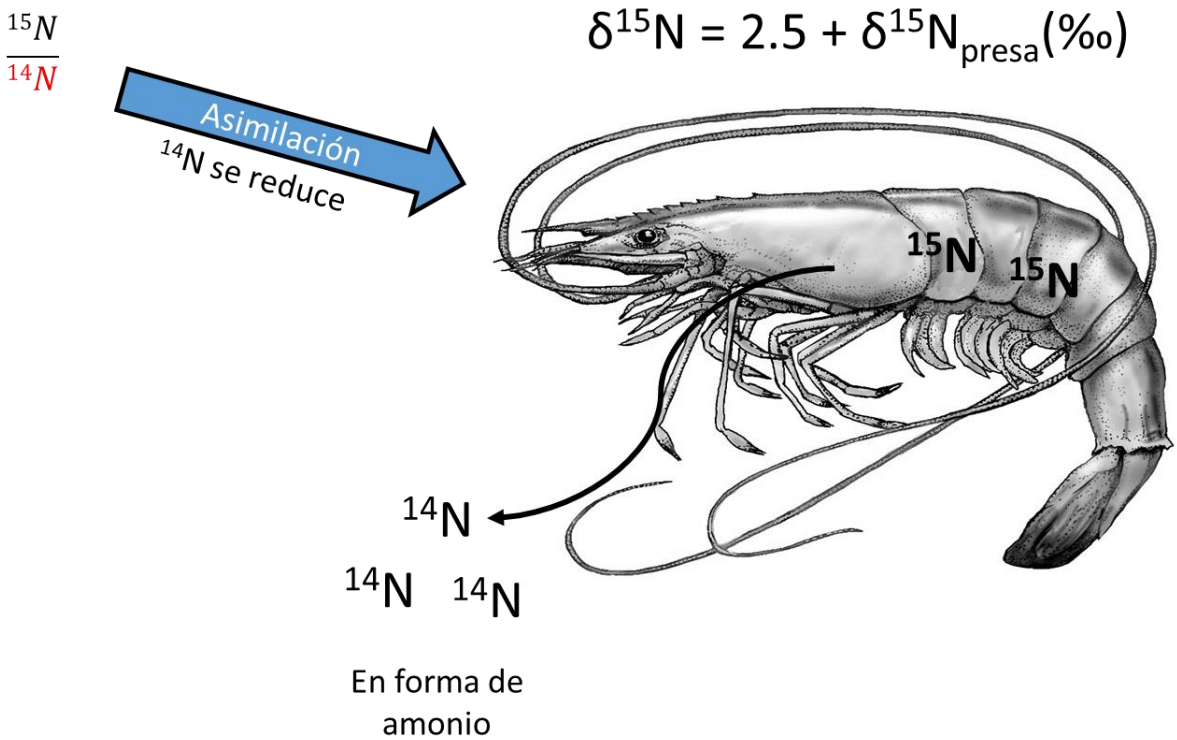

Biofloc and food contribution to grow-out and broodstock of *F. brasiliensis* (Latreille, 1817) determined by stable isotopes and fatty acids

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Resumen

El objetivo de este estudio fue utilizar marcadores dietéticos naturales (isótopos estables y ácidos grasos) durante la engorda en un sistema biofloc y para la producción de huevos de *F. brasiliensis*. La producción de huevos fue comparada para dos orígenes de reproductores: biofloc y silvestres. Para delinear la contribución relativa al músculo de los camarones y huevos, el programa IsoSource fue utilizado. La fuente alimenticia más importante que contribuyó a la engorda de los camarones fue el biofloc >250 µm. De acuerdo al análisis de componentes principales (ACP) aplicado al perfil de ácidos grasos de las fuentes alimenticias, el primer componente explica el 84.4% de la variabilidad, y la fuente más importante de ácidos grasos fue el biofloc >250 µm. El alimento fresco más importante que contribuyó a la producción de huevos fue la biomasa de *Artemia*, poliquetos y alimento semi-húmedo para ambos orígenes de reproductores. De acuerdo al ACP del perfil de ácidos grasos, los alimentos frescos más importantes fueron los poliquetos y alimento semi-húmedo. En conclusión, tanto la señal isotópica como el perfil de ácidos grasos de las fuentes alimenticias pueden ser utilizados exitosamente para determinar la integración de carbono en las dietas de camarones.

Abstract

The aim of this study was to use the natural dietary markers (stable isotopes and fatty acids) during grow-out in a biofloc system and for the egg production of *Farfantepenaeus brasiliensis* shrimp. Egg production was compared for two broodstock origins: biofloc and a wild origin. To delineate the relative contribution to shrimp muscle and eggs, IsoSource software was used. The most important source that contributed to grow-out shrimp was biofloc ≥250 lm. According to the principal component analysis (PCA) applied to the fatty acid profile of food sources, the first component explains 84.4% of the variability, and the most important source of fatty acids for this component was biofloc ≥250 lm. The most important fresh food sources that contributed to egg production were Artemia biomass, polychaetes and semi-moist feed for both broodstock origins. According to a PCA analysis of the fatty acid profiles, the most important fresh foods were polychaetes and semi-moist feed. In conclusion, both isotopic signature and fatty acid profile of the food sources can be used successfully to determine the integration of carbon in the diets of shrimp.

Introducción

La tecnología biofloc es un sistema de cultivo que fue diseñado para el mantenimiento de la calidad del agua. No obstante, los efectos de esta tecnología van más allá, debido a que los camarones tienen la capacidad de ramonear, digerir y asimilar los flóculos microbianos. Por esta razón, no es raro que los camarones cultivados en biofloc tengan mayores tasas de crecimiento y una mejor historia nutricional, lo cual, les brinda la capacidad de tener mejor calidad de su progenie.

A pesar de esto, ha sido documentado que el alimento comercial es la principal fuente de nutrientes para camarones peneidos. Sin embargo, los trabajos que han sostenido esto, han tomado al biofloc como una entidad indivisible, incluso cuando se ha demostrado que diferentes tamaños de partícula tienen diferente valor nutricional. Además, no está claro si los reproductores cultivados en biofloc, podrían mejorar su desempeño reproductivo comparado con reproductores de origen silvestre.

Con el fin de direccionar estas interrogantes el presente estudio utilizó una serie de indicadores productivos y bioquímicos. En cuanto a los productivos destacan la tasa de crecimiento, supervivencia y factor de conversión alimenticia. En cuanto a los bioquímicos, se basó principalmente en el uso de isótopos estables y ácidos grasos con el fin de direccionar la contribución a la generación del músculo de *F. brasiliensis.*

Objetivo de estudio:

Evaluar el efecto del biofloc en la historia nutricional de *F. brasiliensis* en cuanto a engorda y reproducción, a partir de indicadores como isótopos estables, ácidos grasos y análisis químico-proximales de las fuentes alimenticias.

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ORIGINAL ARTICLE

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Biofloc and food contribution to grow-out and broodstock of *Farfantepenaeus brasiliensis* (Latreille, 1817) determined by stable isotopes and fatty acids

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Abstract

The aim of this study was to use the natural dietary markers (stable isotopes and fatty acids) during grow-out in a biofloc system and for the egg production of *Farfantepenaeus brasiliensis* shrimp. Egg production was compared for two broodstock origins: biofloc and a wild origin. To delineate the relative contribution to shrimp muscle and eggs, IsoSource software was used. The most important source that contributed to grow-out shrimp was biofloc ≥250 μm. According to the principal component analysis (PCA) applied to the fatty acid profile of food sources, the first component explains 84.4% of the variability, and the most important source of fatty acids for this component was biofloc ≥250 μm. The most important fresh food sources that contributed to egg production were *Artemia* biomass, polychaetes and semi-moist feed for both broodstock origins. According to a PCA analysis of the fatty acid profiles, the most important fresh foods were polychaetes and semi-moist feed. In conclusion, both isotopic signature and fatty acid profile of the food sources can be used successfully to determine the integration of carbon in the diets of shrimp.

KEYWORDS
biofloc, fatty acids, relative contribution, shrimp, stable isotopes

1 | INTRODUCTION

Biofloc technology is a culture system that helps to control the nitrogenous compounds in the shrimp culture water (Avnimelech, 2009). However, the effects of biofloc technology go farther, and the particles formed are grazed upon by shrimp, with the third maxillipeds as a nutritive source (Burford, Thompson, McIntosh, Bauman, & Pearson, 2004; Cardona et al., 2015; Kent, Browdy, & Leffler, 2011). Then, it is not rare that growth and reproductive performance of peneid shrimp improve when animals are raised on biofloc as has been proven (Emerenciano, Cuzon, Arevalo, & Gaxiola, 2014; Suita et al., 2016).

Nevertheless, it has been suggested that commercial feed is the main source of nutrients for shrimp grown in biofloc (Cardona et al., 2015; Ray & Lotz, 2017; Suita et al., 2016). However, these trials have considered the biofloc to be an indivisible entity, even when biofloc particles have different sizes and nutritional values, and could therefore contribute to the shrimp nutrition in different proportions (Ekasari et al., 2014). Additionally, it is not clear whether the broodstock cultured in biofloc, due to an improvement of its nutritional history, could improve reproductive performance compared with a wild broodstock batch and if this culture in biofloc could affect the use of food sources by broodstock for the egg production.

The use of the isotopic signal ($\delta^{13}C$ and $\delta^{15}N$) of a consuming organism reflects the isotopic value of its assimilated diet and allows the estimation of the dietary contribution of each food source with the help of mathematical models (Adams & Sterner, 2000; Phillips & Gregg, 2003; Suita et al., 2016). However, the isotopic models just give information about the carbon and nitrogen retained, not about the main nutrients used for growth or egg formation. Then, characterization of the fatty acid profile of the shrimp diet could be useful since shrimp is a direct reflection of its diet, and different sources have characteristic profiles (Kharlamenko, Kiyashko, Imbs, & Vyshkvartzev, 2001; Rooker, Turner, & Holt, 2006). Furthermore, measurement of the fatty acids could delineate the importance of one food source with respect to another (Rosas, 2003).

Therefore, the aim of this study was to use the natural dietary markers (stable isotopes and fatty acids) during grow-out in a biofloc system and for the egg production in *Farfantepenaeus brasiliensis* shrimp. Egg production was compared for two broodstock origins, biofloc and wild origin. During the grow-out period, the relative contribution of different biofloc size classes and commercial feed with the stable isotopes of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) were used to delineate which food sources were the main sources of fatty acids. Subsequently, when the shrimp reached reproductive size, the main fresh food sources contributing carbon and nitrogen as well as the fatty acids for the egg production were determined.

2 | MATERIALS AND METHODS

2.1 | Shrimp management

This study was performed at Unidad Multidisciplinaria de Docencia e Investigación (UMDI, FC-UNAM) in Sisal, Yucatán, México. Juveniles of *F. brasiliensis* were obtained from wild adults captured in Isla Contoy (21°29′2N, 86°47′30W), Quintana Roo, México (Emerenciano, Cuzon, Arevalo, Mascaro, & Gaxiola, 2013; Emerenciano et al., 2014). Larvae were reared with live food (Gaxiola, Gallardo, Simôes, & Cuzon, 2010). Subsequently, the juveniles of *F. brasiliensis* in the postlarval stage were transferred to four biofloc grow-out circular tanks of 20,000 L under constant aeration through plastic tubes with a 5-hp blower and were grown for 12 months, until they reached reproductive size. To enhance the welfare of the shrimp during its culture, stock densities were reduced to 300, 150 and 5 shrimp/m^2 during 1–3, 3–11 and 11–12 months respectively. Tanks were exposed to coastal climate conditions, and the water exchange was limited to just compensate for evaporation. Filtered saltwater was added as needed without exceeding 0.5% daily exchange. A high C/N ratio of 20/1 was maintained throughout the study period by addition of sugarcane molasses (Emerenciano, Ballester, Cavalli, & Wasielesky, 2012) to ensure optimal heterotrophic bacterial growth (Avnimelech, 1999; Crab, Kochva, Verstraete, & Avnimelech, 2009). The carbon source was added daily until the biofloc volume reached 5 ml/L (biofloc volume was measured with Imhoff cones). Once biofloc volume achieved this concentration, the carbon source addition was stopped. When the biofloc volume decrease of 5 ml/L or total ammonia nitrogen (TAN) reached 1 mg/L, the carbon addition was restarted (Emerenciano, Cuzon, Paredes, & Gaxiola, 2013; Serra, Gaona, Furtado, Poersch, & Wasielesky, 2015). Sludge was removed daily from each tank by a central drain.

2.2 | Grow-out shrimp

At the third month, shrimp of 1.9 ± 0.2 g in the biofloc grow-out tanks at a density of 150 animals/m^2 (3000 shrimp per tank) were evaluated for 45 days in terms of growth performance, isotopic and fatty acid analysis. Shrimp were fed at 2%–3% biomass, using a commercial feed (Api-Camaron, Malta Cleyton®, 35% crude protein) with biweekly adjustments of the daily ration. Fifty individual shrimp were sampled from each tank to adjust the daily ration. The daily ration was divided into five feeding times at 0:00, 4:00, 9:00, 16:00 and 20:00 hours.

2.3 | Water quality analysis

Salinity (g/L) was measured at 8:00 hours with a refractometer Atago (model Maste-S/Mill). Temperature (°C), pH and dissolved oxygen (mg/L) were measured at 04:00, 08:00, 16:00, 20:00 and 24:00 hours throughout the experimental period with a multiparameter probe (Hach Co. model HQD40). Total ammonia nitrogen (TAN, mg/L) and nitrites (mg/L) were measured once a week by a colorimetric method (Grasshoff, Kremling, & Ehrhardt, 1983; Strickland & Parsons, 1972). Biofloc volume (ml/L) was determined according to Avnimelech (2007). Chlorophyll-a (mg/L) was determined using acetone extraction and spectrophotometry at 750, 664, 647 and 630 nm (Aminot & Rey, 2000) once a week in each culture tank.

2.4 | Sampling of feed sources

Biofloc samples were taken every week by filtering tank water through five mesh sizes of nylon: 10, 50, 100, 250 and 500 μm. Samples were pooled by size at the end of the experiment. Size classes then included biofloc of 10 < 50 μm, biofloc ≥50 < 100 μm, biofloc ≥100 < 250 μm, biofloc ≥250 < 500 μm and biofloc ≥500 μm. Fifty grams of commercial feed at the start of the trial was homogenized with a mortar and pestle until a fine powder was obtained. All food sources were stored in Falcon® tubes, frozen with liquid nitrogen and stored at −80°C for further analysis.

2.5 | Shrimp performance indicators and sampling

At the end of the 45-day period, fifty shrimp per tank were collected and weighed individually with an Ohaus balance (0.1 mg) to obtain the final body weight. Then, grams gained per week (g/week) was estimated as follows = (final body weight − initial body weight)/number of weeks. The specific growth rate (SGR % day) was calculated $(\ln(W_f) - \ln(W_i)) \times 100/t$. Where W_f, final weight; W_i, initial weight; t, time. The feed conversion ratio (FCR) was calculated as follows: FCR = feed provided/shrimp biomass gained (Becerra-

Dórame et al., 2012). At the end of the trial, 20 juveniles per tank were collected for isotopic analysis, but only the muscle of those in the intermolt stage C were used (Bourgeois & Cuzon, 1975).

2.6 | Proximate analysis of food sources

Food sources analysed were commercial feed Api-Camaron, Malta Cleyton® (n = 3) and all biofloc size classes (n = 3 per size class). To determine protein content in the samples, nitrogen content was measured using an Elemental Combustion System 4010 (Costech Analytical Technol., Inc. USA), and then protein content was calculated by multiplying the elemental N content by 6.25 (16% N content in protein). Lipid content of the biofloc size classes was measured according to the procedure of Folch, Lees and Stanley (1957). To determine the lipid content of the commercial feed, method 920.39 of AOAC (1996) was used.

2.7 | Broodstock

The broodstock used for this experiment had two origins. Domesticated shrimp origin: from the grow-out experiment, a batch of shrimp that reached 20 g were transferred to a maturation room for reproduction. The animals were selected based on morphological integrity (Emerenciano, Cuzon, Mascaro, et al., 2012). Wild shrimp origin: adults of *F. brasiliensis* were caught from water surrounding Isla Contoy, Quintana Roo, Mexico (21°29′2N, 86°47′30W). Shrimp were transported to the maturation room and acclimated a week before beginning the experiment (Emerenciano, Cuzon, Mascaro, et al., 2012).

2.8 | Experimental conditions

The procedures used for this experiment have previously been reported and were used for both domesticated and wild broodstock (Emerenciano, Cuzon, Goguenheim, & Gaxiola, 2012; Emerenciano, Cuzon, Arevalo, et al. 2013). The experiment lasted 45 days. The maturation room with a 12-hr photoperiod consisted of two round lined tanks of 12,000 L capacity, with closed seawater recirculation. One tank corresponded to domesticated shrimp and the other tank to wild shrimp. Four animals per square metre were stocked in each tank at a 1:1 male:female ratio. Before the beginning of the trial, females were weighed and unilaterally eyestalk ablated to accelerate gonad maturation. Additionally, each female was labelled with elastomers on different segments to register and identify the spawn. Daily change in ovarian development and the behaviour of the broodstock were recorded; females with mature ovaries were selected at 19:30 hours by direct observation of the gonads.

Fresh food was distributed at 20% of the biomass of each tank at a ratio 1:1:1:1 of *Artemia* biomass, polychaetes, squid and mussels at 08:00, 12:00, 16:00 and 20:00 hours respectively. Semi-moist feed was provided at 3% of the tank biomass at 24:00 hours (Ortiz-Guillén, 2015).

2.9 | Water quality analysis

Temperature (°C), pH and dissolved oxygen (mg/L) were measured at 08:00 and 20:00 hours throughout the experimental period with a multiparameter probe (Hach Co. model HQD40). Salinity (g/L) was monitored once a day at 08:00 with a refractometer (Atago, model Master-S/Mill). Total ammonia nitrogen (TAN; mg/L) and nitrites (mg/L) were measured twice a week (Grasshoff et al., 1983; Strickland & Parsons, 1972).

2.10 | Spawning and egg collection

Females ready to spawn (stage IV) were placed in 100-L tanks filled with seawater treated by UV and EDTA (10 mg/L). Water was continuously aerated. At the end of spawning, females were returned to their respective maturation tanks (Emerenciano, Cuzon, Arevalo, et al. 2013). Recently, spawned eggs (90%) were harvested with a 100-μm mesh net; the remaining 10% of the eggs were left for reproductive purposes. Samples were set on blotting paper and preweighed, placed in labelled Eppendorff® tubes with liquid nitrogen, weighed again and stored at −80°C for stable isotope analysis.

2.11 | Reproductive performance indicators

The reproductive performance was evaluated in terms of latency periods (interval between eyestalk ablation and first spawn), number of eggs per spawn, number of eggs per gram of female body weight, number of nauplii per spawn, fertilization rate (%) and hatching rate (%). Eggs and nauplii were estimated from five replicates of 4.7 ml collected from spawning tanks (Emerenciano, Cuzon, Arevalo, et al. 2013).

2.12 | Fresh food and muscle parent preservation

A pool of 50 grams for both fresh food and semi-moist feed was taken and preserved. For the domesticated animals, 12 muscles were taken from organisms of the biofloc tank. For wild animals, five muscles were taken. All animals were in intermolt stage C (Bourgeois & Cuzon, 1975). The muscles were taken as a reference to the origin of the animals and to determine whether they have an effect on the contribution to egg production. All samples were stored in Falcon® tubes, frozen with liquid nitrogen and stored at −80°C for further analysis.

2.13 | Stable isotope analysis

The $\delta^{13}C$ and $\delta^{15}N$ analysis was performed on shrimp muscle tissue, all biofloc size classes, commercial feed, fresh food, semi-moist feed and eggs. All samples were freeze-dried to a constant weight and then homogenized with mortar and pestle (Aragón-Axomulco et al., 2012). Five to fifty milligrams of sample was packed into tin cups to be analysed for C and N content and isotope ratio using a Dumas combustion elemental analyser coupled to a Thermo Finnigan Mat

253 isotope ratio mass spectrometer. Stable isotopes were expressed in δ notation as the proportional deviation (in parts per thousand, ‰) of the sample isotope ratio from a standard:

$$\delta X = (R_{sample}/R_{standard} - 1) \times 1000 \quad (1)$$

where X is ^{13}C or ^{15}N, and R_{sample} and $R_{standard}$ are the $^{13}C/^{12}C$ and $^{15}N/^{14}N$ absolute ratios of the sample and standard respectively. The multiplication by 1,000 allows us to express the values in parts per thousand. A sample is enriched when the ratio of heavy to light isotopes in the sample is higher than that ratio in the standard, and a sample is depleted when the ratio in the sample is lower than that ratio in the standard. Vienna Peedee Belemnite (VPDB) and atmospheric nitrogen were used as reference standards for $\delta^{13}C$ or $\delta^{15}N$ respectively. Because VPDB is derived from a sedimentary limestone and contains high quantities of ^{13}C, most organic samples are depleted relative to it and thus will be expressed in negative numbers. Therefore, negative δ values do not imply negative amounts of isotopes but rather a smaller percentage of heavy isotopes in the sample than the standard. The atmospheric nitrogen has been used as the reference material because its value is highly constant and close to 0‰. Animals tend to incorporate the heavy nitrogen (^{15}N) through the trophic chain because of metabolic processes. Therefore, when the δ values are expressed, these values are enriched in the heavy isotope and positive values are displayed. During the analysis, glycine ($\delta^{13}C_{VPDB} = -42.66 \pm 0.03$‰ and $\delta^{15}N_{AIR} = 38 \pm 0.03$‰) and L-serine ($\delta^{13}C_{VPDB} = 6.79 \pm 0.03$‰ and $\delta^{15}N_{AIR} = -7.91 \pm 0.07$‰) were used to quantify the analytical results; these amino acids were used as internal laboratory standards at the Institute of Geology (UNAM; Manzanilla, 2012). This procedure is very common in the laboratories because the stock of some formal standards is depleted or expensive, and use of this procedure allows us to monitor accuracy, repeatability and machine linearity (Ben-David & Flaherty, 2012). The EA-IRMS precision was 0.2‰ for both $\delta^{13}C$ and $\delta^{15}N$ (Coplen et al., 2006).

2.14 | Modelling food source contribution to shrimp grow-out tissue and eggs

The relative contribution of the food sources to the shrimp grow-out tissue from different biofloc sizes (ranging between 10 and 500 μm) and commercial feed (Api-Camaron, Malta Cleyton® 35% CP) were estimated. Additionally, the relative contributions of shrimp parents, fresh food and semi-moist feed sources to eggs were computed. The model considers the isotopic signal of the consumer defined as the mixture (grow-out shrimp or eggs) and the food sources (biofloc sizes and commercial feed for grow-out shrimp and fresh food for eggs). However, because the number of food sources is higher than the number of stable isotopes measured in the mixture ($\delta^{13}C$ and $\delta^{15}N$), the model becomes mathematically undetermined (Phillips & Gregg, 2003). For this, the model proposed by Phillips and Gregg (2003) where $n > 1$ could be calculated was used, where n is the number of stable isotopes measured in the mixture. Therefore, to delineate the relative contribution of the food sources to shrimp tissue and eggs, the IsoSource routine developed by Phillips and Gregg (2003) was used. This method determines all feasible combinations of the food sources to the mixture (shrimp tissue and eggs) in 1% increments. The frequency of feasible combinations created by the IsoSource software is presented as range. However, because the range (minimum and maximum) is sensitive to small numbers of observations on the tails of the distribution, the trimmed 1–99th percentile range was used, although the average is presented.

Because food intake accounts for approximately 2.5‰ of ^{15}N isotope enrichment in herbivorous/detritivorous consumers (Abed-Navandi & Dworschak, 2005; Abreu et al., 2007), 2.5‰ was subtracted from shrimp and egg $\delta^{15}N$ values before the IsoSource analysis; no corrections were made to $\delta^{13}C$ values because dietary C represents a negligible isotopic fraction of assimilated C (Post, 2002).

2.15 | Fatty acid analysis

Fatty acid (FA) profile analysis was performed using the food source samples collected from the grow-out and maturation trials. All samples were freeze-dried to a constant weight and then homogenized with a mortar and pestle (Aragón-Axomulco et al., 2012), and 50–100 mg was taken. Lipids were extracted with methylene chloride:methanol (2:1, v/v) according to a modification of the Folch extraction procedure (Folch et al., 1957). Lipid extracts were saponified with 20% KOH:methanol (w:v), and free FA were recovered in hexane from the acidified saponifiable fraction (pH 1–2). Fatty acid methyl esters (FAMEs) were obtained by esterification with 10% BF_3 in methanol (Fluka-Boron trifluoride-methanol solution, 15716, Sigma-Aldrich Co., St Louis, MO, USA) for 60 min at 80°C. FAMEs were analysed by capillary gas chromatography on a PerkinElmer Clarus 500 GC (PerkinElmer Inc., Shelton, CO, USA) equipped with a PerkinElmer Elite-WAX capillary column (30 m × 0.25 mm × 0.25 μm film thickness, cross-bond-PEG, PerkinElmer, Inc., Shelton, CO, USA) and a flame ionization detector (FID). Hydrogen was used as the carrier gas with a flow rate of 40 mL/min. Injector and detector temperatures were programmed to 280 and 250°C respectively. Column temperature was programmed from 40 to 200°C at 20°C/min and from 200 to 250°C at 2.5°C/min. FAMEs were identified by comparing retention times with reference standards (Supelco 37 Component FAME Mix, 47885-U and Fluka-Nonadecanoic acid, 72332, Sigma-Aldrich Co., St Louis, MO, USA), and the results were reported as area percentages.

2.16 | Statistical analysis

An ANOVA was performed for the crude protein, $\delta^{13}C$ and $\delta^{15}N$ of the food sources supplied to the grow-out shrimp. Shrimp reproductive performance was compared using Student's t test, applied to find differences among domesticated and wild broodstock, if any. For the modelling of the contribution of the food sources to the grow-out shrimp and eggs, the IsoSource software was used (Phillips & Gregg, 2003). The routine requires the isotopic values of the sources (represented by all the food provided to the shrimp) and the mixtures (represented by the shrimp grow-out tissue and eggs).

TABLE 1 Proximate composition (mean ± *SE*) of biofloc size classes and commercial feed (g/kg dry weight)

Composition (g/kg)	≥10 < 50 μm	≥50 < 100 μm	≥100 < 250 μm	≥250 < 500 μm	≥500 μm	Commercial feed
Crude lipid	16.1	77	76	61	81	90 ± 5
Crude Protein	11.8^{na}	308 ± 31^{a}	330 ± 40^{a}	319 ± 46^{a}	291 ± 47^{a}	390 ± 46^{a}

Within rows, superscript letters indicate significant differences by the Post hoc Tukey test ($p < .05$). na, not applicable.

Principal component analysis (PCA) was applied to find the main food contribution in grow-out shrimp and eggs in terms of fatty acids. PCA was performed using the factoextra package in the Rstudio program version 0.99.896 (RStudio Team 2012). PCA is a multivariate method of ordination, and its goal is to explain the highest variance with the lowest number of principal components. PCA transforms the original measured variables into new, uncorrelated variables called principal components. The first principal component represents the greatest proportion of the total variance, the second accounts for the residual variance, etc., until the total variance is accounted for. However, it is usual to use the first few principal components that explain 80% of the total variation (Zuur, Ieno, & Smith, 2007). The PCA biplot shows the ordination of the food variables represented by arrows (Zuur et al., 2007).

3 | RESULTS

3.1 | Grow-out shrimp

The results of water quality analysis were dissolved oxygen at 7.4 ± 0.1 mg/L; pH was 8.7; temperature of 24.6°C and salinity of 35.0 ± 0.4 g/L. The TAN and NO_2-N were 0.2 ± 0.0 and 0.1 ± 0.3 mg/L respectively. Chlorophyll-a was 378.1 ± 60.4 mg/m^3 and biofloc volume was 9.6 ± 1.4 ml/L.

The survival of the shrimp was 69.0 ± 1.7%. At the end of the experimental period, the final body weight was 3.3 ± 0.3 g, and the weight gain was 0.2 g/week. The SGR was 1.3 ± 0.1% per day. The FCR obtained was 1.5 ± 0.7.

The crude lipid of the different biofloc size classes was in the range of 16.1–81 g/kg; the commercial feed Api-Camaron, Malta Cleyton® obtained 90 ± 5 g/kg. For crude protein, no significant differences were found among any food sources ($p > .05$; Table 1).

TABLE 2 Reproductive performance (mean ± *SE*) of domesticated and wild origin broodstock shrimp

Indicators	Domesticated	Wild
Female body weight (g)	21.9 ± 0.5^{a}	39.5 ± 0.8^{b}
Female mortality (%)	7	4
Latency period (days)	16	11
Number of eggs per spawn (×10^3)	123.3 ± 21.8^{a}	175.8 ± 12.2^{b}
Number of eggs per spawn (×10^3) per g of female's body weight	5.6 ± 1.0^{a}	4.4 ± 0.3^{b}

Within rows, superscript letters indicate significant differences by Student's *t* test ($p < .05$).

3.2 | Broodstock trial

The temperature was 27.7 ± 0.4°C, pH was 8.3 ± 0.2, salinity was 36 ± 0.4 g/L, and dissolved oxygen was 8.4 ± 0.2 mg/L. The TAN and NO_2-N were 0.6 ± 0.0 mg/L and 0.3 ± 0.0 mg/L respectively.

Table 2 displayed results during the maturation phase and after egg collection, where low mortality occurred (7% and 4% in biofloc and wild broodstock respectively). The latency period was lower in wild broodstock (11 days) than from biofloc origin (16 days). Number of eggs per spawn (×10^3) per gram of female's body weight was higher for biofloc than wild origin with mean values of 5.6 and 4.4 respectively ($p < .05$).

3.3 | Isotopic analysis ($\delta^{13}C$ and $\delta^{15}N$) of food sources, shrimp grow-out tissue and eggs

Stable isotopes of $\delta^{13}C$ and $\delta^{15}N$ in the food sources of the grow-out trial are shown in Table 3. The most enriched food sources in terms of $\delta^{13}C$ were biofloc ≥250 < 500 μm and ≥500 μm, followed by the commercial feed. The most depleted was the biofloc ≥50 < 100 μm and ≥100 < 250 μm. An ANOVA performed on these food sources identifies three groups, isotopically different: biofloc ≥50 < 100 μm and ≥100 < 250 μm in the first group, the second group was formed by biofloc ≥250 < 500 μm and ≥500 μm and the

TABLE 3 Isotopic signals and carbon and nitrogen percentages (mean ± *SE*) of biofloc size classes and commercial feed

	≥10 < 50 μm	≥50 < 100 μm	≥100 < 250 μm	≥250 < 500 μm	≥500 μm	Commercial feed
$\delta^{13}C$ (‰)	−22.9^{na}	−24.7 ± 0.8^{a}	−24.4 ± 0.9^{a}	−20.4 ± 0.9^{b}	−20.6 ± 0.8^{b}	−22.5 ± 0.1^{c}
$\delta^{15}N$ (‰)	8.6^{na}	3.4 ± 0.3^{a}	5.1 ± 0.4^{a}	4.0 ± 03^{a}	4.2 ± 0.3^{a}	3.8 ± 0.1^{a}
C (%)	10.9	31.8 ± 9	32.7 ± 10	32.6 ± 10	32.6 ± 9	43.2 ± 10
N (%)	1.9	5.4 ± 4	5.7 ± 4	5.6 ± 4	5.0 ± 4	6.3 ± 1
C/N	5.7	6.1 ± 0.5	5.9 ± 0.5	6.0 ± 0.5	6.9 ± 0.5	6.9 ± 0.1
n	1	4	3	3	4	3

Within rows, superscript letters indicate significant differences by Post hoc Tukey test ($p < .05$). na= not applicable. *n*= number of samples analysed.

TABLE 4 Isotopic signals and carbon and nitrogen percentages of fresh food and semi-moist feed offered to shrimp broodstock

	Polychaetes	*Artemia* biomass	Squid	Mussels	Semi-moist feed
$\delta^{13}C$ (‰)	−22.6	−21.2	−17.9	−17.3	−22.5
$\delta^{15}N$ (‰)	9.2	9.9	10.3	10.2	8.6
C (%)	43.6	40.3	46.0	42.7	45.2
N (%)	10.1	9.0	12.1	7.1	6.9
C/N	4.3	4.5	3.8	6.0	6.5

n = 1 for all food sources.

third group was the commercial feed. In terms of $\delta^{15}N$, no significant differences were found.

The isotopic signals of the broodstock food sources are summarized in Table 4. The more enriched food source in terms of $\delta^{13}C$ was mussel with −17.3‰, while the most depleted was polychaetes with −22.6‰. The $\delta^{15}N$ ranged between 8.6‰ and 10.3‰ for the food sources.

The shrimp muscle tissue of the grow-out and broodstock as well as eggs are described in Table 5. The mean of $\delta^{13}C$ for shrimp grow-out muscle tissue was −20.1 ± 0.1‰ and the $\delta^{15}N$ was 6.7‰. For broodstock muscle tissue, the most enriched $\delta^{13}C$ and $\delta^{15}N$ were obtained by the wild origin. For eggs, the values of $\delta^{13}C$ were −20.4‰ and −20.1‰ for domesticated and wild origin, while the $\delta^{15}N$ mean value was 11.5‰ for both origins.

3.4 | Modelling the relative contribution of food sources to shrimp grow-out tissue and eggs

The biofloc size classes were grouped in terms of its $\delta^{13}C$ before the computation of the model to calculate its contribution to the grow-out shrimp (Table 3). The IsoSource routine showed the feasible contribution for biofloc ≥250 μm in a range of 95% to 100% followed by the commercial feed with 0% to 5%. The lowest contribution was obtained by the biofloc ≥10 < 50 μm with a range of 0% to 4% and the biofloc ≥50 < 250 μm with 0% to 2% (Table 6).

The most important food sources for the eggs of the domesticated broodstock were *Artemia* biomass (0%–93%) followed by polychaetes (0%–73%) and semi-moist feed (0%–73%) while the two sources that shared the lowest ranges were squid (0%–62%) and mussels (0%–55%). The muscle of *F. brasiliensis* broodstock cultured in biofloc had a contribution of 0%–47% (Table 6).

For the eggs of wild broodstock origin, the most important food sources were polychaetes (0%–77%), semi-moist feed (0%–77%) and *Artemia* biomass (0%–76%). The squid and mussel had relative contributions of 0%–62% and 0%–55% respectively. The muscle of wild *F. brasiliensis* broodstock shared a range of 0%–46% (Table 6).

3.5 | Fatty acid analysis

For the grow-out shrimp, the most representative fatty acids for all biofloc size classes and commercial feed were myristic acid (C14:0), palmitic acid (C16:0), palmitoleic acid (C16:1), stearic acid (C18:0) and oleic acid (C18:1 n-9). Linolenic acid (ALA, C18:3 n-3) was high in the commercial feed and biofloc sizes of 10 μm and 100 μm. Arachidonic acid (ARA, C20:4 n-6) was similar in all biofloc sizes and commercial feed. Eicosapentaenoic acid (EPA, C20:5 n-3) and docosahexaenoic acid (DHA, C22:6 n-3) were higher in the commercial feed than in the biofloc size classes. The sum of n-3 and n-6 was augmented as the biofloc size increased, but the highest content of n-3 was found in commercial feed (Table 7).

The PCA performed for food sources of the grow-out shrimp showed that the first component represents 84.4% of the variability (Figure 1a). The most important food sources that explain the variability of the first component were the biofloc size classes of 250 and 500 μm with 18.6% and 18.9% respectively (Figure 1b).

For the food sources offered to the broodstock of domesticated and wild origin, the linoleic acid concentrations were highest in the artemia biomass, polychaetes and semi-moist feed at 6.0%, 4.0% and 3.4% respectively (Table 8). ARA was highest in artemia biomass, mussels and squid. EPA and DHA were higher in mussels and squid than in artemia biomass, polychaetes and semi-moist feed (Table 8). The n-3/n-6 ratio indicated that artemia biomass, polychaetes and semi-moist feed had a better balance of n-3 and n-6 fatty acids than mussels and squid (Table 8).

TABLE 5 Isotopic signals and carbon and nitrogen percentages (mean ± *SE*) of shrimp of grow-out, broodstock tissue (domesticated and wild) and eggs

	Grow-out muscle tissue	Domesticated muscle tissue	Wild muscle tissue	Domesticated eggs	Wild eggs
$\delta^{13}C$ (‰)	−20.1 ± 0.1	−19.7 ± 0.1	−16.3 ± 0.2	−20.4 ± 0.4	−20.1 ± 0.3
$\delta^{15}N$ (‰)	6.7 ± 0.1	6.1 ± 0.1	10.0 ± 0.0	11.5 ± 0.3	11.5 ± 0.3
C (%)	44.4 ± 0.2	43.6 ± 0.1	45.1 ± 0.1	50.4 ± 0.1	50.2 ± 0.3
N (%)	13.9 ± 0.1	13.8 ± 0.0	13.7 ± 0.1	9.9 ± 0.1	9.9 ± 0.2
C/N	3.2 ± 0.0	3.2 ± 0.0	3.3 ± 0.0	5.1 ± 0.1	5.1 ± 0.1
n	12	12	5	5	5

n, number of samples analysed.

TABLE 6 Relative contribution of the food sources to the nutrition of the grow-out shrimp and eggs according to its origin

Food source	Grow-out (%)	Eggs BFT (%)	Eggs WILD (%)
Biofloc >10 < 50 μm[a]	0–4 (1)	–	–
Biofloc >50 < 250 μm[a]	0–2 (0.3)	–	–
Biofloc >250 μm[a]	95–100 (97.3)	–	–
Commercial feed	0–5 (1.3)	–	–
Artemia biomass	–	0–93 (19.2)	0–76 (15.6)
Polychaetes	–	0–73 (18.5)	0–77 (22.7)
Semi-moist feed	–	0–73 (18.5)	0–77 (25.0)
Squid	–	0–62 (15.1)	0–62 (12.8)
Mussel	–	0–55 (14.0)	0–55 (12.3)
Broodstock muscle[b]	–	0–47 (14.7)	0–46 (11.6)

Data are range (mean).
[a]Biofloc corresponds to different size classes.
[b]Broodstock muscle according to its origin (from biofloc or wild). (–) not tested due to not belonging to that experimental phase.

The PCA performed for food sources of the broodstock showed that the first component of the analysis represents 75.8% and 78.7% of the variability for the domesticated and wild origin respectively (Figure 2a,c). The most important fresh food sources that explain the highest variability for the first component of the domesticated broodstock were semi-moist feed (18.95%), polychaetes (17.40) and the muscle of *F. brasiliensis* broodstock cultured in the biofloc systems (17.32%). For the eggs produced by the wild broodstock, the most important food sources were the muscle of *F. brasiliensis* broodstock (19.78%), semi-moist feed (18.85%) and polychaetes (17.80%; Figure 2b,d).

4 | DISCUSSION

4.1 | Grow-out shrimp: shrimp performance and food source contributions

The water quality of the present work was in the acceptable range for *F. brasiliensis* (Campos, Furtado, & Poersch, 2015; Van Wyk & Scarpa, 1999). Relative to the grow-out trial, the survival (69 ± 1.7%) was in the same range (50%–88%) reported by

TABLE 7 Biofloc size classes and commercial feed fatty acid profile

Fatty acids (% of total FA)	10 μm	50 μm	100 μm	250 μm	500 μm	Commercial feed
C14:0	11.44	34.01	10.73	26.89	24.89	5.4
C14:1	0.64	0.62	2.83	0.77	0.72	0.1
C15:0	1.59	1.92	6.12	1.65	1.61	0.4
C15:1	0.00	0.01	1.64	0.03	0.05	0.0
C16:0	42.81	26.60	24.31	34.17	35.11	24.8
C16:1	11.87	23.21	10.35	20.74	18.79	8.3
C17:0	1.06	0.45	6.22	0.52	0.57	0.5
C18:0	7.30	3.70	5.94	4.32	5.14	5.3
C18:1 n-9	10.63	2.37	9.19	2.76	3.09	22.0
C18:2 n-6 (LA)	3.10	1.00	2.10	1.40	1.70	15.5
C18:3 n-6	0.00	0.30	5.09	0.33	0.37	0.2
C18:3 n-3 (ALA)	3.29	0.43	1.10	0.31	0.53	1.3
C20:0	0.56	0.18	0.00	0.27	0.42	0.3
C20:1 n-9	0.58	0.11	0.00	0.14	0.15	0.9
C20:2	0.15	0.07	0.00	0.07	0.06	0.2
C20:3 n-6	0.00	0.15	0.00	0.13	0.15	0.1
C20:4 n-6 (ARA)	0.33	0.82	0.00	0.90	1.04	0.8
C20:3 n-3	0.00	0.00	0.00	0.02	0.00	0.1
C20:5 n-3 (EPA)	0.75	2.40	1.79	2.93	3.63	8.7
C22:0	0.70	0.19	0.00	0.24	0.28	0.2
C22:1 n-9	0.46	0.05	0.00	0.05	0.07	0.2
C22:6 n-3 (DHA)	0.16	0.06	0.00	0.10	0.14	3.5
Σ Saturated	65.46	67.05	53.32	68.06	68.01	37.11
Σ Monounsaturated	24.18	26.37	24.02	24.50	22.86	31.42
Σ n-3	4.21	2.89	2.89	3.36	4.30	13.51
Σ n-6	3.43	2.26	7.19	2.76	3.26	16.59
(n-3)/(n-6)	1.23	1.28	0.40	1.22	1.32	0.81

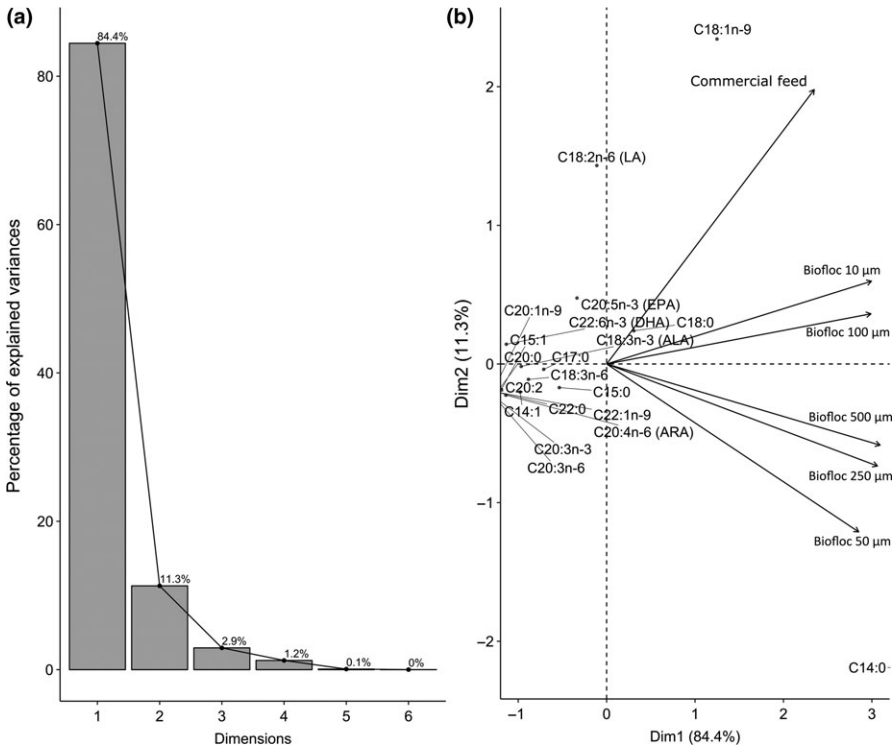

FIGURE 1 Principal component analysis of the food sources supplied to the grow-out shrimp. (a) Percentage of the variance explained by each component, and (b) principal components 1 and 2 of the food sources supplied to the grow-out shrimp

Emerenciano, Ballester, et al. (2012), Viau et al. (2013) and de Souza, Suita, Romano, Wasielesky and Ballester (2014). The SGR (1.3 ± 0.1% per day) was higher than the value reported by de Souza et al. (2014) with 0.03 ± 1.06% per day. The FCR value (1.5 ± 0.07) was similar to the value obtained for *F. brasiliensis* by Lopes, Wasielesky, Ballester and Peixoto (2009) at 1.4. This growth performance could be reached by the capacity of *F. brasiliensis* to ingest and digest biofloc particles of high nutritional value.

The biofloc particles have been characterized by size classes (Table 1), and changes in the nutritional composition of the biofloc particles have been reported, implying that its contribution to the shrimp growth could be different (Ekasari et al., 2014). For this experiment, the biofloc particles >250 μm represent 95%–100% of the relative contribution to shrimp growth, while the commercial feed just represents a contribution of 0%–5% (Table 6). Additionally, the biofloc >10 < 50 μm and >50 < 250 μm represent a source of nutrients but to a lesser degree (Table 6). Then, shrimp reared in biofloc plus commercial feed allowed juveniles to benefit from a "multiphasic diet", representing the phases of the different biofloc size classes (Becerra-Dórame et al., 2012; Cardona et al., 2015; Chim et al., 2014; Ekasari et al., 2014). For *F. paulensis*, the biofilm could represent up to 49% and 70% of carbon and nitrogen retained by the animals (Abreu et al., 2007). Furthermore, reports for *L. vannamei* indicated that biofloc contributed 72% of the carbon and 42% of the nitrogen in shrimp (Ray, 2012). Contrasting results have been reported, indicating that the main source of nutrients for shrimp growth is the commercial feed instead of biofloc (Cardona et al., 2015; Ray & Lotz, 2017; Suita et al., 2016). However, most of this work quantifies the contribution of the biofloc as an indivisible entity, which is well mixed with no clear variations, as it is composed of homogenous particles in suspension. A problem associated with taking the biofloc as an indivisible entity is that the isotopic signal of different sizes may eclipse the isotopic signal of each size fraction, resulting in misunderstandings when the contributions of different foods are determined. For this reason, the stable isotope analysis should be used, in addition to other criteria, as a complement.

In support to stable isotopes, the PCA of the food sources with respect to its fatty acid profile revealed that the biofloc explains the highest variability of the data, specifically the biofloc >250 μm (Table 6). The biofloc is a very chaotic medium with several successions over time, and multiple microorganisms emerge, depending on conditions, forming a microbial loop, where primary producers are consumed by zooplankton, which retain and transfer nutrients to the shrimp (Emerenciano, Cuzon, Paredes, et al., 2013). For this reason, the largest biofloc particles represent a good medium to transfer nutrients as fatty acids, amino acids and vitamins to the shrimp, justifying the high contribution to *F. brasiliensis*, as indicated by the stable isotope analysis (Table 6). Then, the nutritional value of the biofloc in terms of FA, specially EPA and DHA, is interesting, and the contribution to shrimp nutrition in the grow-out and pre-maturation phase could be significant (Ekasari, Crab, & Verstraete, 2010; Emerenciano et al., 2014; Martínez-Córdova, Emerenciano, Miranda-Baeza, & Martínez-Porchas, 2014). Additionally, the digestibility of the food sources could be a relevant factor, and the commercial feed

TABLE 8 Fresh food and semi-moist feed fatty acid profile supplied to breeding shrimp *Farfantepenaeus brasiliensis* of both wild and domesticated origin

	Food sources				
Fatty acids (% of total FA)	*Artemia* biomass	Polychaetes	Mussels	Squid	Semi-moist Feed
C14:0	2.6	2.0	2.9	2.8	8.8
C14:1	0.0	0.2	0.0	0.0	0.0
C15:0	0.5	0.8	0.5	0.6	0.9
C15:1	0.0	0.0	0.0	0.1	0.0
C16:0	23.4	40.2	23.2	23.8	30.3
C16:1	15.2	8.8	5.0	1.9	9.9
C17:0	1.4	1.0	0.8	1.5	1.1
C18:0	11.1	8.2	4.7	9.8	6.5
C18:1n-9	23.6	14.1	3.6	4.3	15.6
C18:2n-6 (LA)	6.0	4.0	1.9	0.6	3.4
C18:3n-6	0.3	0.0	0.0	0.4	0.1
C18:3n-3 (ALA)	5.9	0.7	2.3	2.7	0.0
C20:0	0.0	0.0	0.3	0.7	0.3
C20:1n-9	0.1	3.7	5.2	4.9	5.5
C20:2	0.1	7.9	1.1	0.8	0.2
C20:3n-6	0.0	0.0	2.3	0.4	0.1
C20:4n-6 (ARA)	1.7	0.8	1.9	7.5	0.5
C20:3n-3	0.0	0.3	0.0	0.0	0.1
C20:5n-3 (EPA)	6.8	5.3	15.1	13.1	4.9
C22:0	0.3	0.0	0.1	0.4	0.2
C22:1n-9	0.0	0.8	1.6	1.4	4.1
C22:6n-3 (DHA)	0.0	0.7	25.1	21.2	6.5
Σ Saturated	39.36	52.12	32.47	39.61	48.19
Σ Monounsaturated	38.86	27.63	15.50	12.63	35.21
n-3	12.71	7.09	42.53	37.07	11.53
n-6	8.01	4.80	6.12	8.82	3.99
(n-3)/(n-6)	1.59	1.48	6.95	4.20	2.89

could be less digestible than the biofloc particles (Abreu et al., 2007; Ekasari et al., 2014; Gamboa-Delgado, 2014), explaining why even its high levels of EPA and DHA are not efficiently incorporated into the muscle in contrast to biofloc (Table 7). Therefore, applying such methods (stable isotopes and fatty acids), it has been possible to discern the dietary contribution of multiple food sources to shrimp (Gamboa-Delgado, 2014).

4.2 | Reproductive performance and contribution of food sources to eggs

As an effect of the nutritional history and natural selection, wild shrimp broodstock have better reproductive performance than domesticated shrimp (Braga, Lopes, Krummenauer, Poersch, & Wasielesky, 2011; Emerenciano, Cuzon, et al. 2012; Regunathan, 2008). However, the biofloc technology could be seen as a pre-maturation system where animals obtain benefits of the nutritive biofloc particles improving its nutritional history (Cardona et al., 2016; Chim et al., 2014; Emerenciano et al., 2014). In fact, the benefits obtained from biofloc in grow-out or pre-maturation appear to persist in the broodstock and hence in the progeny (Emerenciano, Cuzon, et al. 2012; Emerenciano, Cuzon, Arevalo, et al. 2013). For this work, the wild broodstock had a lower mortality, shorter latency period and higher number of eggs per spawn, which could be related to the age and weight of the animals used in this study, as has been proven in other peneid shrimp (Braga et al., 2011; Peixoto et al., 2004). However, when the number of eggs is expressed as the number of eggs per gram of body weight, the females of domesticated origin produced more eggs than wild ones (Table 2). Then, an impact of the biofloc on pre-maturation was obtained as in other studies performed with *L. vannamei*, *L. stylirostris* and *F. duorarum* (Cardona et al., 2016; Emerenciano, Cuzon, et al. 2012; Emerenciano, Cuzon, Arevalo, et al. 2013; Emerenciano, Ballester, et al., 2012).

Since broodstock diet is very expensive, the knowledge of the food sources most important to egg production is of practical interest for farmers (Wouters, Lavens, Nieto, & Sorgeloos, 2001). Eggs are derived ultimately from the diet of the female and as a consequence, an isotopic discrimination during the egg formation occurs,

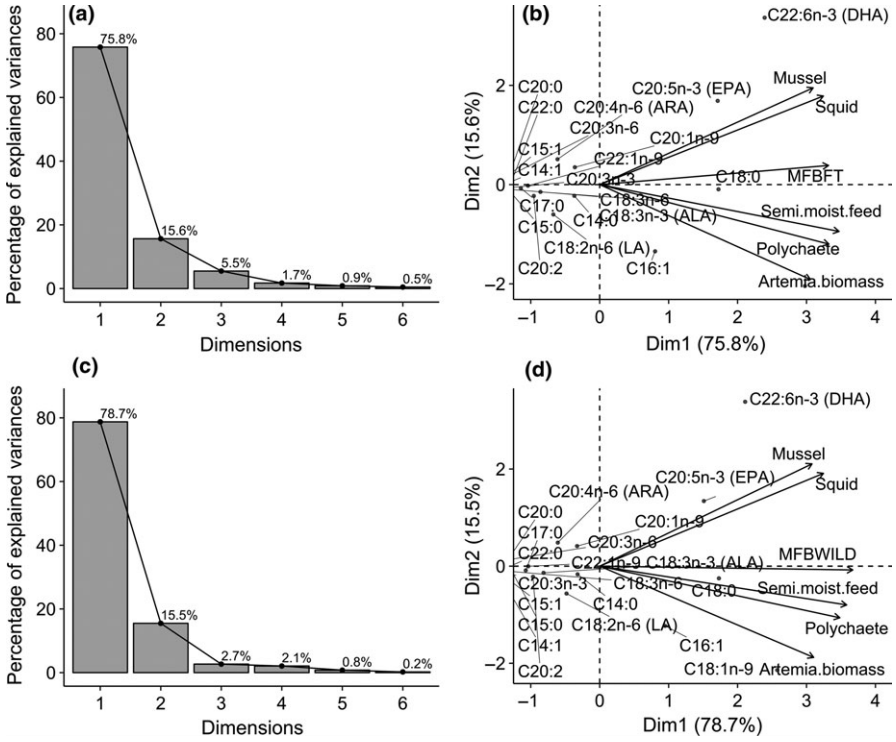

FIGURE 2 Principal component analysis of the fresh food and semi-moist feed sources supplied to the breeding shrimp. (a, b) Percentage of the variance explained by each component for domesticated and wild origin. (c, d) Principal component 1 and 2 of the food sources supplied to the breeding shrimp of wild and domesticated origin

making it possible to discern the contribution of different food sources and even the contribution of parents with different origins (Hobson, 1995). For this work, the higher δ^{15}N signal in eggs than in parental tissue and fresh food sources (Tables 4 and 5) indicated a direct transfer of nutrients from parent to eggs (DeNiro & Epstein, 1978).

The isotopic signature of domesticated and wild eggs showed a larger contribution from *Artemia* biomass, polychaetes and semi-moist feed (Table 6). Muscle of the parents did not contribute much, as it is well described that gonads received neutral and polar lipids from the hepatopancreas directly via haemolymph (Quackenbush, 2001; Teshima, Kanasawa, Horinouchi, & Koshio, 1988). However, a similar contribution irrespective of the origin of the broodstock was found, indicating the importance of the available microbiota during the initial stages of gonadal development (Campos-Ramos, Garza-Torres, Guerrero-Tortolero, Maeda-Martínez, & Obregón-Barboza, 2006). It has been found that shrimp cultured with in situ natural food (microorganisms) begin gonad development before (in month 10) the transfer of proteins from the hepatopancreas to the ovaries, in contrast with those without natural productivity availability (in month 12; Campos-Ramos et al., 2006). This difference could explain the reproductive performance improvements found when comparing the wild and biofloc origin broodstock.

In support of the isotopic analysis, the PCA of the broodstock food sources and the muscle of the broodstock with respect to their fatty acids revealed that polychaetes, semi-moist feed and the broodstock muscle (from both origins) explain the highest variability of the first component (Figure 2). The food sources could represent an important reservoir of carbon for egg production, while the broodstock muscle could provide some nutrients to the egg formation and explain the importance of the nutritional history of shrimp broodstock. As indicated by the isotopic analysis, the most important food sources were the polychaetes and semi-moist feed, which have a better balance between n-3 and n-6 fatty acids, as indicated by the n-3/n-6 ratio found in these foods (Wouters et al., 2001). Additionally, the artemia biomass, polychaetes and semi-moist feed have the highest concentration of LA, which is an important fatty acid associated with the fecundity and survival of the progeny (Table 8; Palacios et al., 2001). The mussels and squid had the highest concentrations of EPA and DHA and could be an important reservoir of these fatty acids, although they are present in the other broodstock food sources (Table 8). These fatty acids play important roles in improving the fecundity, hatching, ovary development and embryogenesis (Wouters et al., 2001). Therefore, the present results confirm that the pre-maturation of pre-breeders under biofloc conditions could help to improve the nutritional status of the broodstock, helping the females to mature in clear water sequence, and the use of stable isotopes and fatty acids could help to discern the contribution and importance of some broodstock sources to egg production.

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CAPÍTULO IV

Artículo III

Nutritional contribution of biofloc within the diet of growout and broodstock of *Litopenaeus vannamei*, determined by stable isotopes and fatty acids

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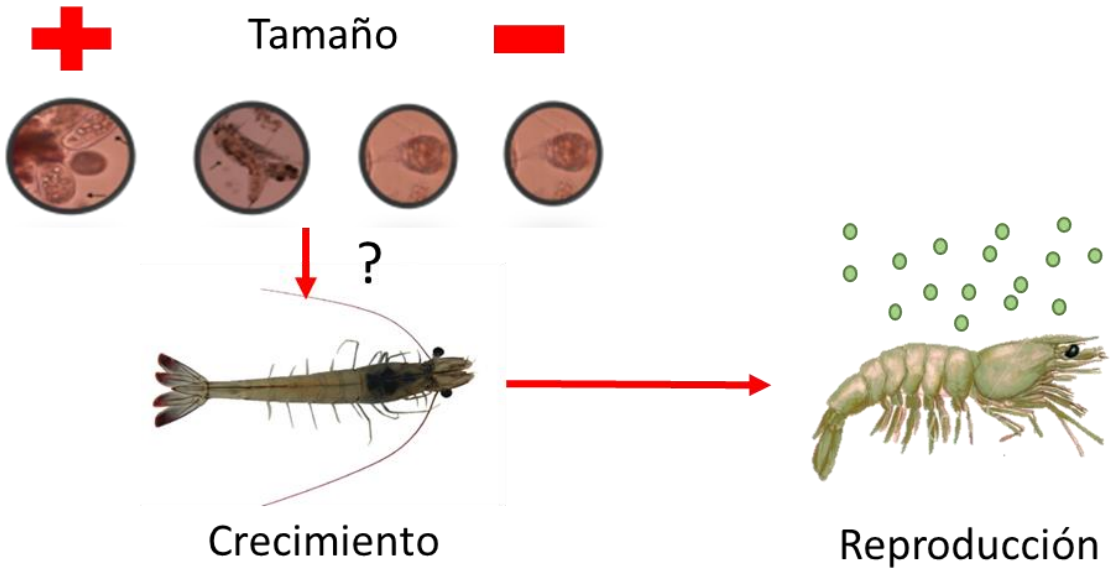

Nutritional contribution of biofloc within the diet of grow-out and broodstock of *Litopenaeus vannamei*, determined by stable isotopes and fatty acids

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Resumen

Las contribuciones relativas de las fuentes alimenticias fueron determinadas a través de la señal isotópica ($\delta^{13}C$ y $\delta^{15}N$) y perfil de ácidos grasos de varios alimentos, músculo de camarón y huevos de *L. vannamei* cultivados en un sistema biofloc. En la fase de engorda, el análisis de isótopos estables mostró que el biofloc ≥250 µm contribuyó entre 55-100%; el biofloc ≥50<250 µm contribuyó 0-22%; y el alimento artificial 0-45%. El análisis de componentes principales de los ácidos grasos mostró que el biofloc ≥250 µm y el alimento artificial fueron las entidades alimenticias durante la engorda. Para la producción de huevos, el análisis de isótopos estables sugirió que las fuentes alimenticias más importantes fueron los poliquetos (0-100%), seguido por la biomasa de *Artemia* (0-86%) y el alimento semi-húmedo (0-66%). En términos de ácidos grasos, las fuentes más importantes fueron la *Artemia*, poliqueto y alimento semi-húmedo. Este trabajo clarifica la importancia de las fuentes alimenticias durante el cultivo en sistemas biofloc y durante la reproducción. El análisis de isótopos estables y ácidos grasos pueden ser exitosamente utilizados para trazar la asimilación de los nutrientes durante la nutrición del camarón.

Abstract

The relative contributions of feed sources were determined through the isotopic signal ($\delta^{13}C$ and $\delta^{15}N$) and fatty acid profile of feed items, shrimp muscle, and eggs of *L. vannamei* reared in a biofloc system. In the grow-out phase, the isotope analysis showed the biofloc particle size class ≥250 µm contributed 55-100%; size ≥50<250 µm contributed 0-22% and artificial feed contributed 0-45%. Principal component analysis applied to fatty acid profiles showed that biofloc ≥250 µm and artificial feed were the most important items in shrimp grow-out. For the egg production, isotope analysis suggested that the most important feed sources according to their relative contributions were polychaetes (0-100%), followed by *Artemia* biomass (0-86%) and semi-moist feed (0-66%), with lower contributions from squid, mussel and the muscle of *L. vannamei* broodstock that had been cultured in biofloc. In terms of fatty acids, the most important items were *Artemia*, polychaete and semi-moist feed. This work clarified the importance of feed sources for shrimp during culture in biofloc systems and during reproduction. Analysis of stable isotopes and fatty acids can be successfully used to trace the assimilation of nutrients during the nutrition of shrimp.

Introducción

El uso de la tecnología biofloc genera agregados microbianos de alto valor nutricional para la nutrición del camarón tanto durante la engorda como en la pre-maduración. Estos agregados microbianos pueden tener diferentes tamaños y pueden ser ingeridos por los camarones como una fuente alimenticia. Sin embargo, el grado al cual el biofloc contribuye a la nutrición de los camarones en comparación con el alimento natural ha sido pobremente evaluado, y si ha sido, el biofloc se ha tomado como una entidad indivisible, incluso cuando se ha demostrado que los diferentes tamaños de biofloc pueden tener diferente valor nutricional. Asimismo, no ha sido determinado si la señal isotópica de los padres se transfiere a los huevos, ni cuál es la contribución relativa de varias fuentes alimenticias en la producción de huevos.

Con el fin de evaluar la contribución del biofloc y varias fuentes alimenticias, en este trabajo se utilizaron las herramientas de isótopos estables en combinación con modelos de mezcla basados en balance de masas. Asimismo, se utilizó el perfil de ácidos grasos de las fuentes alimenticias con el fin de evaluar la importancia. La combinación de ambas herramientas puede direccionar la contribución de las fuentes alimenticias así como evaluar cuales fuentes alimenticias podrían ser los principales reservorios de carbono.

Objetivo de estudio:

Evaluar el efecto del biofloc en la historia nutricional de *L. vannamei* en cuanto a engorda y producción de huevos, a partir de indicadores productivos, de isótopos estables, ácidos grasos y análisis químico-proximales de las fuentes alimenticias.

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Nutritional Contribution of Biofloc within the Diet of Growout and Broodstock of *Litopenaeus vannamei*, Determined by Stable Isotopes and Fatty Acids

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Abstract

The relative contributions of feed sources were determined through the isotopic signal ($\delta^{13}C$ and $\delta^{15}N$) and fatty acid profile of feed items, shrimp muscle, and eggs of *Litopenaeus vannamei* reared in a biofloc system. In the growout phase, the isotope analysis showed the biofloc particle size class ≥250 µm contributed 55–100%; size ≥50 < 250 µm contributed 0–22%; and artificial feed contributed 0–45%. Principal component analysis applied to fatty acid profiles showed that biofloc ≥250 µm and artificial feed were the most important items in shrimp growout. For the egg production, isotope analysis suggested that the most important feed sources according to their relative contributions were polychaetes (0–100%), followed by artemia biomass (0–86%) and semi-moist feed (0–66%), with lower contributions from squid, mussel, and the muscle of *L. vannamei* broodstock that had been cultured in biofloc. In terms of fatty acids, the most important items were artemia, polychaetes, and semi-moist feeds. This work clarified the importance of feed sources for shrimp during culture in biofloc systems and during reproduction. Analysis of stable isotopes and fatty acids can be successfully used to trace the assimilation of nutrients during the nutrition of shrimp.

KEYWORDS

biofloc, broodstock, feed sources, multiphasic diet, shrimp, stable isotopes

Biofloc technology is a culture system that was developed for the control of water quality by the addition of carbon sources (Ebeling et al. 2006; Avnimelech 2007). As a byproduct, a particulate biomass known as biofloc of different sizes and rich in nutrients is formed (Avnimelech 1999; Ekasari et al. 2014). Such flocculated particles can be consumed (biofloc size classes >10 µm) and assimilated in varying proportions (Kent et al. 2011; Ekasari et al. 2014). However, the degree to which biofloc contributes to weight gain in comparison with artificial feed during growout requires additional study (Cardona et al. 2015), recognizing the influence of genotype and

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feed source on the maturation of *Litopenaeus vannamei* toward egg production (Emerenciano et al. 2012b). Also, it has not been determined whether the isotopic signal of parents is transferred to the eggs, nor what are the relative contributions of the feeds supplied.

The use of stable isotopes (e.g., $\delta^{13}C$ and $\delta^{15}N$) can trace the contribution of different feeds to an animal's diet (Gamboa-Delgado 2014). This technique is used in combination with mass-balance and mixing models to calculate the contributions of the feed sources to tissues (Post 2002; Phillips and Gregg 2003); however, these models offer no insights into which biomolecules could represent important reservoirs of carbon, for example. An alternative approach is to characterize the fatty acids of the feeds, because shrimp biochemical composition is a direct reflection of its diet and fatty acids could be a direct carbon source (Kharlamenko et al. 2001; Rooker et al. 2006).

The aim of this study was to use the natural dietary markers (stable isotopes and fatty acid profile) in a biofloc system during the growout phase and egg production of *L. vannamei* shrimp. During growout, the relative contributions of different biofloc size classes and an artificial feed were determined with the stable isotopes of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$), and the feeds that were the main source of fatty acids were identified. Subsequently, when the shrimp reached reproductive size, the main feed sources contributing carbon and nitrogen, as well as fatty acids for egg production, were traced.

Materials and Methods

Shrimp Origin

Litopenaeus vannamei (donated by La Marca farm, located in Sisal) came from breeders cultured previously in biofloc at UMDI Sisal FC-UNAM located at Sisal Beach (21°09′5N and 90°02′5W), Yucatán, Mexico. The larvae were reared and fed until postlarva (Treece and Yates 1990). Once the shrimp reached postlarva stage, they were transferred to four outdoor circular biofloc growout tanks of 20,000 L under constant aeration through a plastic tube using a 5-hp blower. Shrimp were grown for 12 mo until they reached reproductive size. Stocking density was limited: 100 shrimp/m during Months 1–6, 40/m during Months 6–11, and 4/m during the prematuration phase, Months 11–12 (Emerenciano et al. 2013a). Tanks were exposed to coastal climate conditions and water exchange was limited to compensation from evaporation. Filtered saltwater was added as needed without exceeding a 0.5% daily exchange. A C/N ratio of 20/1 was maintained throughout by daily addition of sugarcane molasses until the settling solids (biofloc volume; measured with Imhoff cones) reached 5 mL/L (Avnimelech 1999; Emerenciano et al. 2013a). When settling solids fell below 5 mL/L or total ammonia nitrogen (TAN) reached 1 mg/L, carbon addition was resumed (Emerenciano et al. 2013b; Serra et al. 2015). Sludge was removed daily from each tank through a central drain.

Growout Shrimp and Samples Collection

From the beginning of the seventh month, shrimp (21 ± 0.2 g) in the biofloc growout tanks at a density of 40 shrimp/m^2 (1000 per tank) were surveyed for 45 d in terms of growth performance and isotope ratios. They were fed at 4–5% biomass, with 35% crude protein artificial feed (Api-Camarón; Malta Cleyton®, Culiacan, Sinaloa, Mexico) biweekly adjusted for daily ration. Fifty individual shrimp were sampled from each tank to adjust the daily ration, which was divided into five portions corresponding to 0000, 0400, 0900, 1600, and 2000 h. Every day, temperature (C), pH, and dissolved oxygen (mg/L) were measured at 0400, 0800, 1600, 2000, and 2400 h with a multiparameter probe (Hach HQ40d, Hach Company, Loveland, CO, USA); salinity (g/L) was monitored once a day at 0800 h with a refractometer (Atago Master-S/Mill, Co. Ltd., Tokyo, Japan). TAN (mg/L) and nitrites (mg/L) were measured twice a week, following a colorimetric method (Strickland and Parsons 1972; Grasshoff et al. 1983). The settling solids (biofloc volume; mL/L) were measured with Imhoff cones weekly in 1 L water from each tank after 30-min sedimentation (Avnimelech 2007). Chlorophyll-*a* (mg/L)

was determined by acetone extraction and spectrophotometry at 750, 665, 645, and 630 nm (Aminot 2000) once a week in each tank. For the feed source sampling, 50 g of artificial feed at the start of the trial were homogenized with mortar and pestle until a fine powder was obtained. Biofloc samples were taken every week by filtering tank water through nylon mesh of four successive sizes, 50, 100, 250, and 500 µm, and the samples were pooled at the end into the following size classes: ≥50 < 100 µm; ≥100 < 250 µm; ≥250 < 500 µm; and ≥500 µm. All feed sources were stored in Falcon® tubes, frozen with liquid nitrogen and stored at −80 C until further analysis. To evaluate the shrimp performance, at the end of the experiment, all shrimp were counted; final individual live wet weight from a sample of 50 individuals per tank was determined on an Ohaus balance (0.1 g readability). Growth rate (g/week) was deduced from the difference between final and initial body weights, divided by the number of weeks. A total of 20 juveniles from each tank were taken for isotopic analysis, but only those in intermolt stage C were used (Bourgeois and Cuzon 1975).

Broodstock Condition and Samples Collection

One 20,000-L outdoor tank was stocked at four shrimp/m^2 with shrimp from the four grow-out tanks until they reached 35–40 g (Emerenciano et al. 2012b). The following procedures were performed according to Emerenciano et al. (2013a). Broodstock shrimp (35.2 ± 0.2 g) grown at a density of four shrimp/m^2 in a male–female ratio of 1:1 were transferred to a maturation room for 45 d in one 12,000-L tank. Seawater was replaced 20% daily and recycled through mechanical, biological, and physical filters. Females were unilaterally eyestalk-ablated to accelerate secondary vitellogenesis. They were labeled with elastomer tags on different segments to identify each specimen and its spawn (Emerenciano et al. 2013a). Daily change in ovarian development and the behavior of breeders was recorded; females with mature ovaries were collected at 1900 h after direct observation of gonads. Animal feeds used to feed shrimp were adult artemia biomass, polychaetes, squid, mussels, and semi-moist feed fed (Ortiz-Guillén 2015) at 0800, 1200, 1600, 2000, and 2400 h, respectively; Artemia biomass, polychaetes, mussels, and squid were distributed at 20% biomass in a 1:1:1:1 ratio, but semi-moist feed at 3% of total biomass. For the water quality analysis, the temperature (C), pH, and dissolved oxygen (mg/L) were daily recorded as in the grow-out period, but at 0800 and 2000 h. TAN and nitrites were determined twice a week. For the spawning, every day, the females ready to spawn (stage IV) were placed in 100-L tanks filled with seawater that had been treated with ultraviolet and ethylenediaminetetraacetic acid (10 mg/L); water was continuously aerated. At the end of spawning, females were returned to their maturation tank. Of the freshly spawned eggs (independent of spawn order), 90% were collected with a 100-µm mesh net, and 10% were left for reproductive purposes; samples were set out on blotting paper and preweighed in labeled Eppendorff® tubes with liquid nitrogen, weighed again, and stored at −80 C. For the feed source sampling, 50 g of pooled animal feed and 50 g of pooled semi-moist feed were stored separately in Falcon tubes. Muscle tissue was taken from shrimp from the biofloc tank but only from those in intermolt stage C (Bourgeois and Cuzon 1975). All samples were frozen with liquid nitrogen and then stored at −80 C for further stable isotopes analysis. The evaluation of the reproductive performance was evaluated in terms of the latency period (interval between eyestalk ablation and first spawn), number of eggs per spawn, number of eggs per gram body weight of the female, number of nauplii per spawn, fertilization rate (%), and hatching rate (%). Eggs and nauplii were estimated from five replicates of 4.7 mL collected from spawning tanks (Emerenciano et al. 2013a).

Proximate Analysis of Feeds

Analyses involved the artificial feed and the biofloc in each size class. For protein content, nitrogen was measured by an Elemental Combustion System 4010 (Costech Analytical Technologies Inc., Valencia, CA, USA), and protein

content was calculated from N × 6.25 (assuming 16% N in protein). Lipid content was measured in each biofloc size class (Folch et al. 1957) and in the feed (920.39) (AOAC 1996).

Stable Isotopes Analysis

The $\delta^{13}C$ and $\delta^{15}N$ analysis was performed on shrimp muscle tissue, each biofloc size class, feed, animal feeds, semi-moist feed, and eggs. Each sample was freeze-dried to a constant weight and then homogenized with mortar and pestle (Aragón-Axomulco et al. 2012). A 5–50-mg sample was packed into a tin cup to be analyzed for C and N content, and for the isotope ratio, by coupling a Dumas combustion elemental analyzer to a Thermo Finnigan Mat 253 isotope-ratio mass spectrophotometer. Stable isotopes were expressed in δ notation as the proportional deviation (in parts per thousand, ‰) of the sample isotope ratio from that of a standard:

$$\delta X = (\text{Rsample}/\text{Rstandard} - 1) \times 1000$$

where X is ^{13}C or ^{15}N, Rsample and Rstandard are the $^{13}C/^{12}C$ and $^{15}N/^{14}N$ ratios of sample and standard, respectively. The multiplication by 1000 allows one to express the values in parts per thousand. A sample is enriched when the ratio of heavy to light isotopes in the sample is higher than that of the standard and a sample is depleted when the ratio in the sample is lower than the ratio in the standard. Vienna Peedee Belemnite (VPDB) and atmospheric nitrogen were used as reference standards for $\delta^{13}C$ or $\delta^{15}N$, respectively. During the analysis process, glycine ($\delta^{13}C_{VPDB} = -42.66 \pm 0.03$‰ and $\delta^{15}N_{AIR} = 38 \pm 0.03$‰) and L-serine ($\delta^{13}C_{VPDB} = 6.79 \pm 0.03$‰ and $\delta^{15}N_{AIR} = -7.91 \pm 0.07$‰) were used to quantify the analyzed results; these amino acids were used as internal laboratory standards at the Institute of Geology (UNAM) (Manzanilla 2012). This is a common laboratory procedure because the stocks of some formal standards are depleted or are expensive and allows accuracy to be monitored, repeatability, and machine linearity. EA-IRMS precision was 0.2‰ for both $\delta^{13}C$ and $\delta^{15}N$ (Coplen et al. 2006).

Modeling Feed Source Contributions to Shrimp Tissue in Grow-out and Eggs

The relative contributions of the feed sources to the grow-out shrimp tissue were calculated for biofloc sizes (range 50–500 µm) and feed. The relative contributions of breeders and animal feeds or semi-moist feed sources to eggs were computed. The model takes into account the isotopic signal of the consumer known as the mixture (shrimp grow-out tissue or eggs) and the feed sources (e.g., grow-out: biofloc sizes and artificial feed). However, because the number of feed sources is higher than the number of stable isotopes measured in the mixture ($\delta^{13}C$ and $\delta^{15}N$), the model turns were mathematically undetermined (Phillips and Gregg 2003). Therefore, the model proposed by Phillips and Gregg (2003) was used where $n > 1$ could be calculated, and where n is the number of stable isotopes measured in the mixture. To delineate the relative contribution of the feed sources to shrimp tissue and eggs, the IsoSource routine developed by Phillips and Gregg (2003) was used. This method determines all feasible combinations of the feed sources to the mixture (shrimp tissue and eggs) in 1% increments. The frequency of feasible combinations created by the IsoSource software is presented as histograms. However, because the range (minimum and maximum) is sensitive to small numbers of observations on the tails of the distribution, the trimmed 1–99th percentile range was used, although the average is presented.

As the feed intake accounts for approximately 2.5‰ of 15N isotope enrichment in herbivorous/detritivorous consumers (Abed-Navandi and Dworschak 2005; Abreu et al. 2007), 2.5‰ was subtracted from $\delta^{15}N$ values for shrimp and eggs before IsoSource analysis; no corrections were made to $\delta^{13}C$ values because dietary C represents a negligible isotopic fraction of assimilated C (Post 2002).

Fatty Acid Profile

Analysis used the samples of feeds collected from the grow-out and maturation trials. All samples were freeze-dried to a constant weight, homogenized with mortar

and pestle (Aragón-Axomulco et al. 2012), and then 50–100 mg samples were taken. Lipids were extracted with methylene chloride: methanol (2:1, v/v) according to a modification of the Folch extraction procedure (Folch et al. 1957). Lipid extracts were saponified with 20% KOH : methanol (w : v) and free fatty acids were recovered in hexane from the acidified saponifiable fraction (pH 1–2). Fatty acid methyl esters were obtained by esterification with 10% boron trifluoride in methanol (Fluka-Boron trifluoride-methanol solution, 15716, Sigma-Aldrich Co., St Louis, MO, USA) for 60 min at 80 C. Samples were analyzed by capillary gas chromatography in a Perkin Elmer Clarus 500 GC (Perkin Elmer Inc., Shelton, CT, USA) equipped with a Perkin Elmer Elite-WAX capillary column (30 m × 0.25 mm × 0.25 µm film thickness, crossbond-polyethylene glycol) and a flame ionization detector. Hydrogen was used as a carrier gas with a flow rate of 40 mL/min. Injector and detector temperatures were programmed to 280–250 C, respectively. Column temperature was programmed from 40 to 200 C at 20 C/min and from 200 to 250 C at 2.5 C/min. The fatty acid methyl esters were identified by comparing retention times with reference standards (Supelco 37 Component FAME Mix, 47885-U and Fluka-Nonadecanoic acid, 72332; Sigma-Aldrich Co., St Louis, MO, USA) and the results were reported as area percentages.

Statistical Analysis

An ANOVA was performed for the $\delta^{13}C$ and $\delta^{15}N$ of the feed sources supplied to the grow-out shrimp. For the modeling of the contribution of the feed sources to the grow-out shrimp and eggs, the IsoSource software was used (Phillips and Gregg 2003); the routine procedure requires the isotopic values of the sources (represented by all the feeds provided to the shrimp) and the mixtures (represented by the shrimp grow-out tissue and eggs). Principal component analysis (PCA) was applied to determine which were the main feeds in grow-out and egg production in terms of fatty acids. PCA was implemented using FactoMineR (Factor analysis and data mining with R) package in the Rstudio program version 0.99.896 (RStudio Team 2012). PCA is a multivariate method of ordination and its goal is to explain the highest variance with the lowest number of principal components. PCA transforms the original measured variables into new, uncorrelated variables called principal components. The first principal component represents the greatest proportion of the total variance, the second accounts for the residual variance, and so on, until the total variance is accounted for. However, it is typical to use the first few principal components to explain 80% of the total variation (Zuur et al. 2007). The PCA biplot shows the ordination of the feed variables represented by arrows (Zuur et al. 2007).

Results

Grow-out Shrimp

The following water chemistry parameters were maintained throughout the experiment: temperature was 23.8 ± 0.1 C, pH 8.3 ± 0.0, salinity 39.3 ± 0.3 g/L, dissolved oxygen 8.8 ± 0.1 mg/L, TAN 0.2 ± 0.0 mg/L, and NO_2-N 1.2 ± 0.2 mg/L. Chlorophyll-*a* was calculated at 271.4 ± 50.8 mg/m^3 and settling solids were 5.5 ± 1.4 mL/L. The shrimp survival was calculated at 89 ± 1.7%, and the final body weight was 25.2 ± 0.2 g, with a weight gain of 0.6 ± 0.03 g/week. The proximate analysis revealed that crude protein and lipid concentration of biofloc was greater as the biofloc was larger and almost equal to the artificial feed (Table 1).

Broodstock Shrimp

The following water chemistry parameters were maintained throughout the experiment: the temperature was 28 ± 0.7 C, pH 8.4 ± 0.1, salinity 36 ± 0.4 g/L, and dissolved oxygen 4.9 ± 0.2 mg/L. TAN was 0.6 ± 0.0 mg/L and NO_2-N was 0.3 ± 0.0 mg/L. According to the reproductive performance, the latency period was 20 d. The number of eggs per spawn (×10^3) was 114.7 ± 46.9 and the number of eggs per spawn (×10^3) per gram of female was 3.2 ± 1.3. Fertilization rate (%) was 95.7 ± 3.7 and the

TABLE 1. *Crude lipid and protein composition (g/kg dry weight) for four biofloc size classes and artificial feed.*[1]

Composition (g/kg)	≥50 < 100 µm	≥100 < 250 µm	≥250 < 500 µm	≥500 µm	Artificial feed
Crude lipid	105	77	74	81	90
Crude protein	349	330	349	350	391

[1] $n = 1$ for each biofloc size class and artificial feed. $n = 1$ for all feed sources. Value corresponds to pooled samples.

TABLE 2. *Isotopic signals and carbon and nitrogen percentages of the grow-out feed sources and grow-out tissue of* Litopenaeus vannamei.[1]

	Grow-out feed sources					Grow-out tissue
	Biofloc ≥50 < 100 µm	Biofloc ≥100 < 250 µm	Biofloc ≥250 < 500 µm	Biofloc ≥500 µm	Artificial feed	Shrimp muscle
$\delta^{13}C$ (‰)	-25.3 ± 1.1^{a}	-24.3 ± 0.3^{a}	-20.5 ± 0.1^{b}	-20.1 ± 1.6^{b}	-22.5 ± 0.0^{c}	-20.3 ± 0.0
$\delta^{15}N$ (‰)	3.4 ± 0.2^{a}	5.3 ± 0.9^{a}	4.0 ± 0.2^{a}	4.4 ± 0.3^{a}	3.84 ± 0.1^{a}	7.0 ± 0.0
C (%)	30.7 ± 1.1^{a}	31.8 ± 1.3^{a}	31.6 ± 2.1^{a}	33.6 ± 2.3^{a}	43.2 ± 0.0^{b}	44.9 ± 0.1
N (%)	5.6 ± 0.3^{a}	5.9 ± 0.3^{a}	5.8 ± 0.4^{a}	5.9 ± 0.5^{a}	6.3 ± 0.1^{a}	13.7 ± 0.1
C/N	5.5 ± 0.1^{a}	5.4 ± 0.1^{a}	5.5 ± 0.1^{a}	5.7 ± 0.1^{a}	6.9 ± 0.1^{b}	3.3 ± 0.1
n	4	4	4	3	3	12

[1] Values are the mean ± SE. Different superscript letters within rows indicate significant differences among feed sources according to ANOVA test ($P < 0.05$) among grow-out feed sources. n = number of samples analyzed.

hatching rate (%) was 62.4 ± 19.7. The number of nauplii per spawn ($\times 10^3$) was 72.3 ± 45.6.

Isotopic Analysis ($\delta^{13}C$ and $\delta^{15}N$) and the Relative Contribution of Feed

The feeds supplied in the grow-out trial formed three groups that differed significantly in their $\delta^{13}C$ values: biofloc size classes ≥50 < 100 µm and ≥100 < 250 µm; biofloc ≥250 < 500 and ≥500 µm; and the artificial feed. No significant differences in $\delta^{15}N$ were detected (Table 2). For the muscle tissue from shrimp in the grow-out trial, the $\delta^{13}C$ was -20.3 ± 0.0‰ and the $\delta^{15}N$ was 7.0 ± 0.0‰ (Table 2).

The relative contribution of different feed sources to the shrimp grow-out muscle tissue was computed with reference to three feeds that represented the three isotopically different groups: biofloc ≥250 µm (≥250 < 500 + ≥500 µm), artificial feed, and biofloc ≥50 < 250 µm (≥50 < 100 µm + ≥100 < 250 µm) (Fig. 1). According to the isotopic signatures, biofloc particles ≥250 µm had contributed most (55–100%) to the muscle, followed by artificial feed (0–45%) and biofloc particles ≥50 < 250 µm (0–22%) (Fig. 1).

Among the feeds supplied to shrimp broodstock (Table 3), the highest in $\delta^{13}C$ was mussel with −17.3‰ and the lowest was polychaete with −22.6‰. The $\delta^{15}N$ ranged between 8.6 for semi-moist feed and 10.3‰ for polychaete. The mean of $\delta^{13}C$ for eggs was -22.1 ± 0.1‰ and the $\delta^{15}N$ was 12.4 ± 0.1‰ (Table 3), with the $\delta^{15}N$ value being higher than in the muscle tissue of the grow-out tissue or the animal feeds.

Modeling indicated that the most important contributors to egg formation were polychaetes (0–100%), followed by artemia biomass (0–86%) and semi-moist feed (0–66%), while squid, mussel, and the muscle of the parent female cultured in biofloc contributed the least (Fig. 2).

Fatty Acid Profile

For the grow-out shrimp, the most representative fatty acids for all biofloc size classes and the artificial feed (Table 4) were myristic acid (C14:0), palmitic acid (C16:0), palmitoleic acid (C16:1), stearic acid (C18:0), and oleic acid (C18:1n-9). Linolenic acid (ALA; C18:3n-3) and arachidonic acid (ARA; C20:4n-6) were each similar across all biofloc sizes and the feed. Eicosapentaenoic acid (EPA; C20:5n-3)

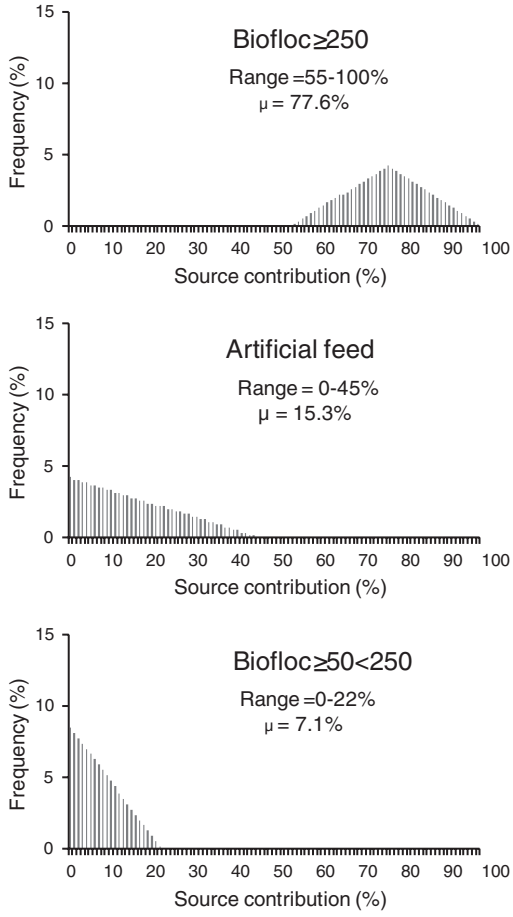

FIGURE 1. *Contribution of three feed sources to muscle tissue grow-out in* Litopenaeus vannamei. μ, *mean. Artificial feed: Api-Camaron, Malta Cleyton®. Biofloc ≥ 50 < 250 μm corresponds to biofloc >50 μm + 100 μm + <250 μm. Biofloc ≥250 corresponds to biofloc ≥250 μm+ > 500 μm.*

and docosahexaenoic acid (DHA; C22:6n-3) were individually higher in the feed than in biofloc. The ratio n-3/n-6 decreased according to the biofloc size (Table 4).

In the PCA for feed sources of the grow-out shrimp, the first component represents 93.4% of the variability (Fig. 3). Within this component, the biofloc regardless of its size, explained the highest variability of the data (84%), while the commercial feed just 16% (Fig. 3).

For the broodstock shrimp, the linoleic acid concentrations were highest in artemia biomass, polychaete, and semi-moist feed with 6.0, 4.0, and 3.4%, respectively (Table 5). ARA was highest in artemia biomass, mussel, and squid. EPA and DHA were higher in mussel and squid than in artemia biomass, polychaete, and semi-moist feed (Table 5). The n-3/n-6 ratio indicated that artemia biomass, polychaete, and semi-moist feed had a better balance of n-3 and n-6 fatty acids than mussel and squid (Table 5).

For the PCA of the fatty acids of the broodstock feed sources the first component explained the 75.5% of the variability and the second component 15.5% of the variability, with both components explaining the 91% of the variability. According to this exploratory analysis, the two variables that explain the highest variability of the first component were the semi-moist feed (19.1%) and polychaete (17.7%), while for the second component were the mussel (31.6%) and artemia biomass (27.3%).

Discussion

Grow-out Shrimp: Shrimp Performance and Feed Sources Contribution

Physicochemical parameters remained within the acceptable range for *L. vannamei* culture throughout the trial (Van Wyk and Scarpa 1999; Lin and Chen 2001, 2003; Taw 2010). High survival rate in this grow-out trial (89.7%) is consistent with other reports of >85% survival in biofloc (Burford et al. 2004; Serra et al. 2015). The juvenile growth rates of 0.6 g/week were lower than the >1 g/week reported with *L. vannamei* in biofloc (Ray et al. 2011; Xu et al. 2016) but similar to the potential growth rate of *L. vannamei* when it reaches 20 g (FAO 2004). The potential to ingest and digest particles of various sizes due to the third maxillipeds could explain survival and growth in biofloc (Moss et al. 1999; Kent et al. 2011).

In this respect, the isotopic, proximate, and fatty acid analysis of the feed sources has the potential to discern the dietary contribution of multiple feed sources in shrimp. In the present work, the main feed source for shrimp was biofloc ≥250 μm, followed by artificial feed and particles between 50 and <250 μm, where these provided a constant supply of nutrients (Table 1; Fig. 1) (Ekasari et al. 2014; Cardona et al. 2015).

TABLE 3. *Isotopic signals and carbon and nitrogen percentages of animal feed and semi-moist feed offered to shrimp broodstock as well as the eggs produced.*[1]

	Polychaetes	Artemia biomass	Squid	Mussel	Semi-moist feed	Eggs
$\delta^{13}C$ (‰)	−22.6	−21.2	−17.9	−17.3	−22.5	−22.1 ± 0.1
$\delta^{15}N$ (‰)	9.2	9.9	10.3	10.2	8.6	12.4 ± 0.1
C (%)	43.6	40.3	46.0	42.7	45.2	64.2 ± 0.5
N (%)	10.1	9.0	12.1	7.1	6.9	9.7 ± 0.1
C/N	4.3	4.5	3.8	6.0	6.5	6.6 ± 0.0

[1] $n = 1$ for all animal feed sources. $n = 5$ for the eggs produced by *Litopenaeus vannamei*.

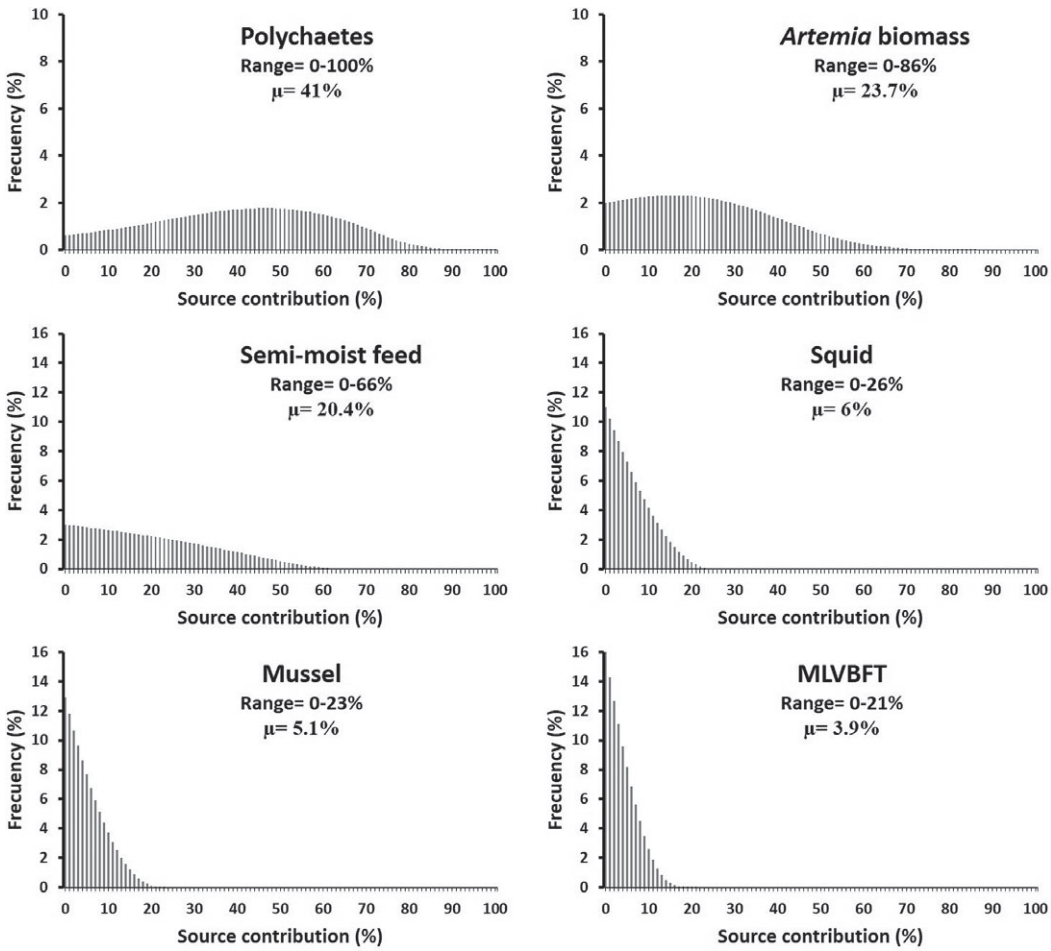

FIGURE 2. *Contribution of five animal feed sources and parental muscle (MLVBFT = muscle of* Litopenaeus vannamei *broodstock cultured in biofloc systems) to eggs.* μ, *mean.*

For example, Cam et al. (1991) reported that natural productivity contributed to 85% of tissue buildup for *Metanephrops japonicus*, but the contribution of dry feed increased as the meiofauna decreased. Also, shrimp may retain carbon and nitrogen from the biofloc, which can contribute 72% of carbon and 42% of nitrogen in shrimp (Ray 2012), although lower contributions (39.8% of C and 36.9% of N) have been reported for *Litopenaeus stylirostris* (Cardona

TABLE 4. *Fatty acid profiles (% of total fatty acids) of biofloc size classes and artificial feed.*[1]

Fatty acids (% of total fatty acid)	50 µm	100 µm	250 µm	500 µm	Artificial feed
C14:0	13.27	10.51	11.61	9.87	5.4
C14:1	0.26	0.31	0.29	0.13	0.1
C15:0	1.23	1.16	1.11	1.05	0.4
C15:1	0.04	0.05	0.04	0.01	0.0
C16:0 (palmitic acid)	42.77	43.41	42.32	41.63	24.8
C16:1	11.15	9.41	11.00	9.45	8.3
C17:0	0.86	0.87	0.82	0.92	0.5
C18:0	7.14	8.71	7.14	8.88	5.3
C18:1n-9	9.14	8.95	11.52	15.93	22.0
C18:2n-6 (linoleic acid)	3.60	3.90	4.40	5.0	15.5
C18:3n-6	0.18	0.24	0.15	0.00	0.2
C18:3n-3 (linolenic acid)	2.84	3.73	2.58	1.13	1.3
C20:0	0.50	0.59	0.52	0.56	0.3
C20:1n-9	0.47	0.40	0.56	0.72	0.9
C20:2	0.14	0.16	0.15	0.16	0.2
C20:3n-6	0.08	0.08	0.07	0.05	0.1
C20:4n-6 (arachidonic acid)	0.79	1.06	0.69	0.34	0.8
C20:3n-3	0.11	0.12	0.14	0.17	0.1
C20:5n-3 (eicosapentaenoic acid)	2.70	3.49	2.04	1.06	8.7
C22:0	0.50	0.58	0.54	0.56	0.2
C22:1n-9	0.27	0.26	0.23	0.23	0.2
C22:6n-3 (docosahexaenoic acid)	0.22	0.32	0.25	0.35	3.5
Σ Saturated	66.27	65.83	64.07	63.46	37.11
Σ Monounsaturated	21.34	19.37	23.64	26.47	31.42
Σ n-3	5.88	7.67	5.00	2.71	13.51
Σ n-6	4.65	5.28	5.30	5.39	16.59
(n-3)/(n-6)	1.30	1.50	0.94	0.50	0.82

[1] $n = 1$ for all feed sources. Value corresponds to pooled samples.

et al. 2015). The present results are slightly higher than those reported elsewhere but this could be related to the species, the ontogenetic stage, and the sources of the artificial feed, as well as the analysis of different size classes of biofloc (Ekasari et al. 2014; Cardona et al. 2015; Suita et al. 2016).

In support for stable isotopes, the exploratory analysis (PCA) of the feed sources respect to their fatty acids revealed that the biofloc (regardless its size) explained the highest variability of the data (Fig. 3). This means that all biofloc size have fatty acids of high relevance to the *L. vannamei* nutrition and are linked by a trophic chain formed in the tank. For example, the biofloc sizes of 50 and 100 µm have EPA and DHA concentrations higher than the biofloc >250 µm, indicating there is a production of these fatty acids by the phytoplankton (Table 4). However, these fatty acids could be more easily transferred by the biofloc >250 µm to the shrimp, as the shrimp is the final consumer in the tank. The concentrations of ARA (0.34–1.06%) and EPA (1.06–3.49%) in biofloc measured in this study were higher than the 0.4 and 0.5% reported by Emerenciano et al. (2014); these fatty acids are important in the development of nervous tissue, as well as being essential components of cell membranes and precursors of prostaglandins (Table 4) (Glencross 2009). In fact, it has been determined that the biofloc have a microbial loop, where primary producers are consumed by rotifers, copepods, and nematodes, which retain the nutrients and transfer them through the food chain (Emerenciano et al. 2013b). For this reason, the biofloc >250 µm could represent a good medium to ingest particles rich in fatty acids and other biomolecules as amino acids and vitamins justifying the high contribution of these particles to the grow-out as indicated by the stable isotope analysis. Applying such methods, it has been possible to discern the dietary

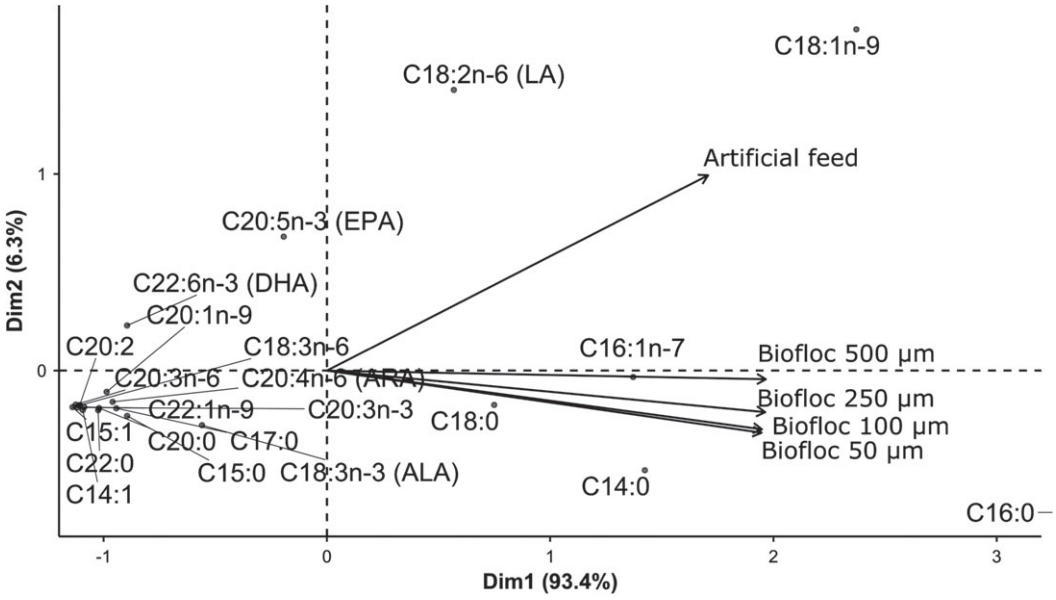

FIGURE 3. *Biplot of the principal component analysis of feeds supplied to grow-out shrimp respect to their fatty acids.*

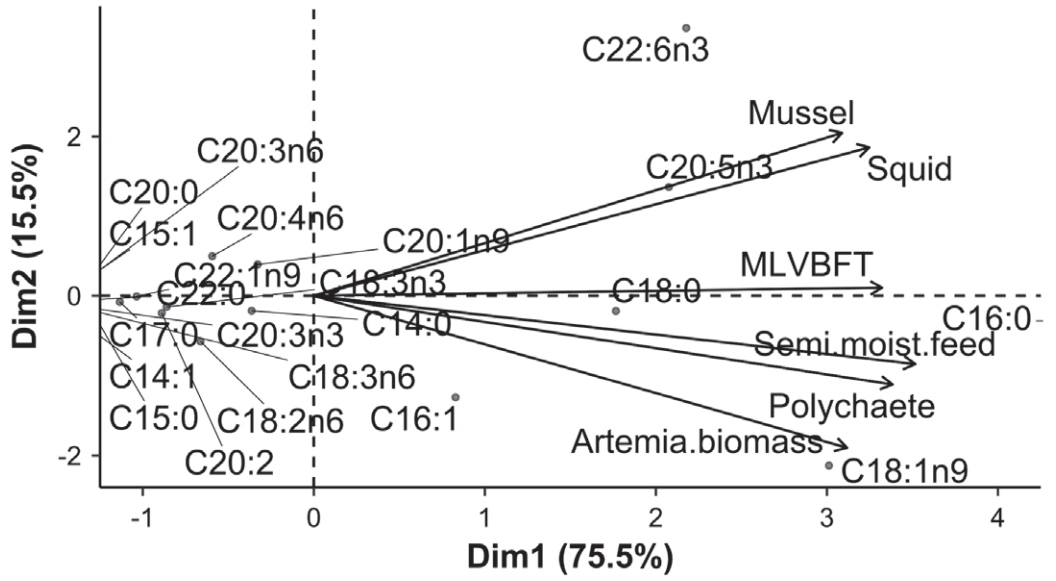

FIGURE 4. *Principal component analysis of the feeds supplied to the broodstock shrimp respect to their fatty acids. MLVFT = muscle of* Litopenaeus vannamei *broodstock cultured in biofloc system.*

contribution of multiple feed sources to shrimp (Gamboa-Delgado 2014). Therefore, the biofloc can be seen as a multiphasic system with phases represented by different particle sizes that nourish the shrimp.

Broodstock Shrimp: Reproductive Performance and Contribution of Feed Sources to Eggs

Biofloc could be seen as a breeding or pre-maturation medium from which shrimp take up particles to obtain native sources of nutrients

TABLE 5. *Fatty acid profile of animal feeds and semi-moist feed supplied to breeding shrimp* Litopenaeus vannamei.[1]

	Feed sources				
Fatty acids (% of total fatty acid)	Artemia biomass	Polychaete	Mussel	Squid	Semi-moist feed
C14:0	2.6	2.0	2.9	2.8	8.8
C14:1	0.0	0.2	0.0	0.0	0.0
C15:0	0.5	0.8	0.5	0.6	0.9
C15:1	0.0	0.0	0.0	0.1	0.0
C16:0 (palmitic acid)	23.4	40.2	23.2	23.8	30.3
C16:1	15.2	8.8	5.0	1.9	9.9
C17:0	1.4	1.0	0.8	1.5	1.1
C18:0	11.1	8.2	4.7	9.8	6.5
C18:1n-9	23.6	14.1	3.6	4.3	15.6
C18:2n-6 (linoleic acid)	6.0	4.0	1.9	0.6	3.4
C18:3n-6	0.3	0.0	0.0	0.4	0.1
C18:3n-3 (linolenic acid)	5.9	0.7	2.3	2.7	0.0
C20:0	0.0	0.0	0.3	0.7	0.3
C20:1n-9	0.1	3.7	5.2	4.9	5.5
C20:2	0.1	7.9	1.1	0.8	0.2
C20:3n-6	0.0	0.0	2.3	0.4	0.1
C20:4n-6 (arachidonic acid)	1.7	0.8	1.9	7.5	0.5
C20:3n-3	0.0	0.3	0.0	0.0	0.1
C20:5n-3 (eicosapentaenoic acid)	6.8	5.3	15.1	13.1	4.9
C22:0	0.3	0.0	0.1	0.4	0.2
C22:1n-9	0.0	0.8	1.6	1.4	4.1
C22:6n-3 (docosahexaenoic acid)	0.0	0.7	25.1	21.2	6.5
Σ Saturated	39.4	52.1	32.5	39.6	48.2
Σ Monounsaturated	39.9	27.7	15.5	12.6	35.2
n-3	12.7	7.1	42.5	37.1	11.5
n-6	8.0	4.8	6.1	8.8	4.0
(n-3)/(n-6)	1.6	1.5	6.9	4.2	2.9

[1] $n = 1$ for all feed sources.

(Chim et al. 2014; Ekasari et al. 2014). The benefits obtained from biofloc in grow-out or prematuration appear to persist in the broodstock and hence in the progeny (Emerenciano et al. 2012b, 2013a). In the present work, the latency period and the number of eggs are in agreement with other findings (Emerenciano et al. 2012a, 2013a), but the fertilization rate and the number of nauplii per spawn were higher than those reported by Emerenciano et al. (2013a). Hence, reproductive performance of *L. vannamei* raised in biofloc was at least as good as previous results (Emerenciano et al. 2013a), as has been noted in *L. vannamei* and *L. stylirostris* prior to secondary vitellogenesis (Emerenciano et al. 2013a; Chim et al. 2014).

The higher $\delta^{15}N$ signal in eggs than in parental tissue and animal feed sources indicated a direct transfer from parent to eggs owing to a discrimination of heavy isotopes in the process of digestion, assimilation, and subsequent transfer of nutrients to eggs (Table 3) (DeNiro and Epstein 1978). In the present study, artemia biomass and polychaetes were the main contributors of carbon and nitrogen to egg formation (Fig. 2); they are needed by broodstock for high numbers of eggs and spawns, and high larval quality because they are rich in n-3 fatty acids (Wouters et al. 2001). Muscle of the parents did not contribute much as it is well described that gonads received neutral and polar lipids from hepatopancreas directly through hemolymph (Teshima et al. 1988; Quackenbush 2001).

In support of the isotopic analysis, the PCA of the broodstock feed sources with respect to their fatty acids revealed that the semi-moist feed and polychaetes explained the highest variability of the first component, while mussel and

artemia biomass were the most important for the second component (Fig. 4). These sources could represent an important reservoir of carbon for egg production. According to Wouters et al. (2001) the n-3/n-6 ratio must be between 1 and 2, indicating a good balance between the n-3 and n-6 fatty acids, which is well represented for artemia biomass, polychaete, and semi-moist feed (Table 5) and could explain their high contribution to egg production as indicated by the isotopic analysis. Also, the artemia biomass, polychaetes, and semi-moist feed have the highest concentration of linoleic acid, which is an important fatty acid associated with the fecundity and survival of the progeny (Palacios et al. 2001) (Table 5). The mussel and squid had the highest concentrations of EPA and DHA and could be an important reservoir of these fatty acids to egg production, although these fatty acids are presented in the other broodstock feed sources (Table 5). These fatty acids are important for reproduction because they improve the fecundity, hatching, and ovary development, and play an important role in embryogenesis (Wouters et al. 2001). Then, applying the isotopic analysis and the PCA of the fatty acids for the broodstock it was possible to discern the contribution and importance of some broodstock feed sources to egg production.

Conclusion

Biofloc may be regarded as a multiphasic feed source because different size classes could contribute in different proportions. The important contribution of the particles >250 µm could be related to their nutritional value, because this size has the highest concentrations of crude lipids and proteins. Both the stable isotopes and the fatty acid profiles showed that the artificial feed was a less important feed during grow-out in terms of assimilation for shrimp, despite its nitrogen contribution during biofloc system development. The results also indicated that the main feed sources for eggs were artemia biomass, polychaetes, and semi-moist feed. These two analytical methods delineate the assimilation and origin of carbon and fatty acids and clarify the importance of multiple feed sources for shrimp nutrition.

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CAPÍTULO V

Artículo IV

Does unilateral eyestalk ablation affect the quality of the larvae of the pink shrimp *Farfantepenaeus brasiliensis* (Latreille, 1817) (Decapoda: Dendrobranchiata: Penaeidae)?

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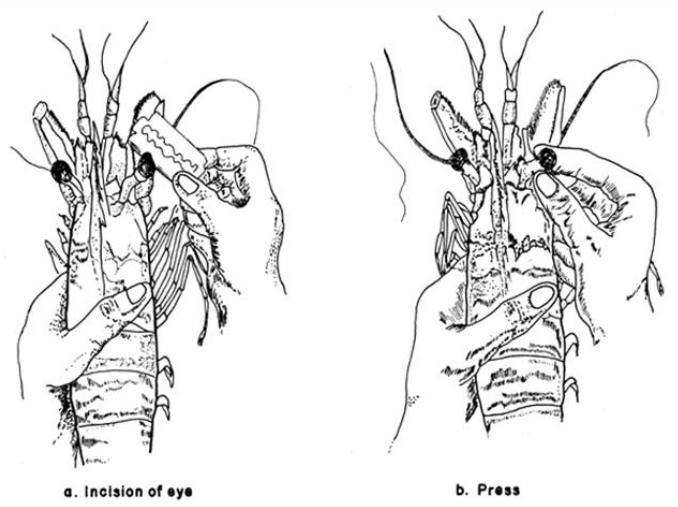

Does unilateral eyestalk ablation affect the quality of the larvae of the pink shrimp *Farfantepenaeus brasiliensis* (Latreille, 1817) (Decapoda: Dendrobranchiata: Penaeidae)?

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Resumen

La ablación unilateral del pedúnculo ocular ha sido ampliamente utilizada en granjas de reproducción de camarones peneidos para acelerar la maduración de las hembras, pero ha sido asociada con alta mortalidad de los reproductores y pobre calidad de la progenie. Nosotros estudiamos el desempeño reproductivo y la calidad de los huevos y nauplios de hembras reproductoras con y sin ablación de *F. brasiliensis*. El análisis bioquímico de los acilglicéridos, glucosa, colesterol y proteína total soluble se llevó a cabo en hepatopáncreas, ovarios, hemolinfa, huevos y nauplios. Encontramos que las hembras con ablación presentaban una mayor velocidad de maduración gonádica pero alta mortalidad. La tasa de fertilización y el número de nauplios viables, sin embargo, fue mejorada en las hembras no ablacionadas. Los huevos de hembras no ablacionadas eran más pequeños, no obstante, los nauplios fueron mayores en comparación de hembras ablacionadas. La concentración del colesterol y proteína total soluble en la hemolinfa fue mayor en hembras no ablacionadas que ablacionadas. Una mayor concentración de proteína total soluble, así como la proporción acilglicéridos:colesterol (AG:C), fue encontrada en los huevos de hembras no ablacionadas que en aquellas ablacionadas. El índice de condición naupliar, indicó que la progenie de los reproductores no ablacionados fue mayor que en aquellas hembras con ablación, lo cual confirma que la ablación unilateral del pedúnculo ocular afecta no solamente la mortalidad de las hembras si no también la calidad de la progenie.

Abstract

Unilateral eyestalk ablation has been widely used in commercial hatcheries of penaeid shrimps to accelerate female maturation, but has been associated with high broodstock mortalities and poor-quality offspring. We studied the reproductive performance and the quality of eggs and nauplii larvae of unilaterally ablated eyestalks and non-ablated *Farfantepenaeus brasiliensis* (Latreille, 1817) broodstock. The biochemical analysis of triglycerides, glucose, cholesterol, and total soluble protein was performed in the hepatopancreas, ovaries, hemolymph, eggs, and nauplii. We found that females with unilaterally ablated eyestalks showed a better reproductive performance but higher mortality than non-ablated females. The fertilization rate and the number of viable nauplii, however, were improved in non-ablated females. Eggs were smaller in the non-ablated than in ablated females, but the length of the nauplii was higher in non-ablated than in ablated

females. The concentration of cholesterol and total soluble protein in hemolymph was higher in non-ablated than in ablated females. A higher concentration of total soluble protein, as well as the ratio of acylglycerides:cholesterol (AG:C), was found in the eggs of non-ablated females than in those of ablated females. The nauplii-condition index, calculated from the level of nauplii acylglycerides, percentage of viable nauplii, and length of nauplii, indicated that the index of offspring of the non-ablated broodstock was higher than in those of ablated females, which confirms that unilateral eyestalk ablation affects not only the mortality of females but the quality of the offspring as well.

Introducción

La ablación unilateral del pedúnculo ocular ha sido ampliamente utilizada en la acuacultura para acelerar la maduración gonádica y consecuentemente aumentar la producción de semilla en los centros de reproducción. Sin embargo, esta práctica ha sido asociada con altas mortalidades de los reproductores, pobre calidad de la progenie y con maltrato animal.

Por lo anterior, es importante llevar a cabo estudios que demuestren el efecto de la ablación sobre la progenie de los camarones peneidos. Ya que larvas de mala calidad pueden incrementar el riesgo de infección vertical en las granjas. Con el fin de evaluar esto, una serie de indicadores reproductivos, morfométricos y bioquímicos fueron utilizados en combinación con índices que califican la calidad de la progenie.

Objetivo de estudio:

Evaluar el efecto de la ablación unilateral del pedúnculo ocular aplicado a *F. brasiliensis* y su relación con la calidad de la progenie, mediante el uso de indicadores productivos, morfométricos y bioquímicos.

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Does unilateral eyestalk ablation affect the quality of the larvae of the pink shrimp *Farfantepenaeus brasiliensis* (Letreille, 1817) (Decapoda: Dendrobranchiata: Penaeidae)?

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ABSTRACT

Unilateral eyestalk ablation has been widely used in commercial hatcheries of penaeid shrimps to accelerate female maturation, but has been associated with high broodstock mortalities and poor-quality offspring. We studied the reproductive performance and the quality of eggs and nauplii larvae of unilaterally ablated eyestalks and non-ablated *Farfantepenaeus brasiliensis* (Latreille, 1817) broodstock. The biochemical analysis of triglycerides, glucose, cholesterol, and total soluble protein was performed in the hepatopancreas, ovaries, hemolymph, eggs, and nauplii. We found that females with unilaterally ablated eyestalks showed a better reproductive performance but higher mortality than non-ablated females. The fertilization rate and the number of viable nauplii, however, were improved in non-ablated females. Eggs were smaller in the non-ablated than in ablated females, but the length of the nauplii was higher in non-ablated than in ablated females. The concentration of cholesterol and total soluble protein in hemolymph was higher in non-ablated than in ablated females. A higher concentration of total soluble protein, as well as the ratio of acylglycerides:cholesterol (AG:C), was found in the eggs of non-ablated females than in those of ablated females. The nauplii-condition index, calculated from the level of nauplii acylglycerides, percentage of viable nauplii, and length of nauplii, indicated that the index of offspring of the non-ablated broodstock was higher than in those of ablated females, which confirms that unilateral eyestalk ablation affects not only the mortality of females but the quality of the offspring as well.

Key Words: aquaculture, eggs, nauplii larvae, offspring quality

INTRODUCTION

Unilateral eyestalk ablation has been widely implemented in aquaculture to accelerate the gonadal maturation and consequently increase the seed production of shrimp broodstock (Racotta *et al.*, 2003; Kannan *et al.*, 2015). This technique removes one eyestalk, which hosts the sinus-organ X that produces hormones such as the gonad inhibiting hormone. These hormones are responsible for accelerating maturation and leads to predictable peaks of maturation and spawning that facilitates the setting up of production schedules (Primavera, 1985; Swetha *et al.*, 2011). This practice, however, has been associated with high broodstock mortalities and poor-quality offspring (Palacios *et al.*, 1998; 1999b), and it has also been under criticism by consumers due to animal welfare concerns (Munkongwongsiri *et al.*, 2015). Unilateral eyestalk ablation has been shown to improve shrimp reproductive performance but to decrease the quality of the offspring and the broodstock.

The quality of the nauplius larva of shrimp is affected by nutrition, origin, and endocrine manipulation of the broodstock and is of particular interest to aquaculturists because the viability of the culture could be compromised (Primavera, 1985; Racotta *et al.*, 2003). Several biochemical and morphological indicators, or a combination of both, have been used for estimating larval

quality (Palacios *et al.*, 1999b). Triglycerides, cholesterol, total soluble protein, and glucose are biomolecules of high relevance for the offspring and their importance has been widely demonstrated (Palacios *et al.*, 1998; Palacios & Racotta, 1999; Emerenciano *et al.*, 2012c). Egg size (volume or diameter) is an approximate equivalent of the quantity of reserves. The length of the nauplii larvae reflects the degree of development at a given time (Racotta *et al.*, 2003). The evaluation of larval quality and reproductive performance of broodstock with unilaterally ablated and non-ablated eyestalks will help determine if this technique is warranted for the pink shrimp, *Farfantepenaeus brasiliensis* (Latreille, 1817), which is commonly farmed.

Farfantepenaeus brasiliensis is naturally distributed from Florida, USA to Rio Grande do Sul, Brazil. With good growth and survival rates, it is an alternative to the culture of *Litopenaeus vannamei* (Boone, 1931) in coasts where it naturally occurs (Gaxiola *et al.*, 2010; Emerenciano *et al.*, 2012a). A wild broodstock batch of 250 individuals was used in this study because the nutritional history and reproductive performance are better than in the domesticated populations (Regunathan, 2008; Emerenciano *et al.*, 2012c). The aim of this study was to evaluate the reproductive performance and larval quality of wild *F. brasiliensis* with and without unilateral eyestalk ablation in order to evaluate this practice.

MATERIALS AND METHODS

Collection and acclimation of specimens

A total of 250 *F. brasiliensis* broodstock were collected from northwest Contoy Island, Mexico (21°29′2″N, 86° 47′30″W) and transferred to the maturation room at Unidad Multidisciplinaria de Docencia e Investigación de Sisal, Sisal Beach (21°09′5″N, 90°02′5″W), Yucatán, Mexico. Two 12,000 l round, lined maturation tanks (4.0 m in diameter) connected to a water-recirculation system with a chiller Delta Star DSHP-9 (Aqualogic, San Diego, CA, USA) for temperature control, and with continuous aeration, were used for the broodstock acclimation for one week before starting the experiment (Emerenciano *et al.*, 2012c). Water temperature was 28 ± 1 °C, salinity 35 g l^{-1}, pH of 9.1 ± 0.1, and dissolved oxygen 7.9 mg l^{-1}. The daily water exchange rate was 200%. Shrimp were stocked at 10.4 shrimp m^{-2} per tank. Fresh food was distributed at 20% of the biomass of each tank at a ratio 1:1:1:1 of *Artemia*, polychaete, squid, and mussels biomass at 0800, 1200, 1600 and 2000 h (Emerenciano *et al.*, 2013). Semi-moist feed (51.1% crude protein, 14.4% crude lipid) was provided at 3% of the tank biomass at 2400 h (Ortiz-Gullén, 2015). A 2.4% mortality rate was calculated for both tanks.

During acclimation, the shrimp broodstock were selected by morphological integrity of the body (exoskeleton in good condition, without necrosis or melanization, complete appendices, and reproductive system without external alterations) as well as size (Braga *et al.*, 2010; Emerenciano *et al.*, 2012c). Females (stage III, $N = 40$ per treatment) and males ($N = 20$ per treatment) with gonadal development were transferred to the experimental tanks five days before the beginning the experiment. All females were tagged with elastomers.

Experimental design

Two 45-day treatments were designed, shrimp with unilaterally ablated eyestalks and shrimp with non-ablated eyestalks. At the beginning of the experiment (day 1), 10 females of each treatment were sampled and those in stage IV, ready to spawn according to Guitart & Quintana (1978), were used for further analysis; leading a group of 30 females and 20 males (female to male ratio, 1.5:1; density, 4.16 shrimp m^{-2}) until the end of the experiment. Females in the ablated-eyestalk treatment were unilaterally ablated immediately after tagging to minimize manipulation (Emerenciano *et al.*, 2012c). Each treatment involved a tank with the same characteristics as the acclimation tanks. The temperature was maintained with a chiller at 28.0 ± 1 °C and oxygen was 6.0 ± 0.3 mg l^{-1} throughout both treatments; oxygen was within the acceptable range for the culture of penaeid shrimps (Van Wyk & Scarpa, 1999). Daily change in ovarian development and the behavior of the broodstock was recorded. Females with mature ovaries (Guitart & Quintana, 1978) were selected at 1930 h by direct observation of the gonads. The broodstock diet was the same as in the acclimation period.

Sampling

Females of each treatment with ovaries in stage IV were sampled on day 45 and the hemolymph, hepatopancreas, and ovaries extracted for biochemical analysis. Hemolymph (400 µl) was withdrawn from base of the pereiopod of the first abdominal segment using a sterile 1 ml syringe containing a cold shrimp-isotonic solution of SIC-EDTA anticoagulant (NaCl 450 mM, KCl 10 mM, Hepes 10 mM + EDTA-Na2 10 mM, pH 7.3). After hemolymph sampling, the gonad and hepatopancreas were removed from the cephalothorax and dorsal region. These tissues were weighed, placed in a 2.0 ml Eppendorf® tube, immediately frozen with liquid nitrogen, and stored at –80 °C for further biochemical analysis. The hepatosomatic and gonadosomatic indexes were calculated as the percentage of the shrimp body weight.

Throughout the experimental period, selected females with mature ovaries were identified and transferred into a separate 100 l tank (28–29 °C) filled with seawater (salinity of 35 g l^{-1}) treated with UV and EDTA (10 mg l^{-1}) (Emerenciano *et al.*, 2012c). The water was continuously aerated. After spawning, the females were returned to their respective maturation tanks. Half of the spawned eggs were harvested with a 100 µm mesh net, placed in labeled Eppendorf® tubes, and frozen with liquid nitrogen; the samples were then stored at –80 °C. The remaining eggs were transferred to a hatching tank and the nauplii biomass was collected for biochemical analysis. A sample of 30 eggs and nauplii at stage IV from each spawn (when spawns were fertile) were collected for morphological analysis. Egg diameter and the length of nauplii were measured under a light microscope provided with a micrometer.

Reproductive performance

Reproductive performance was evaluated in terms of the number of eggs per spawn ($\times 10^3$), number of eggs ($\times 10^3$) per gram of body weight of the female, total viable nauplii, fertilization rate (%), and hatching rate (%). Eggs and nauplii were estimated from five 4.7 ml replicates collected from the spawning tanks. The percentage of viable nauplii was estimated as the number of nauplii obtained after positive phototropism in relation to the number of eggs spawned. A nauplii-condition index (NCI) was estimated as: NCI = (nauplii AG) × (% viable nauplii) × (nauplii length)/ (100) (Palacios *et al.*, 1998).

Biochemical analysis

Changes in acylglycerides, glucose, and cholesterol were measured using commercial kits for medical diagnosis (Randox©, Tlalnepantla, Mexico) (Hernández-López, 2001; Martínez-Porchas *et al.*, 2013). Total soluble protein was measured, according to Bradford (1976), in hemolymph, ovaries, hepatopancreas, eggs, and nauplii (see Emerenciano *et al.*, 2012b). The hemolymph in the anticoagulant solution was centrifuged at 800 g for 3 min at 4 °C and the supernatant transferred to another Eppendorf ® tube; aliquots of 10 µl for the analysis of acylglycerides, glucose, cholesterol, and total soluble protein were used for each analysis. The ovaries, hepatopancreas, eggs, and nauplii were homogenized

in 500 µl of distilled water for 2 min at 4 °C, and the acylglycerides and cholesterol were measured in aliquots of 10 µl (Emerenciano *et al.*, 2012b). The remaining sample was centrifuged at 800 g for 20 min at 4 °C, and the supernatant was transferred to another Eppendorf ® tube; aliquots of 10 µl were used for the analysis of glucose and total soluble protein (Emerenciano *et al.*, 2012b). All determinations were made with 200 µl of the reactive solution and incubated for 5 min in darkness at 26 °C. Samples were subsequently read in an ELISA reader (Bio-Rad Laboratories, Richmond, CA, USA) at 490 nm for acylglycerides, glucose, and cholesterol, and at 595 nm for total soluble protein. The indexes for acylglycerides/total soluble protein (AG:TSP) and acylglycerides/cholesterol (AG:C) were calculated in ovaries, hepatopancreas, eggs, and nauplii using the metabolite data following specifications given by Palacios *et al.* (1998).

Statistical analysis

Two-way ANOVA followed by a Tukey test for unequal N post-hoc mean comparations were performed to assess significant differences in biochemical composition of hepatopancreas, hemolymph, ovaries, hepatosomatic, and gonadosomatic index, using ablation (A, unilaterally ablated eyestalks and non-ablated eyestalks) and day (D, day 1 and 45) as categorical factors in the model. A Student´s t-test was applied to data on reproductive performance and biochemical composition of eggs and nauplii. All data were previously checked for homogeneity of variance and normality. Statistical analyses were carried out using the RStudio program version 1.1.383 (RStudio Team, 2016) with the Rcmdr package (Fox, 2005).

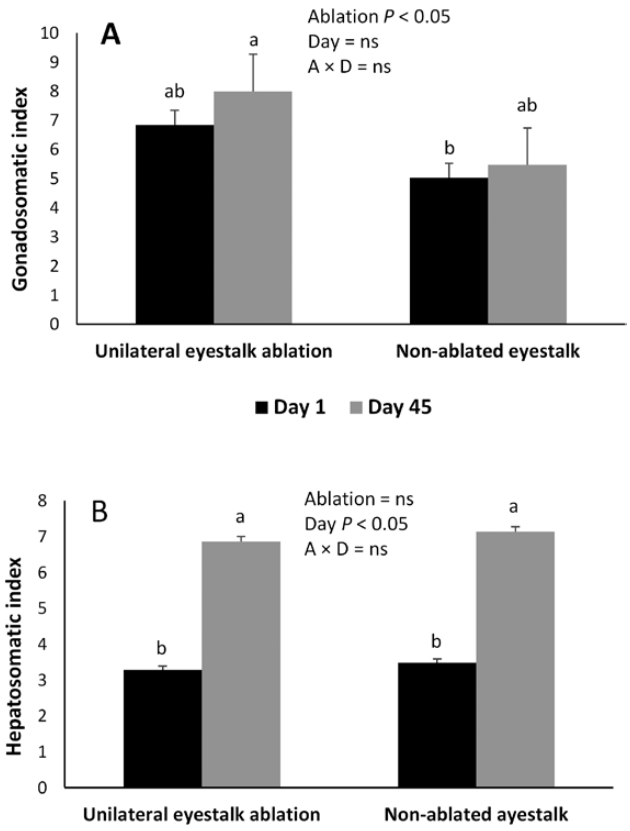

Figure 1. Gonadosomatic (**A**) and hepatosomatic (**B**) indexes (mean ± SE) of females of *Farfantepenaeus brasiliensis* broodstock with unilateral eyestalk ablation and non-ablated eyestalks in a 45-day experimental period. Bi-factorial ANOVA; different letters indicate significant differences (post-hoc Tukey test, $P = 0.05$); ns, not significant.

RESULTS

Reproductive performance

The gonadosomatic index was higher for the unilaterally-ablated-eyestalk spawners than the non-ablated spawners ($P < 0.05$). The hepatosomatic index was higher for day 45 than for day 1 for both treatments ($P < 0.05$; Fig. 1).

A higher mortality was detected in the ablated than in the non-ablated females. No males died during the experimental period in both treatments. Ablated spawners achieved better results ($P < 0.05$) in terms of the number of consecutive maturations per female. Furthermore, the number of females that spawned at least once was higher in the ablated than in the non-ablated treatment. Conversely, the non-ablated spawners had better performance in terms of the fertilization rate and viable nauplii than in the ablated spawners (Table 1). The total viable nauplii produced was highest for the ablated treatment. The weight of the broodstock was similar between treatments ($P > 0.05$). The egg diameter was higher for the ablated than in the non-ablated treatment ($P < 0.05$), but the length of the nauplii was highest in the non-ablated treatment ($P < 0.05$).

Biochemical composition and nauplii-condition index

The highest level of acylglycerides in hepatopancreas was found in non-ablated females at day 45 (Table 2). The lowest levels of cholesterol were found for the ablated and non-ablated females at day 1; the highest concentration was in the non-ablated females at day 45. The AG:TSP ratio was significantly higher ($P < 0.05$) in non-ablated females at day 45.

The highest level of acylglycerides in hemolymph was found in non-ablated females at day 45, and the total soluble protein was the highest in non-ablated females at day 1 ($P < 0.05$; Table 2). The highest level of acylglycerides in ovary was found in ablated females at day 45; the lowest in non-ablated females at day 45 ($P < 0.05$). The highest level of glucose was found in ablated females ($P < 0.05$). The AG:TSP ratio was significantly the highest ($P < 0.05$; Table 2) in the ablated females at day 45.

Table 1. Reproductive performance and morphometric parameters (mean ± SE) of females of *Farfantepenaeus brasiliensis* broodstock with unilaterally ablated and non-ablated eyestalks in a 45-day experimental period. Superscript letters indicate significant differences (Student´s t-test, $P < 0.05$); * stage IV nauplius larva.

Variable	Ablated	Non-ablated
Reproductive performance		
Female mortality (%)	33.3	10
Number of eggs per spawn (×10³)	241.9 ± 13.3	269.21 ± 8.5
Number of eggs per spawn (×10³) per g of spawners body weight	6.0 ± 0.21	6.6 ± 0.34
Number of consecutive maturations per female	3.8^{a} ± 0.52	1.33^{b} ± 0.2
Females that spawned at least once (%)	83.3%	20%
Fertilization rate (%)	45.1^{a} ± 0.9	71.6^{b} ± 4.6
Hatch rate (%)	34.5 ± 5.35	37.4 ± 12.8
Viable nauplii (%)*	14.8^{a} ± 2.9	33.7^{b} ± 8.6
Total viable nauplii /tank (×10³)*	911.5	269.8
Morphometric parameters		
Female weight (g)	40.8 ± 0.6	40.2 ± 0.7
Male weight (g)	24.9 ± 0.5	24.2 ± 0.5
Egg diameter (µm)	249^{a} ± 0.3	240^{b} ± 0.1.4
Nauplii length (µm)	438.3^{a} ± 1.1	448.2^{b} ± 1.4

Eggs from the non-ablated females had higher total soluble protein and AG:C than those form the ablated females ($P < 0.05$), but AG:TSP higher for eggs from ablated females than those from non-ablated females ($P < 0.05$; Table 3). The nauplii-condition index was higher for larvae from non-ablated females than in the ablated females ($P < 0.05$; Fig. 2).

DISCUSSION

This study confirms earlier findings that females with unilaterally ablated eyestalks increase spawn frequency and production of eggs and nauplii in penaeid shrimps (Aktaş & Kumlu, 1999; Browdy & Samocha, 1985; Browdy *et al.*, 1986; Palacios *et al.*, 1999a) but decreases the quality of offspring (Primavera & Posadas, 1981; Chamberlain & Gervais, 1984; Munkongwongsiri *et al.*, 2015). A decrease in larval quality has been observed because the unilateral eyestalk ablation is associated with low fecundity and a low hatch rate in penaeids (Chamberlain & Gervais, 1984; Browdy *et al.*, 1986; Palacios *et al.*, 1999a). Risks associated with vertical transmission of disease are consequently increased (Emerenciano *et al.*, 2012b). New strategies to improve larval quality, such as environmental control, genetics, nutrition, and endocrine manipulation must be tested (Vaca & Alfaro, 2000; Racotta *et al.*, 2003).

The gonadosomatic index was higher in ablated than in non-ablated females (Fig. 1A), a trend previously reported in investigations on the decrease of the gonad-inhibiting hormone causing ovarian growth (Anilkumar & Adiyodi, 1985; Palacios *et al.*, 1999a). The hepatosomatic index was higher at day 45 than at day 1 in both treatments, indicating that females had stored reserves of proteins and lipids to be transported to the ovaries (Fig. 1B). This trend has been reported in wild females of *Farfantepenaeus duorarum* (Burkenroad, 1939), where the hepatosomatic index increases as the number of spawns increases (Emerenciano *et al.*, 2012c). A high-energy demand due to females diverting all their energy to producing more spawns might compromise survival (Zacharia & Kakati, 2002; Racotta *et al.*, 2003). This study confirms that the ablated females had lower survival than non-ablated ones (Table 1).

Non-ablated females produced smaller eggs (Table 1) than those from ablated females, as reported in penaeids such as *Penaeus monodon* (Fabricius, 1798) and *Litopeneaus vannamei* (Boone, 1931) with non-ablated eyes, where oocytes were smaller than those produced by ablated females (Anilkumar & Adiyodi, 1985; Tan-Fermin, 1991; Palacios *et al.*, 1999a). Ablation increased egg size and consecutive maturations in *P. monodon* but decreased the number of eggs per spawn, even when the number of consecutive maturations is higher (Tan-Fermin, 1991). Such a trend occurs in *F. brasiliensis*, where there is a smaller number of eggs, but the ablated females spawn more frequently (Table 1).

The non-ablated females had a higher fertilization rate that ablated females (Table 1). The spawn quality and the subsequent survival of larvae were nevertheless negatively affected when obtained from ablated *L. vannamei* females (Palacios *et al.*, 1999a). Furthermore, the oocytes of non-ablated *L. vannamei* females are reabsorbed before secondary vitellogenesis begins, which could be related to fecundity (Palacios *et al.*, 1999a).

Inversely, the oocytes of ablated *L. vannamei* females develop abruptly, causing malformations or a reduction in the transfer of some energy-rich biomolecules to the eggs (Tan-Fermin, 1991; Palacios *et al.*, 1999a). The total number of viable nauplii produced by ablated females was higher than in the non-ablated females (Table 1), indicating that ablation accelerates the production of nauplii in farms (Browdy & Samocha, 1985; Browdy *et al.*, 1986; Palacios *et al.*, 1999a).

The highest values for total soluble protein and cholesterol were found in non-ablated females at day 45. These biomolecules are involved in the development of eggs and nauplii. The total soluble protein is essential constituent of tissue and cholesterol is an important cell constituent and a component of lipoproteins (e.g., vitellin) involved in acylglyceride transfer into the ovaries of decapods (Babu, 1987; Anger, 2001; Regunathan, 2008; Emerenciano *et al.*, 2012c). Cholesterol and total soluble protein in hemolymph

Table 2. Biochemical analysis (wet weight; mean ± SE) of hepatopancreas, hemolymph, and ovary (stage IV) of females of *Farfantepenaeus brasiliensis* broodstock with unilaterally ablated and non-ablated eyestalks at day 1 and 45. Superscript letters indicate significant differences (post-hoc Tukey test, $P = 0.05$); $^{+}$bi-factorial ANOVA where $*P < 0.05$. TSP, total soluble protein; AG, acylglycerides; C, cholesterol.

Variable	Ablated		Non-ablated		Significant level^{+}		
	Day 1	Day 45	Day 1	Day 45	Ablation	Day	*A* × *D*
Hepatopancreas	(*N* = 9)	(*N* = 6)	(*N* = 8)	(*N* = 4)			
Acylglycerides (mg g^{-1})	49.0^{b} ± 7.5	35.7^{b} ± 10.6	43.5^{b} ± 11.5	93.6^{a} ± 11.8	*	ns	*
Cholesterol (mg g^{-1})	1.8^{b} ± 0.5	3.1^{ab} ± 0.6	1.4^{b} ± 0.5	5.3^{a} ± 0.8	ns	*	*
Glucose (mg g^{-1})	2.5^{a} ± 0.3	2.9^{a} ± 0.4	2.4^{a} ± 0.3	2.0^{a} ± 0.5	ns	ns	ns
Total soluble protein (mg g^{-1})	20.7^{a} ± 1.5	26.2^{a} ± 1.8	20.8^{a} ± 1.6	23.9^{a} ± 2.5	ns	ns	ns
AG: TSP	2.5^{b} ± 0.4	1.6^{b} ± 0.5	2.0^{b} ± 0.2	3.3^{a} ± 0.6	*	ns	*
AG: C	34.0 ± 5.6	16.7 ± 6.8	23.1 ± 7.4	17.0 ± 8.3	ns	ns	ns
Hemolymph	(*N* = 4)	(*N* = 6)	(*N* = 6)	(*N* = 4)			
Acylglycerides (mg ml^{-1})	83.4 ± 0.7	158.2 ± 39.8	88.2 ± 13.6	94.2 ± 44.5	ns	ns	ns
Cholesterol (mg ml^{-1})	20.3^{b} ± 6.9	24.3^{b} ± 5.7	43.3^{ab} ± 4.3	50.3^{a} ± 6.4	*	ns	ns
Glucose (mg ml^{-1})	32.4^{a} ± 3.4	33.6^{a} ± 5.7	32.3^{a} ± 6.9	35.0^{a} ± 6.4	ns	ns	ns
Total soluble protein (mg ml^{-1})	68.7^{b} ± 22.5	103.1^{ab} ± 26.2	212.7^{a} ± 37.7	122.4^{ab} ± 30.2	*	ns	*
Ovary	(*N* = 10)	(*N* = 6)	(*N* = 8)	(*N* = 4)			
Acylglycerides (mg g^{-1})	6.3^{ab} ± 0.5	7.4^{a} ± 0.7	5.3^{ab} ± 0.6	4.2^{b} ± 0.8	*	ns	ns
Cholesterol (mg g^{-1})	1.6^{a} ± 0.3	1.0^{a} ± 0.4	1.8^{a} ± 0.3	2.1^{a} ± 0.5	ns	ns	ns
Glucose (mg g^{-1})	1.5^{b} ± 0.2	2.8^{a} ± 0.2	1.3^{b} ± 0.2	0.9^{b} ± 0.3	*	ns	*
Total soluble protein (mg g^{-1})	45.5 ± 4.1	49.1 ± 5.3	45.2 ± 4.6	53.4 ± 6.5	ns	ns	ns
AG:TSP	0.2^{b} ± 0.1	1.4^{a} ± 0.1	0.2^{b} ± 0.1	0.4^{b} ± 0.2	*	*	*
AG:C	3.8 ± 0.7	3.3 ± 0.9	3.3 ± 0.7	2.4 ± 0.6	ns	ns	ns

Table 3. Biochemical analysis (wet weight; mean ± SE) of the eggs and nauplii (stage IV) of females of *Farfantepenaeus brasiliensis* broodstock with unilaterally ablated and non-ablated eyestalks. Superscript letters indicate significant differences (Student´s t-test, $P < 0.05$). TSP, total soluble protein; AG, acylglycerides; C, cholesterol.

Variable	Ablated	Non-ablated
Eggs	(*N* = 27)	(*N* = 4)
Acylglycerides (mg g^{-1})	9.3 ± 0.3	8.3 ± 1.1
Cholesterol (mg g^{-1})	1.6 ± 0.0	1.3 ± 0.1
Glucose (mg g^{-1})	2.9 ± 0.2	2.3 ± 0.2
Total soluble protein (mg g^{-1})	32.0^{a} ± 3.0	56.1^{b} ± 10.0
AG:TSP	0.4^{a} ± 0.0	0.1^{b} ± 0.0
AG:C	2.5^{a} ± 0.1	4.7^{b} ± 0.1
Nauplii	(*N* = 18)	(*N* = 4)
Acylglycerides (mg g^{-1})	3.7 ± 0.5	2.4 ± 0.4
Cholesterol (mg g^{-1})	2.3 ± 0.2	1.7 ± 0.2
Glucose (mg g^{-1})	3.6 ± 0.4	2.5 ± 0.3
Total soluble protein (mg g^{-1})	27.6 ± 2.2	19.2 ± 1.8
AG:TSP	0.1 ± 0.0	0.1 ± 0.0
AG:C	0.3 ± 0.1	0.1 ± 0.0

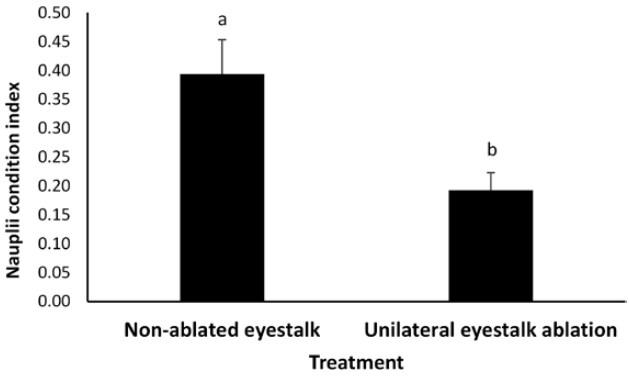

Figure 2. Nauplii-condition index (mean + SE) for the nauplii of females of *Farfantepenaeus brasiliensis* broodstock with unilateral eyestalk ablation and non-ablated eyestalks in a 45-day experimental period. Letters indicate significant differences by Student´s t-test ($P < 0.05$).

were highest in the non-ablated females at days 45 and 1, respectively, indicating a higher transport of these nutrients to the ovaries for the development of eggs.

The acceleration of metabolism in ablated females was also evident in the acylgliceride content in ovary (Table 2); however, there were no differences in the quantities of eggs and nauplii in both treatments.

Glucose was highest in ablated females at day 45, indicating a rapid transfer to the eggs. Glucose is essential for larval survival, but the source of glucose in eggs remains unknown although it could be a byproduct of the metabolism of carbohydrates and triglycerides (Palacios *et al.*, 1998). The AG:TSP is used as a bioenergetic-condition index and could be used to calculate the amount of energy in eggs and nauplii. The highest AG:TSP was found in the ovary of the non-ablated females at day 45, with no differences when compared to the ratio at day 1, indicating that females maintain constant the transfer of nutrients to the eggs in contrast to the ablated females (Table 2).

The total soluble protein and the AG:C of eggs were higher in non-ablated females than in ablated females. Conversely, the AG:TSP was higher in eggs from ablated females than from non-ablated females. This indicates that energy reserves in the eggs change when the females are ablated, with important implications on the number of viable nauplii, which is highest for the non-ablated females. A higher proportion, not quantity, of the main energy biomolecules (AG:TSP and AG:C) is vital for the quality of the offspring (Anger, 2001; Regunathan, 2008; Emerenciano *et al.*, 2012b). Furthermore, the length of nauplii from non-ablated females was higher than in ablated females (Table 1), which is related to the highest concentration of total soluble protein of eggs (Table 2). This finding could be related to the offspring viability because larger nauplii could improve the chance of ingesting large food items by the protozoea I, when exogenous feeding begins (Anger, 2001).

The nauplii-condition index is an indicator that summarizes the use of acylglycerides as energy source through the ontogeny, length, and hatching rate of the larvae; therefore, it is a predictor of the viability of the spawns (Palacios *et al.*, 1998; Regunathan, 2008). We obtained a higher index in the non-ablated than in the ablated females (Fig. 2) in spite that the total number of nauplii was higher in the ablated condition (Table 1). These results confirm that the unilateral eyestalk ablation affects not only the broodstock survival, but the offspring quality as well.

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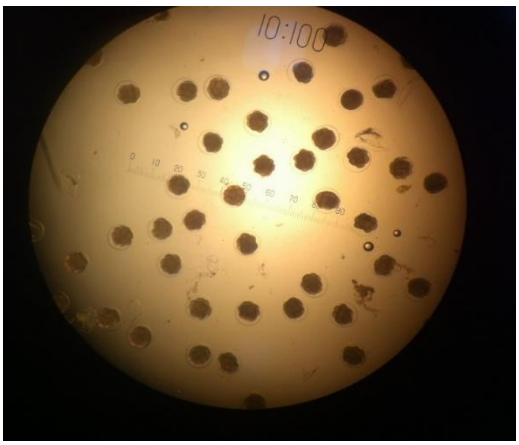
CAPÍTULO VI

Artículo V

Does biofloc improve the production of quality eggs in *Litopenaeus vannamei* (Boone, 1931) (Decapoda: Dendrobranchiata: Penaeidae) without unilateral eyestalk ablation?

Sometido: Journal of Crustacean Biology



Sometido a: Journal of crustacean biology

Does biofloc improve the production of quality eggs in *Litopenaeus vannamei* (Boone, 1931) (Decapoda: Dendrobranchiata: Penaeidae) without unilateral eyestalk ablation?

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Resumen

El objetivo de este estudio fue evaluar la producción y calidad de huevos de hembras de *L. vannamei* (Boone, 1931) con y sin ablación unilateral del pedúnculo ocular. Se utilizaron dos condiciones experimentales, biofloc y agua clara, donde se probó el desempeño reproductivo de los reproductores con y sin ablación de ambos orígenes. Los análisis bioquímicos de acilglicéridos, colesterol, glucosa y proteína total soluble fueron llevados a cabo en hepatopáncreas, ovarios, hemolinfa y huevos. Las hembras de origen biofloc mejoraron el número de huevos en términos huevos por desove y huevos por desove por gramo de hembra reproductora comparados con aquellas de origen agua clara. La ablación incremento la tasa de mortalidad en agua clara comparado a aquellas hembras de biofloc. En términos de la composición bioquímica, los reproductores con y sin ablación de origen biofloc mostraron una mayor concentración de reservas en el hepatopáncreas comparado con reproductores de agua clara. En los ovarios, un mayor contenido de glucosa fue encontrado en organismos con ablación. Los huevos de origen biofloc con y sin ablación tuvieron una mayor concentración de acilglicéridos, colesterol y glucosa; estos resultados indican una mejor calidad de huevos y más reservas nutricionales asociadas con la condición fisiológica de los reproductores de biofloc.

Abstract

The aim of this study was to evaluate the production and quality of eggs from *Litopenaeus vannamei* (Boone, 1931) females with or without unilateral eyestalk ablation. Two experimental conditions, biofloc or clear-water systems, were used to test the reproductive performance of ablated and non-ablated broodstock from both origins. Biochemical analyses of acylglycerides, cholesterol, glucose and total soluble protein were performed in

the hepatopancreas, ovaries, hemolymph and eggs. Females from biofloc improved the number of egg released in terms of eggs per spawn and eggs per spawn per gram of spawner´s body weight compared with that of clear-water broodstock. Ablation increased the mortality rate in clear-water compared to that in biofloc females. In terms of the biochemical composition, biofloc broodstock with or without ablation showed a higher content of hepatopancreas reserves compared to that of clear-water broodstock. In the ovaries, a higher content of glucose was found with ablation. Eggs from biofloc origin with or without ablation had the highest concentration of acylglycerides, cholesterol and glucose; these results indicated a better egg quality and more nutritional reserves were associated with the physiological condition of the broodstock in biofloc.

Introducción

L. vannamei es la especie de cultivo más importante en las Américas. Para su reproducción, la ablación unilateral del pedúnculo ocular ha sido usada. Sin embargo, esta práctica ha sido asociada con altas mortalidades de los reproductores, pobre calidad de la progenie, y con maltrato animal.

Por lo anterior, es importante llevar a cabo estudios que demuestren el efecto de la ablación sobre la progenie de los camarones peneidos. Ya que larvas de mala calidad pueden incrementar el riesgo de infección vertical en las granjas. Con el fin de evaluar esto, una serie de indicadores reproductivos, morfométricos y bioquímicos fueron evaluados.

Objetivo de estudio:

Evaluar el efecto de la ablación unilateral del pedúnculo ocular aplicada a *L. vannamei* proveniente de sistemas de agua clara y biofloc en relación a la calidad de huevos, mediante el uso de indicadores productivos, morfométricos y bioquímicos.

Journal of Crustacean Biology

Does biofloc improve the production of quality eggs in Litopenaeus vannamei (Boone, 1931) (Decapoda: Dendrobranchiata: Penaeidae) without unilateral eyestalk ablation?

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Abstract:	The aim of this study was to evaluate the production and quality of eggs from Litopenaeus vannamei (Boone, 1931) females with or without unilateral eyestalk ablation. Two experimental conditions, biofloc or clear-water systems, were used to test the reproductive performance of ablated and non-ablated broodstock from both origins. Biochemical analyses of acylglycerides, cholesterol, glucose and total soluble protein were performed in the hepatopancreas, ovaries, hemolymph and eggs. Females from biofloc improved the number of egg released in terms of eggs per spawn and eggs per spawn per gram of spawner´s body weight compared with that of clear-water broodstock. Ablation increased the mortality rate in clear-water compared to that in biofloc females. In terms of the biochemical composition, biofloc broodstock with or without ablation showed a higher content of hepatopancreas reserves compared to that of clear-water broodstock. In the ovaries, a higher content of glucose was found with ablation. Eggs from biofloc origin with or without ablation had the highest concentration of acylglycerides, cholesterol and glucose; these results indicated a better egg quality and more nutritional reserves were associated with the physiological condition of the broodstock in biofloc.
Keywords:	aquaculture, broodstock, clear-water systems, shrimp
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Does biofloc improve the production of quality eggs in *Litopenaeus vannamei* (Boone, 1931) (Decapoda: Dendrobranchiata: Penaeidae) without unilateral eyestalk ablation?

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ABSTRACT

The aim of this study was to evaluate the production and quality of eggs from *Litopenaeus vannamei* (Boone, 1931) females with or without unilateral eyestalk ablation. Two experimental

conditions, biofloc or clear-water systems, were used to test the reproductive performance of ablated and non-ablated broodstock from both origins. Biochemical analyses of acylglycerides, cholesterol, glucose and total soluble protein were performed in the hepatopancreas, ovaries, hemolymph and eggs. Females from biofloc improved the number of egg released in terms of eggs per spawn and eggs per spawn per gram of spawner´s body weight compared with that of clear-water broodstock. Ablation increased the mortality rate in clear-water compared to that in biofloc females. In terms of the biochemical composition, biofloc broodstock with or without ablation showed a higher content of hepatopancreas reserves compared to that of clear-water broodstock. In the ovaries, a higher content of glucose was found with ablation. Eggs from biofloc origin with or without ablation had the highest concentration of acylglycerides, cholesterol and glucose; these results indicated a better egg quality and more nutritional reserves were associated with the physiological condition of the broodstock in biofloc.

Key Words: aquaculture, broodstock, clear-water systems, shrimp

INTRODUCTION

Biofloc technology can serve as a pre-maturation medium with minimal water exchange, and it improves the nutritional condition of peneid shrimps while stimulating ovary development and nutrient transfer to the eggs (Chim *et al*., 2014; Magaña-Gallegos *et al*., 2018c). In shrimp aquaculture, unilateral eyestalk ablation has been adopted as a tool to accelerate secondary vitellogenesis of females, leading to predictable peaks of maturation and spawning (Vaca & Alfaro, 2000; Swetha *et al*., 2011). Nevertheless, this practice, associated with high broodstock

mortalities and poor quality eggs, is under criticism by consumers due to animal welfare concerns (Munkongwongsiri *et al*., 2015).

The quality of eggs depends on broodstock origin, nutrition and endocrine manipulation, and egg quality is of particular interest for aquaculturists for progeny viability (Magaña-Gallegos *et al*., 2018a). Acylglycerides, total soluble protein, cholesterol and glucose are important biochemical indicators that support the energy and structural needs of progeny (Palacios *et al*., 1998; Anger, 2001; Emerenciano *et al*., 2012b, 2013). Egg diameter has been shown to be an indicator of quality and of reserve levels (Racotta *et al*., 2003). These criteria, however, have not been studied in ablated or non-ablated female shrimp from biofloc or clear-water systems.

Additionally, it is not clear whether biofloc can significantly improve the nutritional history (Cardona *et al*., 2016; Magaña-Gallegos *et al*., 2018c), egg production and egg quality of broodstock compared to clear-water conditions and whether ablation affects egg quality. The aim of this study was to evaluate the quantity and quality of eggs from ablated and non-ablated *L. vannamei* from biofloc or clear-water conditions.

MATERIALS AND METHODS

Shrimp origin

L. vannamei breeders were conditioned at UMDI Sisal FC-UNAM located at Sisal Beach (21°09′5N and 90°02′5W), Yucatán, Mexico. After spawning, larvae were reared to postlarvae as in Treece & Yates (1990) to reach 1 g. The juveniles were transferred to eight outdoor circular grow-out tanks, each with a 20,000 l capacity (four in biofloc and four in clear-water), under constant aeration through a plastic tube using a 5-hp blower. *L. vannamei* broodstock production followed the methodology reported by Emerenciano *et al*. (2013). Stocking density varied from

100 at the beginning (month 1-6) to 50 shrimp m^{-2} (month 6-11) until the harvest. At the end the grow-out period, the preadults were selected according to aspect integrity (Braga *et al*., 2015) and restocked at a density of 40 shrimp m^{-2} in one biofloc tank and one clear-water tank. Filtered saltwater in the biofloc tank was adjusted to compensate for evaporation but did not exceed 0.5% daily exchange. For clear-water tanks, there was a 100% daily replacement of water (Emerenciano *et al*., 2012a). During the grow-out (month 1-11) and pre-maturation phase (month 11-12), biofloc was maintained at C/N 20/1 by a daily addition of sugarcane molasses (49% of carbon) until the settling solids measured with Imhoff cones reached 5 ml l^{-1}. When settling solids fell below 5 ml l^{-1} or total ammonia nitrogen reached 1 mg l^{-1}, carbon addition resumed (Emerenciano *et al*., 2013; Serra *et al*., 2015; Magaña-Gallegos *et al*., 2018b). For the grow-out phase, shrimp were fed five times per day at 2400, 0400, 0900, 1400 and 2000 h, using a commercial feed (Api-Camaron, Malta Cleyton®, 35% crude protein and 9% crude lipid, Culiacan, Sinaloa, Mexico). Throughout the pre-maturation phase, the same feeding schedule was applied but with an addition of squid at 3% of the biomass of each tank at 2000 h (Emerenciano *et al*., 2013). During the grow-out and pre-maturation phases, water quality parameters were monitored and were maintained within an acceptable range for shrimp culture (Van Wyk & Scarpa, 1999). At the end of month 12, shrimp were sampled, selected by morphological integrity, and transferred to a maturation room.

Experimental design

Four 30-day treatments were designed for ablated and non-ablated shrimp of biofloc and clear-water origins. Each treatment involved a round, lined maturation tank, 4.0 m in diameter, connected to a water-recirculation system with a chiller Delta Star DSHP-9 (Aqualogic, San Diego, CA, USA) for temperature control and continuous aeration; the daily water exchange rate

was set at 200%, 28 ± 1 °C, salinity 35 g l^{-1}, pH 8.1 ± 0.1, and dissolved oxygen 6.1 ± 0.2 mg l^{-1}. Water quality parameters remained within an acceptable range for shrimp culture (Van Wyk & Scarpa, 1999). Groups of 37 females and 37 males were used (female to male ratio, 1:1; density, 5.8 shrimp m^{-2}) in the tanks with non-ablated shrimp, while groups of 20 females and 20 males were used (female to male ratio, 1:1; density, 3.2 shrimp m^{-2}) in the tanks with ablated shrimp. We used more non-ablated than ablated females because we expected the non-ablated shrimp to spawn less frequently (Palacios *et al*., 1999). All females were tagged with an elastomer on different segments to identify them and their spawn (Emerenciano *et al*., 2013) and to limit stress unilateral eyestalk ablation was preformed after tagging. Daily changes in ovarian development and behavior were recorded. Females with mature ovaries (Guitart & Quintana, 1978) were selected at 1930 h by directly observing the gonads. Fresh food was distributed at 20% of the biomass of each tank at a ratio 1:1:1:1 of *Artemia* biomass, polychaetes, squid, and mussels at 0800, 1200, 1600 and 2000 h (Magaña-Gallegos *et al*., 2018c); semi-moist feed (51.1% crude protein, 14.4% crude lipid) was provided at 3% of tank biomass at 2400 h (Ortiz-Gullén, 2015).

Sampling

Ovaries in stage IV (Guitart & Quintana, 1978) were sampled on day 30, and the hemolymph, hepatopancreas and ovaries were extracted for biochemical analysis. The hemolymph (400 µl) was withdrawn from the base of the pereiopod of the first abdominal segment using a sterile 1 ml syringe containing a cold, shrimp-isotonic solution of SIC-EDTA anticoagulant (NaCl 450 mM, KCl 10 mM, Hepes 10 mM + EDTA-Na2 10 mM, pH 7.3). The gonads and hepatopancreas were removed from the cephalothorax and dorsal region, respectively. These tissues were weighed, placed in a 5.0 ml tube, immediately frozen with liquid nitrogen, and stored at –80 °C for further

biochemical analysis. The hepatosomatic and gonadosomatic indexes were calculated as percentage of shrimp body weight.

Throughout the experimental period, select females with mature ovaries were transferred into a separate 100 l tank (28–29 °C) filled with seawater (salinity 35 g l^{-1}) and treated with UV and EDTA (10 mg l^{-1}) according to Emerenciano *et al*. (2012c). The water was continuously aerated. After spawning, females were returned to their respective maturation tanks. Spawned eggs were harvested by a 100 µm mesh net, placed in labeled Eppendorf® tubes, and frozen with liquid nitrogen; samples were then stored at –80 °C until analysis. A sample of 30 eggs from each spawn was collected for morphological analysis. The eggs diameter were measured under a light microscope provided with a micrometer.

Reproductive performance

Reproductive performance was evaluated in terms of the number of eggs per spawn ($\times 10^3$), number of eggs per spawn ($\times 10^3$) per gram of spawner´s body weight, female mortality (%), number of consecutive maturations per female, and females that spawned at least once (%). Egg numbers were estimated from five replicates of 4.7 ml of liquid collected from the spawning tanks.

Biochemical analysis

Changes in acylglycerides, cholesterol and glucose were measured using commercial kits (Kits Elitech Diagnostics, Sees, France; cat. TGML-0427, cat. CHSL-0507 and GPSL-5505, respectively) following previously described procedures (Emerenciano *et al*., 2012b, 2013). Total soluble protein was measured, according to Bradford (1976), in the hemolymph, ovaries, hepatopancreas and eggs (Emerenciano *et al*., 2012b). A solution of hemolymph plus

anticoagulant solution was centrifuged at 800 g for 3 min at 4 °C, and the supernatant was transferred to another Eppendorf ® tube; 10 µl aliquots were used for acylglycerides, glucose, cholesterol, and total soluble protein analyses. Ovaries, hepatopancreas and eggs were homogenized in 500 µl of distilled water for 2 min at 4 °C, and acylglycerides and cholesterol were measured using 10 µl aliquots (Emerenciano *et al*., 2012b). The remaining sample was centrifuged at 800 g for 20 min at 4 °C, and the supernatant was transferred to another Eppendorf ® tube; 10 µl aliquots were used for glucose and total soluble protein analyses (Emerenciano *et al*., 2013). All determinations were made with 200 µl of the reactive solution incubated in the dark for 5 min at 26 °C. Samples were subsequently read in an ELISA reader (Bio-Rad Laboratories, Richmond, CA, USA) at 500 nm for acylglycerides, glucose, and cholesterol, and at 595 nm for total soluble protein. The indexes for acylglycerides/total soluble protein (AG:TSP) and acylglycerides/cholesterol (AG:C) were calculated for the ovaries, hepatopancreas and eggs using previous recommendations (Palacios *et al*., 1998).

Statistical analysis

Two-way ANOVA followed by a Tukey test for unequal N post-hoc mean comparisons was performed to assess significant differences in the biochemical composition of the hepatopancreas, hemolymph, and ovaries, the hepatosomatic and gonadosomatic indexes, reproductive performance and biochemical composition, using ablation (A, unilaterally ablated-eyestalks and non-ablated eyestalks) and origin (O, clear-water and biofloc) as categorical factors in the model. All data were previously checked for homogeneity of variance and normality. Statistical analyses were carried out using the RStudio program version 1.1.383 (RStudio Team, 2016) with the Rcmdr package (Fox, 2005).

RESULTS

Reproductive performance

A higher mortality rate was detected for the unilaterally ablated eyestalk spawners compared to the non-ablated spawners. Compared to non-ablated females, ablated females achieved better results in terms of the number of females that spawned at least once (Table 1). The ablated biofloc spawners produced a higher number of eggs than ablated and non-ablated clear-water spawners ($P < 0.05$). Non-ablated spawners from biofloc or clear-water produced more eggs than ablated spawners in clear-water ($P < 0.05$; Table 1). The number of consecutive maturations was higher for ablated biofloc spawners than clear-water and non-ablated biofloc spawners. During the experiment, non-ablated females spawned more than one time (Table 1).

<Table 1>

Non-ablated spawners produced larger eggs than those of ablated spawners ($P < 0.05$; Fig. 1), but eggs from clear-water origin were larger than those of biofloc origin ($P < 0.05$; Fig. 1).

<Fig. 1>

Biochemical composition

In the hepatopancreas, the highest concentrations of acylglycerides, cholesterol and total soluble protein occurred with non-ablated biofloc spawners ($P < 0.05$). The AG:TSP and AG:C indexes were higher in the ablated and non-ablated biofloc spawners than clear-water spawners ($P < 0.05$; Table 2). In the hemolymph, acylglycerides were higher in ablated biofloc spawners than non-ablated biofloc and clear-water spawners. In the ovaries, the highest concentration of glucose was found with ablated biofloc spawners, while the lowest concentration was found in non-ablated

biofloc spawners. There was a higher concentration of acylglycerides in the eggs produced by biofloc females compared to clear-water females. The AG:TSP and AG:C indexes were higher for eggs of biofloc origin than clear-water origin.

<Table 2>

DISCUSSION

This study confirmed that biofloc conditions affected the nutritional history of broodstock, improving the number and quality of peneid shrimp eggs (Emerenciano *et al*., 2012b, 2014; Chim *et al*., 2014). Unilateral eyestalk ablation, however, increased the percentage of females that spawn (Browdy & Samocha, 1985; Palacios *et al*., 1999; Magaña-Gallegos *et al*., 2018a) but decreased egg quality in terms of the main biomolecules that support the energetic and structural needs of the progeny (Magaña-Gallegos *et al*., 2018a). For this reason, new strategies to improve egg quality must be proven to reduce the risks associated with vertical transmission diseases (Vaca & Alfaro, 2000; Emerenciano *et al*., 2012b).

Female mortality has been associated with ablation (Zacharia & Kakati, 2002; Magaña-Gallegos *et al*., 2018a) because ablated shrimp use their energy sources for reproduction more than maintenance, causing exhaustion (Rosas *et al*., 1993; Palacios *et al*., 1998). In fact, a higher rate of mortality occurred with ablated females regardless of origin (Table 1).

Egg number per spawn helped to evaluate the potential of a shrimp broodstock; clear-water ablated shrimp produced fewer eggs per spawn than ablated biofloc shrimp (Table 1). This result suggests that broodstock origin has an effect on nutritional history. Biofloc provided highly valued nutrients, such as proteins, free amino acids, lipids and vitamins, which could cause pre-breeders and broodstock to exhibit a better nutritional status compared to shrimp cultured in

clear-water (Cardona *et al*., 2016; Magaña-Gallegos *et al*., 2018c). In this experiment, more than one maturation occurred for non-ablated females of biofloc and clear-water origin (Table 1). This phenomenon is interesting because in other works it has been very hard to obtain more than two consecutive spawns for non-ablated female (Aktaş & Kumlu, 1999; Palacios *et al*., 1999). The reason for more than one maturation occurring for non-ablated females could be related to the nutritional history, broodstock management and the diet used prior to and during reproduction.

Eggs from biofloc origin were smaller than those from clear-water (Fig. 1), which is related to the higher number of eggs released by those females (Emerenciano *et al*., 2013). Eggs from non-ablated spawners were larger than eggs from ablated spawners (Fig. 1), indicating that they could contain more reserves. Egg size (as volume or diameter) is an indicator of the yolk content, in terms of reserves (Racotta *et al*., 2003).

Non-ablated biofloc spawners had a higher concentration of acylglycerides, cholesterol and total soluble protein in their hepatopancreas, which is related to their capacity to survive and produce larger eggs than the other treatments (Table 2). In fact, biofloc spawners had better condition and bioenergetics indexes (AG:TSP and AG:C) (Anger, 2001) than clear-water spawners. The concentration of acylglycerides in the hemolymph increased for ablated biofloc and non-ablated clear-water spawners, indicating a greater transport of this nutrient from the ovaries to eggs. Ovaries, however, did not show this trend (Table 2).The level of glucose was highest in ovaries of ablated biofloc spawners, which could be related to an intense need to meet energy requirements for egg production or be a by-product of carbohydrate and triglyceride metabolism (Palacios *et al*., 1998).

The biochemical composition of eggs, particularly of yolk components that support the energetic and structural needs for further larval performance, has been previously indicated as a

predictor of egg quality in *L. vannamei* (Palacios & Racotta, 1999; Palacios *et al*., 2001; Racotta *et al*., 2003). Eggs from biofloc origin with or without ablation had the highest concentration of acylglycerides, cholesterol and glucose, indicating more nutritional reserves associated with optimal physiological conditions for broodstock in biofloc.

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Figure 1. Egg size (mean ± SE) of females of a *Litopenaeus vannamei* broodstock with unilaterally ablated and non-ablated eyestalks from clear-water or biofloc origin during a 30-day experimental period. Bi-factorial ANOVA; different letters indicate significant differences (post-hoc Tukey test, $P = 0.05$.

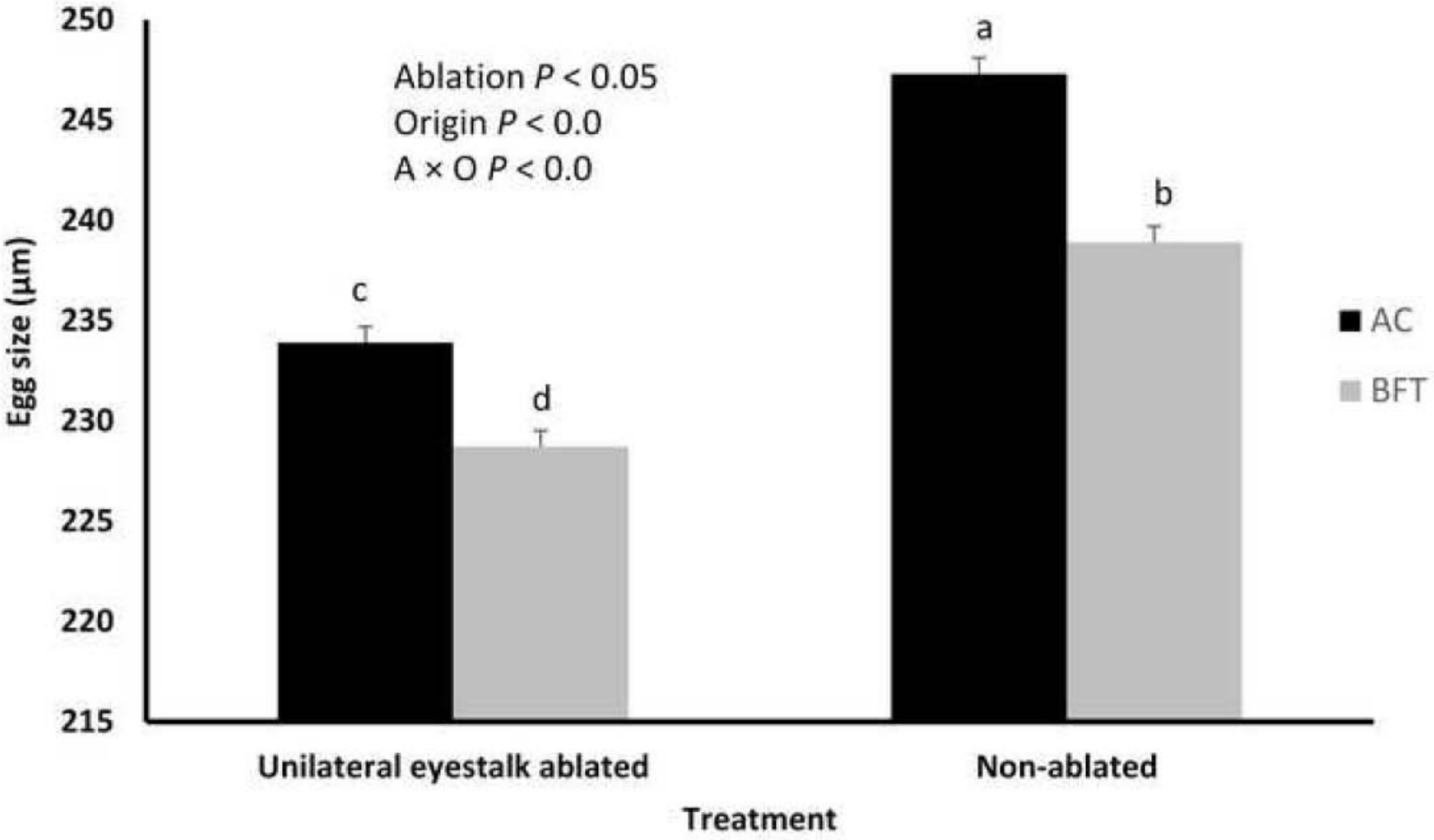
Table 2 Biochemical analyses (mean ± SE) of the hepatopancreas, hemolymph, ovary (stage IV) and eggs of females of a *Litopenaeus vannamei* broodstock with unilaterally ablated and non-ablated eyestalks from clear-water or biofloc origin during a 30-day experimental period. Superscript letters within rows indicate significant differences (post-hoc Tukey test, $P = 0.05$); $^{+}$bi-factorial ANOVA where $*P < 0.05$. TSP, total soluble protein; AG, acylglycerides; C, cholesterol. ns = not significant.

	Clear-water		Biofloc		Significant level^{+}		
Variable	Ablated	Non-ablated	Ablated	Non-ablated	Origin	Ablation	$O \times A$
Hepatopancreas	(N = 8)	(N = 7)	(N = 7)	(N = 5)			
Acylglycerides (mg g^{-1})	$35.4^{b} \pm 4.7$	$27.3^{b} \pm 5.0$	$38.6^{ab} \pm 5.4$	$57.0^{a} \pm 6.0$	*	ns	*
Cholesterol (mg g^{-1})	$8.0^{ab} \pm 0.7$	$6.7^{b} \pm 0.7$	$6.9^{b} \pm 0.7$	$10.4^{a} \pm 0.9$	ns	ns	*
Glucose (mg g^{-1})	3.0 ± 0.3	2.5 ±0.4	2.6 ± 0.4	3.2 ± 0.5	ns	ns	ns
T. soluble protein (mg g^{-1})	$37.7^{ab} \pm 1.9$	$37.1^{ab} \pm 1.8$	$34.0^{b} \pm 2.1$	$43.6^{a} \pm 2.3$	ns	*	*
AG:TSP	$0.7^{b} \pm 0.1$	$0.7^{b} \pm 0.1$	$1.1^{ab} \pm 0.1$	$1.4^{a} \pm 0.1$	*	ns	ns
AG:C	$4.3^{ab} \pm 0.5$	$4.3^{ab} \pm 0.6$	$4.3^{ab} \pm 0.7$	$7.1^{a} \pm 0.7$	*	*	*
Hemolymph	(N = 8)	(N = 7)	(N = 4)	(N = 4)			
Acylglycerides (mg dL^{-1})	$73.7^{c} \pm 9.6$	$82.5^{abc} \pm 10.0$	$116.0^{ab} \pm 15.0$	$50.0^{c} \pm 10.0$	ns	*	*
Cholesterol (mg dL^{-1})	45.3 ± 5.4	47.0 ± 5.8	49.9 ± 8.9	31.0 ± 8.9	ns	ns	ns
Glucose (mg dL^{-1})	22.6 ± 4.7	30.7 ± 5.0	27.9 ± 7.0	19.3 ± 5.7	ns	ns	ns
T. soluble protein (mg mL^{-1})	50.9 ± 6.3	60.3 ± 6.3	65.3 ± 8.2	55.9 ± 8.2	ns	ns	ns
Ovary	(N = 8)	(N = 7)	(N = 7)	(N = 5)			
Acylglycerides (mg g^{-1})	11.4 ± 0.8	11.1 ± 0.8	11.0 ± 0.9	10.7 ± 0.9	ns	ns	ns
Cholesterol (mg g^{-1})	6.2 ± 0.4	6.2 ± 0.4	7.5 ± 0.5	6.6 ± 0.5	ns	ns	ns
Glucose (mg g^{-1})	$4.6^{ab} \pm 0.9$	$2.6^{b} \pm 0.9$	$6.6^{a} \pm 0.9$	$1.2^{b} \pm 0.9$	ns	*	ns
T. soluble protein (mg g^{-1})	76.3 ± 3.8	72.8 ± 3.7	80.5 ± 4.1	76.9 ± 4.1	ns	ns	ns
AG:TSP	0.2 ± 0.0	0.2 ± 0.0	0.3 ± 0.0	0.1 ± 0.0	ns	ns	ns
AG:C	1.8 ± 0.0	1.7 ± 0.0	1.7 ± 0.0	1.7 ± 0.0	ns	ns	ns

Eggs	(*N* = 11)	(*N* = 11)	(*N* = 12)	(*N* = 8)			
Acylglycerides (mg g^{-1})	$22.8^{a} \pm 0.9$	$17.7^{b} \pm 0.9$	$21.3^{ab} \pm 0.9$	$23.9^{a} \pm 1.2$	*	ns	*
Cholesterol (mg g^{-1})	$9.3^{a} \pm 0.4$	$8.0^{b} \pm 0.4$	$8.4^{a} \pm 0.4$	$9.3^{a} \pm 0.5$	ns	ns	*
Glucose (mg g^{-1})	$0.7^{ab} \pm 0.1$	$0.6^{b} \pm 0.1$	$0.6^{b} \pm 0.1$	$1.0^{a} \pm 0.1$	ns	ns	*
T. soluble protein (mg g^{-1})	49.8 ± 2.8	46.5 ± 2.7	46.0 ± 2.6	48.1 ± 3.3	ns	ns	ns
AG:TSP	$0.5^{a} \pm 0.0$	$0.4^{b} \pm 0.0$	$0.5^{ab} \pm 0.0$	$0.6^{a} \pm 0.0$	ns	ns	*
AG:C	$2.5^{ab} \pm 0.1$	$2.2^{b} \pm 0.1$	$2.7^{a} \pm 0.1$	$2.6^{ab} \pm 0.1$	ns	*	ns

Table 1 Reproductive performance (mean ± SE) of females of a *Litopenaeus vannamei* broodstock with unilaterally ablated and non-ablated eyestalks from clear-water or biofloc origin during a 30-day experimental period. Superscript letters within rows indicate significant differences (post-hoc Tukey test, $P = 0.05$); $^{+}$bi-factorial ANOVA where $*P < 0.05$. ns = not significant. na = does not apply.

	Clear-water		Biofloc		Significant level^{+}		
Variable	Ablated	Non-Ablated	Ablated	Non-Ablated	Ablation	Origin	*A* × *O*
Reproductive parameters							
Female mortality (%)	5	2.7	10	0	na	na	na
Number of eggs per spawn ($\times 10^3$)	$99.9^{b} \pm 11.1$	$127.1^{ab} \pm 10.4$	$149.3^{a} \pm 9.1$	$128.5^{ab} \pm 16.1$	*	ns	ns
Number of eggs per spawn ($\times 10^3$) per g of spawner´s body weight	$2.2^{b} \pm 0.3$	$2.9^{ab} \pm 0.2$	$3.6^{a} \pm 0.2$	$3.2^{ab} \pm 0.4$	*	ns	ns
Females that spawn at least once (%)	60	54.1	60	37.8	na	na	na
Number of consecutive maturations per female	2	3	4	2	na	na	na
Female weight	43.9 ± 0.8	42.3 ± 0.8	40.5 ± 0.9	40.2 ± 0.5	ns	ns	ns



CAPÍTULO VII

Discusión general y conclusiones

Discusión

El desarrollo de la acuacultura y el uso del biofloc en el cultivo de camarones peneidos con énfasis en *L. vannamei* y *F. brasiliensis*

Las biotecnologías utilizadas en la acuacultura han avanzado rápidamente en la última década debido al gran incremento de la población mundial y la subsecuente demanda de productos ricos en proteína. De hecho, se estima que para el año 2030, la acuacultura equipare en producción a los productos provenientes de la pesca (FAO, 2018; Msangi *et al.*, 2013). A pesar de esto, es necesario desarrollar estrategias que permitan: i) incrementar las densidades de siembra sin comprometer el bienestar de los organismos, ii) disminuir la dependencia a las harinas y aceites de pescado mediante el uso de fuentes alternativas de proteína y lípidos y iii) mejorar los esquemas de bioseguridad en las granjas ya que el monocultivo es más vulnerable a infecciones parasitarias, bacterianas o virales.

Dentro de las estrategias que han surgido actualmente para incrementar la producción de camarón se encuentra la tecnología biofloc. Esta tecnología tuvo sus inicios en los años 70´s, aunque las bases fundamentales para el manejo de esta técnica se establecieron a finales de 90´s (Avnimelech, 1999). Aunque más recientemente se establecieron las bases para un mejor manejo de las comunidades autotróficas, heterotróficas y fotoautotróficas en el medio de cultivo (Ebeling *et al.*, 2006).

Este sistema ha demostrado ser de gran ayuda en la producción de camarón, como ya hemos visto a lo largo de los capítulos II al VI. El sistema tiene el potencial de impactar notablemente en la historia nutricional de los camarones, reflejado en términos de producción (biomasa ganada en comparación con sistemas de agua clara), menores tasas de conversión alimenticia, bioseguridad (sistema de mínimo recambio de agua, ayuda a prevenir ingreso de patógenos), entre otros.

Cómo el sistema impacta de manera positiva a los camarones, había sido interrogante hasta hace unos años. Incluso, cuando ya se sabía que la productividad natural de los estanques de cultivo contribuía en mayor proporción que los alimentos artificiales. El biofloc comprende la fase de floculación, en la cual las bacterias heterotróficas forman agregados microbianos en conjunto con otros materiales del medio como son exopolisacáridos, cationes disueltos en el agua como calcio y magnesio, partículas suspendidas colonizables

como por ejemplo el salvado de trigo (utilizando como floculante en sistemas biofloc). Una segunda fase, viene dada por una sucesión de microorganismos que ayudan a reciclar la proteína (al formarse un "*loop"* microbiano) y que a su vez colonizan los flóculos. Estos microorganismos, comprenden el bacterioplancton, el cual puede ser de gran valor nutricional para los camarones de cultivo. De hecho, en los capítulos III y IV, la cantidad de lípidos y ácidos grasos poliinsaturados aumenta en las fracciones de tamaño más pequeñas de los flóculos (alrededor de 10 a 50 micras). Esto puede estar ligado a los productores primarios como son las microalgas, las cuales tienen las rutas metabólicas para generar por ejemplo los ácidos eicosapentaenoico y decosahexaenoico, así como el ácido linoleico y alfa-linoleico, los cuales son ácidos grasos esenciales para la nutrición de los camarones, ya que estos no pueden sintetizarlos *de novo*. Además, en cuanto al valor nutricional de los flóculos se observa en los capítulos III y IV, que la cantidad de proteína aumenta en los flóculos de mayor tamaño (alrededor de 250 micras o superiores), indicando que hay microorganismos de alto valor nutricional para los camarones como son los nematodos, copépodos, rotíferos, etc. Esta dinámica microbiana en los sistemas de cultivo, mejora la calidad del agua al reciclar la proteína y evita que esta se convierta en amonio y nitrito, los cuales son tóxicos para los camarones. Además de esto, el paso de nutrientes a través de la cadena trófica (del fitoplancton al zooplancton y finalmente a los camarones) genera que los camarones peneidos tengan una mejor nutrición. De hecho, los camarones cultivados en biofloc pueden disminuir sus requerimientos de proteína, ya que los flóculos microbianos pueden aportar la proteína faltante a los alimentos artificiales. A pesar de esto, es necesario llevar a cabo investigaciones que promuevan la mejora de la calidad nutricional de los flóculos microbianos con el fin de mantener estas ventajas a lo largo del cultivo.

En esta tesis, el biofloc fue un medio de cultivo que mejoró la calidad de *L. vannamei* y *F. brasiliensis* desde el punto de vista de la engorda, pre-maduración y reproducción. De hecho, el desempeño reproductivo de *L. vannamei* y *F. brasiliensis* se ve incrementado respecto a otros trabajos publicados. En el capítulo VI, se realizó el experimento donde se comparaba el desempeño reproductivo de *L. vannamei* de origen biofloc y agua clara, y se demuestra que hay un aumento de los camarones de origen biofloc en términos de la calidad de los huevos producidos, número de huevos por desove y las hembras tienen

menor mortalidad. Todo esto se debe a una mejora en su historia nutricional, ganada a través del cultivo de los camarones en sistemas biofloc. *F. brasiliensis* también se adecuó bien al sistema biofloc, de hecho su tasa de conversión alimenticia y su desempeño reproductivo fue mayor a lo reportado en la literatura, indicando que la historia nutricional de *F. brasiliensis* se ve impactada de manera positiva por los flóculos microbianos. A pesar de esto, es necesario seguir llevando a cabo investigación con el fin de mejorar su desempeño durante la engorda, ya que la supervivencia ronda entre el 60-70% y su crecimiento se encuentra alrededor de los 0.5 a 0.7 gramos por semana, lo cual es bastante contrastante con *L. vannamei* donde la supervivencia se encuentra entre el 85-90% y el crecimiento es mayor a 1.0 gramo por semana. Si bien, *F. brasiliensis* no presenta el mismo potencial de cultivo en términos de engorda, solamente la investigación de sus necesidades nutricias ayudará a mejorar las velocidades de crecimiento. Incluso, esta premisa, no solamente es necesaria para *F. brasiliensis* sino también para *L. vannamei, L. stylirostris, P. monodon,* entre otros camarones peneidos (NRC, 2011). En cuanto a la reproducción, *F. brasiliensis* mostró poder madurar incluso sin el uso de la ablación unilateral del pedúnculo ocular, logrando madurar hasta 2-3 veces una misma hembra en un periodo de 45 días. Esto había sido bastante complicado de lograr, incluso con *L. vannamei* donde la remaduración de las hembras no había sido posible en periodos tan cortos. Esto indica que los protocolos de reproducción seguidos en el laboratorio de maduración de camarones peneidos de la UMDI-SISAL son bastante adecuados.

Por lo tanto, los camarones utilizados en esta tesis, *L. vannamei* y *F. brasiliensis* muestran un alto potencial de adaptarse a la tecnología biofloc, explotarla de manera eficiente y llevar estos beneficios a la etapa de reproducción, donde incluso, la calidad de la progenie es de mejor calidad. Sin embargo, es necesario seguir llevando a cabo investigación que permita mejorar los esquemas de producción para *F. brasiliensis*, ya que con los resultados obtenidos de crecimiento, su inclusión a las granjas comerciales se puede ver limitada. A pesar de esto, *F. brasiliensis* puede ser utilizada exitosamente en zonas donde no se permita la introducción de especies exóticas como *L. vannamei*.

Métodos aplicados para evaluar la contribución nutricional a camarones peneidos

Las dos técnicas fundamentales utilizadas en esta tesis fueron: i) el análisis de isótopos estables en combinación con modelos de mezcla basados en balance de masas, y ii) el uso de los ácidos grasos, ya que los camarones tienden a ser el reflejo de su dieta. Si bien, estas dos técnicas en conjunto, tienen el potencial de direccionar la contribución del carbono y nitrógeno (isótopos de estos elementos utilizados en esta tesis) en la generación de músculo y producción de huevos de *L. vannamei* y *F. brasiliensis*, su interpretación debe ser generada con cuidado. Esto se debe a que hay varias suposiciones para poder utilizar estas herramientas.

En términos de isótopos estables, hay que tomar en cuenta varios factores: i) los organismos deben estar en equilibrio con su dieta, ii) algunos modelos matemáticos toman en cuenta que tanto la asimilación de carbono y nitrógeno se dan a la misma velocidad, cuando esto puede ser más complejo, iii) las señales isotópicas de las fuentes alimenticias deben ser significativamente diferentes con el fin de que los modelos puedan calcular con más claridad la contribución de cada fuente al consumidor, iv) el uso de factores de discriminación deben ser específicos para el experimento o deben ser tomados con cautela, ya que de esto depende fuertemente la adecuación de la señal de los consumidores respecto a las fuentes alimenticias; de hecho este factor puede estar relacionado al estado ontogénico, calidad nutricional del alimento y porcentaje de proteína en la dieta (Gamboa-Delgado & Le Vay, 2009).

Para esta tesis, se asumió que los camarones ya habían alcanzado el "equilibrio con su dieta" ya que el tejido objetivo durante la engorda fue el músculo. Se ha determinado que el músculo de los camarones llega al equilibrio con su dieta en alrededor de 15-30 días, siendo más rápido el equilibrio cuando los organismos son más pequeños, debido a que tienen un intercambio de nutrientes mucho más rápido que los organismos más grandes (Gamboa-Delgado *et al.*, 2013; Gamboa-Delgado & Le Vay, 2009; Suita *et al.*, 2016). Esto quiere decir, que hay que tomar en cuenta el factor de crecimiento, cuando se realizan este tipo de estudios. En términos de la asimilación de carbono y nitrógeno, los modelos asumen que hay una incorporación igual de rápida tanto para carbono y nitrógeno, aunque esto puede variar dependiendo de las fuentes de alimentación, estado ontogénico de los organismos,

temperatura, etc. En cuanto a las señales isotópicas de las fuentes alimenticias de esta tesis, estas fueron significativamente diferentes unas de otras, y cuando no, estas fueron agrupadas, como se ha sugerido (Phillips & Gregg, 2003).

En cuanto al uso de los factores de discriminación, en la literatura, se ha determinado que los organismos tienen un muy bajo o nulo fraccionamiento isotópico respecto al carbono, siendo este de entre 0 y 1 ‰ (Post, 2002). Para esta tesis, los análisis fueron sometidos a factores de discriminación entre 0 y 1 ‰, sin obtener diferencias en las contribuciones calculadas. No obstante, han habido reportes donde el fraccionamiento de carbono puede llegar a ser hasta de 3‰ (Cardona *et al.*, 2015) o 5‰ (Gamboa-Delgado & Le Vay, 2009), no obstante, estos valores se pueden deber al estadio ontogénico, fuente de proteína del alimento y calidad de la proteína. Muchos alimentos artificiales están hechos de harinas marinas y terrestres, las cuales tienen diferentes composiciones de nutrientes, lo que provoca que pueda haber mayores o menores factores de discriminación (Abreu *et al.*, 2007; Gamboa-Delgado & Le Vay, 2009). En cuanto al nitrógeno, el factor de fraccionamiento se ha determinado en alrededor de 2.5‰ para crustáceos y camarones, por lo que este valor fue elegido. A pesar de que la literatura brinda valores de fraccionamiento diferentes, estos muchas veces no reflejan las condiciones reales de lo que ocurre bajo condiciones específicas o bajo múltiples fuentes alimenticias (Cardona *et al.*, 2015; Gamboa-Delgado *et al.*, 2013). Por ejemplo, se han determinado valores de enriquecimiento de entre 2 y 3.5‰ para carbono y de 2.5 a 5‰ para nitrógeno en *L. stylirostris* (Cardona *et al.*, 2015). En la Tabla 1, se presentan diferentes valores para el factor de fraccionamiento para camarones.

Si bien, los isótopos estables nos brindan información sobre las fuentes de carbono y nitrógeno de las cuales los camarones se están alimentando, estos no direccionan de qué biomoléculas podrían estar proviniendo. Como métodos que ayudan a complementar la información brindada por los isótopos estables, se encuentran los ácidos grasos y aminoácidos. El fundamento se basa en que los camarones tienen un perfil de ácidos grasos y aminoácidos muy parecido al de sus fuentes alimenticias que más contribuyen a su nutrición. Es así que podemos direccionar de igual manera, que alimentos son más importantes que otros en términos de las principales biomoléculas.

Tabla 1. Factores de discriminación calculados [(a)] ($\Delta\delta X = \delta X_{consumidor} - \delta X_{alimento}$; $X = \delta^{13}C$ o $\delta^{15}N$) o utilizados [(b)] (basados en la literatura) en experimentos con camarones peneidos. PL = postlarva.

Especie	Valores de fraccionamiento isotópico		Referencia
	$\Delta^{13}C$ (‰)	$\Delta^{15}N$ (‰)	
[a] *L. stylirostris* (1)	2-3.5	2.5-5	Cardona *et al.*, 2015
[b] *L. vannamei PL* (2)	0.4	2.7	Suita *et al.*, 2016
[a] *L. vannamei* (3)	-	0.8-5	Gamboa-Delgado & Le Vay, 2009
[a] *L. vannamei* (4)	1.3	2.1	Ray & Lotz, 2017
[b] *F. paulensis* (5)	0.5	2.5	Abreu *et al.*, 2007

(1) Cultivado en sistema biofloc más alimento artificial. (2) Cultivado en sistema biofloc más alimento artificial y otra miscelánea de alimentos. (3) Cultivados en agua clara con proteína de pescado y soya. (4) Camarones cultivados en biofloc más alimento artificial. (5) Cultivado con biofilm más alimento artificial.

Más allá de este tipo de técnicas, actualmente se está utilizando el análisis de isótopos estables de compuestos específicos. En estos análisis, se detecta la señal isotópica de varios aminoácidos y da una luz de la ruta que siguen estos nutrientes hasta su incorporación en el tejido (O'Brien *et al.*, 2005). Lo mismo está surgiendo con los ácidos grasos, donde se han dado inferencias del uso de ácidos grasos de cadena corta por parte de copépodos y como los convierten en ácidos grasos de cadena larga (PUFA) (De Troch *et al.*, 2012). En acuacultura, esta técnica no ha sido utilizada, por lo que su uso podría brindar más detalles acerca del metabolismo de los organismos acuáticos y con qué eficiencia utilizan los nutrientes de los alimentos.

Conclusión

- El biofloc es un sistema que ayuda a mejorar la engorda y pre-maduración de camarones peneidos.
- Tanto los isótopos estables como ácidos grasos mostraron que el alimento artificial fue menos importante en términos de asimilación para *L. vannamei* durante la engorda, a pesar de que el alimento ayude a promover la creación del biofloc. El biofloc puede representar hasta un 77% en la nutrición de *L. vannamei* mientras que el alimento comercial un promedio de 15.3%. En sí, el alimento comercial puede ser visto como un fertilizante muy caro para la acuacultura, por lo que nuevos esquemas de alimentación, reformulación de los porcentajes de proteína del alimento y uso de

harinas alternativas a la harina de pescado, deben ser tomadas en cuenta para futuros estudios.

- El biofloc para *F. brasiliensis* fue una fuente alimenticia de mayor importancia que para *L. vannamei*. El biofloc representó entre un 95-97% de la contribución de carbono y nitrógeno, mientras que el alimento comercial entre un 0 y 5%. Esto indica que el alimento artificial fue poco o nulamente incorporado en la dieta de esta especie. Por lo tanto el alimento comercial puede ser visto como un complemento en la dieta o como un fertilizante muy caro para promover el biofloc.
- Una ventaja de este estudio, fue dividir el biofloc por tamaño de partícula. Esto aportó resolución en cuanto a la contribución del biofloc y alimento comercial en la dieta de ambos camarones de estudio. Los trabajos convencionales, no habían separado el biofloc por tamaño, lo que podría haber causado "ruido" al momento de evaluar la contribución al músculo. Además, una fortaleza del trabajo es que los camarones fueron cultivados toda su vida en biofloc, confiriéndoles las herramientas para digerir el biofloc y utilizarlo como una fuente de nutrientes.
- *L. vannamei* muestra una mejor adecuación a los sistemas biofloc, presentando mejor crecimiento y supervivencia así como una mejor tasa de conversión alimenticia. Además, esta especie mejora su historia nutricional, lo que provoca tener mejor desempeño reproductivo y mejor calidad de huevos.
- *F. brasiliensis* es una especie poco estudiada. Sin embargo, en la presente tesis se dan algunos aspectos claves para su cultivo desde postlarva hasta reproductor. Además se demuestra que es capaz de asimilar tanto el biofloc como el alimento artificial, mejorando su historia nutricional y proveyendo mejores resultados durante la reproducción en términos de la calidad de su progenie.
- *F. brasiliensis* mostró un gran potencial en cuanto a su reproducción, lo que le confiere la factibilidad de poder contar con postlarvas para establecer granjas comerciales. A pesar de esto, hace falta todavía mucho trabajo en cuanto a su nutrición, ya que la mayoría de los alimentos comerciales del mercado están enfocados en *L. vannamei*. Esto puede explicar su bajo uso por parte de *F. brasiliensis* durante la engorda. Hay que recordar que la nutrición de los organismos es especie-específica y que no debemos asumir que los requerimientos nutricionales

son los mismos para todos los peneidos. Solamente para *F. brasiliensis* se han reportado requerimientos de proteína de hasta un 50% mientras que para *L. vannamei* de entre un 35-40% para las fases juveniles.

- La ablación unilateral del pedúnculo ocular es útil para acelerar la maduración gonádica tanto de *L. vannamei* como de *F. brasiliensis*. No obstante, la calidad de la progenie se ve afectada negativamente.
- La reproducción de *L. vannamei* y *F. brasiliensis* sin ablación puede ser llevada a cabo como se demostró en la presente tesis. Las ventajas son: mejor supervivencia de los reproductores, mayor número de nauplios viables y un mejor estado de condición tanto de los reproductores como de la progenie.
- Esta tesis, demostró que la reproducción de *L. vannamei* y *F. brasiliensis* puede llevarse a cabo múltiples veces en un periodo de 45 días sin el uso de la ablación unilateral del pedúnculo ocular. Por ejemplo, varias hembras de *L. vannamei* remaduraron hasta dos veces en 30 días, mientras que *F. brasiliensis* remaduró hasta tres veces en 45 días. Esto es importante porque, hasta ahora, la reproducción de camarones peneidos sin ablación había sido muy difícil de lograr, y no hay reportes de remaduraciones en un periodo tan estrecho como en esta tesis. Esto indica, que los esquemas de pre-maduración y maduración son de alto impacto en la historia nutricional de los camarones peneidos y tiene importantes efectos durante los periodos de reproducción.
- Es necesario llevar a cabo más estudios con *F. brasiliensis* con el fin de mejorar su biotecnología. Aspectos como su nutrición, palatabilidad de ciertas fuentes proteicas, uso de harinas alternativas a la de pescado, bioquímica, parámetros fisicoquímicos y su reproducción deben ser más profundamente evaluados con el fin de que en un futuro esta especie pueda ser utilizada a escala comercial.
- Como sugerencia, podría ser interesante plantear para futuros estudios el uso de alimentos formulados, con el fin de obtener una señal isotópica lo suficientemente disímil de las otras fuentes alimenticias y así generar intervalos de contribución mucho más estrechos.

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