INSTITUTO POLITÉCNICO NACIONAL

CENTRO INTERDISCIPLINARIO DE INVESTIGACIÓN PARA EL DESARROLLO INTEGRAL REGIONAL.

UNIDAD OAXACA.

EFECTO DE LA CANTIDAD DE QUITINA EN LA DIETA SOBRE EL CRECIMIENTO Y SUPERVIVENCIA DE LANGOSTINOS *Macrobrachium tenellum* CULTIVADOS BAJO DIFERENTES COMBINACIONES DE TEMPERATURA Y FOTOPERIODO.

TESIS

QUE PARA OBTENER EL GRADO ACADÉMICO DE DOCTOR EN CIENCIAS EN CONSERVACIÓN Y APROVECHAMIENTO DE RECURSOS NATURALES

PRESENTA:

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INSTITUTO POLITECNICO NACIONAL

SECRETARIA DE INVESTIGACION Y POSGRADO

ACTA DE REVISION DE TESIS

En la Ciudad de Oaxaca de Juárez siendo las 13:00 horas del día 29 del mes de noviembre del 2016 se reunieron los miembros de la Comisión Revisora de Tesis designada por el Colegio de Profesores de Estudios de Posgrado e Investigación del **Centro Interdisciplinario de Investigación para el Desarrollo Integral Regional, Unidad Oaxaca (CIIDIR-OAXACA)** para examinar la tesis de grado titulada: **"Efecto de la cantidad de quitina en la dieta sobre crecimiento y supervivencia de langostinos *Macrobrachium tenellum* cultivados bajo diferentes combinaciones de temperatura y fotoperiodo"**

Presentada por el alumno

De los Santos	**Romero**	**Rodolfo Benigno**
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Con registro: B 1 2 0 2 3 2

aspirante al grado de: **DOCTORADO EN CIENCIAS EN CONSERVACIÓN Y APROVECHAMIENTO DE RECURSOS NATURALES**

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

LA COMISIÓN REVISORA

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CARTA CESIÓN DE DERECHOS

En la Ciudad de Oaxaca de Juárez el día 28 del mes de noviembre **del año 2016,** el (la) que suscribe Rodolfo Benigno de los Santos Romero, alumno (a) del Programa de **DOCTORADO EN CIENCIAS EN CONSERVACIÓN Y APROVECHAMIENTO DE RECURSOS NATURALES** con número de registro **B120232,** adscrito al Centro Interdisciplinario de Investigación para el Desarrollo Integral Regional, Unidad Oaxaca, manifiesta que es autor (a) intelectual del presente trabajo de Tesis bajo la dirección del **Dr. Marcelo Ulises García Guerrero y Dr. Fernando Vega Villasante** y cede los derechos del trabajo titulado: **Efecto de la cantidad de quitina en la dieta sobre el crecimiento y supervivencia de langostinos *Macrobrachium tenellum* cultivados bajo diferentes combinaciones de temperatura y fotoperiodo,** al Instituto Politécnico Nacional para su difusión, con fines académicos y de investigación.

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CONTENIDO

RESUMEN

En México *Macrobrachium tenellum* es considerado como un candidato para su explotación silvestre y el cultivo porque crece rápido, todavía es común en su ambiente natural, siendo un camarón de agua dulce nativo desde México a Perú, aunque es ampliamente explotado por las pesquerías locales que comprometen su conservación. Exploramos el requerimiento de quitina en las dietas de langostinos cultivados añadiendo 5, 10 15, 20 y 25% para suplementar la dieta de los juveniles durante 60 días. Se midió el crecimiento, la supervivencia y la actividad enzimática (tripsina, quimotripsina, lipasa, amilasa y quitinasa) cada 15 días. El peso final y la tasa de crecimiento específica más altos se observaron en los organismos con dietas suplementadas con 20% de quitina. Los resultados más pobres se obtuvieron de dietas con 5 y 10% de quitina. La quitina en la dieta no tuvo un efecto significativo sobre la supervivencia. No se encontraron diferencias estadísticamente significativas en la actividad enzimática en ningún tratamiento

Estos langostinos toleran altas densidades de cultivo (# ind/m3) y grandes fluctuaciones en los parámetros físico-químicos del agua. Para entender cómo afectan las condiciones ambientales a los parámetros de producción de langostinos juveniles, se realizaron experimentos con temperatura combinada (26ºC y 30ºC) y fotoperíodo (14HL/10HD y 10HL/14HD) asociados a una dieta de quitina de 20% durante 60 días. Los parámetros de crecimiento, supervivencia y actividad enzimática se midieron cada 15 días. El peso final y la tasa de crecimiento específico más altos fueron el resultado de la combinación de fotoperíodo 14HL/10HD con temperaturas de 30ºC y 26°C mostrando diferencias significativas con los resultados de una temperatura ambiental de 22°C con relación a un fotoperiodo 12HL/12HD (control). Los tratamientos con temperatura de 30°C y 26°C no tuvieron un efecto significativo en la supervivencia. Las diferencias estadísticamente significativas se encontraron sólo en la actividad enzimática de la quitinasa en el tratamiento 14HL/10HD a una temperatura de 30°C, y alta actividad enzimática (quitinasa) se produjo cuando las temperaturas eran más bajas.

Los langostinos se caracterizan también por su agresividad y territorialidad, lo que limita su conservación y manejo en cautiverio. Para entender cómo estos organismos responden a la presencia y el número de machos dominantes en una población y el efecto de tres tipos de refugios en la reducción de la presencia de jerarquías y aumentar la supervivencia y el crecimiento, diseñamos tratamientos experimentales para determinar el efecto en el crecimiento de diez juveniles con ausencia

de machos dominantes, la presencia de un macho dominante y la presencia de dos machos dominantes, cada uno asociado con tres tipos de sustrato, tejas de arcilla, tubos de PVC y red de malla de plástico. Se observaron diferencias significativas (P <0,01) en el crecimiento de langostinos en ausencia de machos dominantes, no hubo diferencias (P> 0,05) para los diferentes sustratos utilizados como refugio. Se sugiere que los efectos sobre el crecimiento y la supervivencia causados por la jerarquía social del langostino y el uso de refugios forman parte de una gama más amplia de interacciones que pueden ser ecológicas, etológicas y fisiológicas

Palabras clave: Quitina, fotoperiodo, temperatura, actividad enzimática, langostino

SUMMARY

In Mexico *Macrobrachium tenellum* is considered as a candidate for wild exploitation and cultivation because it grows fast, is still common in their natural environment, is a freshwater prawn native from Mexico to Peru, althought it is extensively exploited by local fisheries jepardizing its conservation. We explored the chitin requirement on cultivated prawns diets by adding 5, 10 15, 20, and 25% to supplement juveniles' diet for 60 days. Growth, survival, and enzyme activity (trypsin, chymotrypsin, lipase, amylase, and chitinase) were measured every 15 days. Highest final weight and specific growth rate were observed on organism with diets supplemented with 20% chitin. Poorest performance resulted from diets with 5 and 10% chitin. Chitin in the diet did not have a significant effect on survival. Statistically significant differences in enzyme activity were not found in any treatment

These prawns tolerates cultivation at high densities (#ind/m3) and wide fluctuations in water physico-chemical parameters. To understand how environmental conditions affect the production parameters of juveniles prawns, experiments were conducted with combined the temperature (26ºC and 30º C) and photoperiod (14HL/10HD and 10HL/14HD) associated to a chitin diet of 20% for 60 days. Growth parameters, survival, and enzymatic activity were measured every 15 days. The higher final weight and specific growth rate were the result of the combination of photoperiod 14HL/10HD with temperatures 30ºC and 26°C showing significant differences from the results of a environment temperature 22°C in relation to a photoperiod 12HL/12HD (control). Treatments with 30°C and 26°C temperature had no significant effect on survival. Statistically significant differences were found only on the enzyme activity of chitinase in the treatment 14HL/10HD at a temperature of 30°C, and high enzymatic activity (chitinase) occurred when temperatures were lower.

The prawns are also characterized by their aggressiveness and territoriality, which limits their conservation and management in captivity. To understand how these organisms respond to the presence and number of dominant males in a population and the effect of three types of shelters on reducing the presence of hierarchies and increase survival and growth, we designed experimental treatments to determine the effect on the growth of ten juveniles with the absence of dominant male, the presence of a dominant male and the presence of two dominant males, each associated with three types of substrate, clay tiles, PVC pipes and plastic mesh net. We observed significant differences ($P < 0.01$) in the growth of prawns in the absence of dominant males, there were no differences ($P > 0.05$)

for the different substrates used as shelter. It is suggested that the effects on growth and survival caused by the social hierarchy of prawns and the use of shelters are part of a wider range of interactions that may be ecological, ethological and physiological

Keywords: Chitin, photoperiod, temperature, enzyme activity, prawns.

CAPITULO I
INTRODUCCIÓN

En México la acuacultura es una actividad que fortalece la soberanía alimentaria y es prioridad para la planeación nacional del desarrollo, donde la investigación científica y tecnológica debe consolidarla como herramienta para definir e implementar instrumentos y decisiones relativas a la conservación, restauración, protección y aprovechamiento sustentable de los recursos acuícolas (DOF, 2007), ha tomado relevancia en los últimos veinte años, donde los beneficios ecológicos y económicos solo se logran con el adecuado manejo de las poblaciones naturales y de los sistemas de cultivo, a través del conocimiento primario de su biología y ecología, y del desarrollo de biotecnologías que permitan su utilización sustentable (Vega-Villasante *et al.*, 2011; Espinosa-Chaurand *et al*., 2011). En la actualidad es difícil promover el desarrollo de proyectos de acuicultura comercial, sin tener en cuenta la relación entre los parámetros de producción de los animales asociados a los factores ambientales involucrados, reconociendo que las relaciones que se establecen entre las diversas funciones productivas y el medio ambiente no son sólo económicas, sino también tecnológicas, ambientales, biológicas y sociales (Montanhini-Neto *et al*, 2015).

En la acuacultura de langostinos nativos del género *Macrobrachium*, los esfuerzos de investigación y desarrollo se han llevado a cabo principalmente en las regiones tropicales, sin embargo, las actividades en las regiones templadas también ofrece oportunidades positivas, a pesar de no poder llevar a cabo el cultivo durante todo el año, lo que se refleja en un crecimiento restringido que impiden alcanzar una producción comercialmente viable. Las áreas de investigación incluyen la evaluación de las dietas, los parámetros ambientales asociados al agua, los efectos de sustratos artificiales en la estructura de la población, el crecimiento de los langostinos, y la clasificación de los animales en los estanques para reducir el crecimiento individual heterogéneo y las interacciones agresivas (Tidwell *et al*., 2005).

Respecto a las dietas, los langostinos necesitan cubrir sus necesidades fisiológicas para tener adecuados niveles de crecimiento, por lo que es necesario que encuentren en su medio las condiciones y materiales indispensables para logarlo (Cortes *et al.*, 2003). Son muchos los estudios que han tratado de establecer el efecto de ciertos insumos en la alimentación de los crustáceos, en especial sobre las necesidades de nutrientes del género *Macrobrachium*, Du y Niu (2003) y Luna *et al.*, (2007) examinaron el efecto de la proteína de soya sobre el crecimiento. Gitte y Indulkar (2005) estudiaron el efecto de diferentes niveles de proteína en el crecimiento y el comportamiento, mientras que García-Ulloa *et al*. (2008) y Espinosa-Chaurand *et al.* (2012) investigaron el efecto de las proteínas alternativas en relación con el comportamiento de alimentación. Araujo y Valenti (2007), Le Vay *et al*. (2001) y Barros y Valenti (2003) estudiaron los hábitos de alimentación y la estabilidad y la

palatabilidad de los alimentos en agua y Siddiqui *et al*. (2011) estudiaron las propiedades organolépticas de los alimentos y los órganos sensoriales de los langostinos. Para especies nativas del Pacífico de México se tienen los trabajos de Ponce-Palafox *et al*. (2006), García-Ulloa *et al.* (2008), Espinosa-Chaurand *et al*. (2011) y Vega-Villasante *et al*. (2011), pero con esto no se tiene aún la información suficiente para preparar un alimento efectivo que cubra sus requerimientos de crecimiento durante su cultivo.

Lo anterior puede deberse a que los langostinos anfídromos se encuentran influenciados por factores ambientales que intervienen de manera cinegética con la alimentación, como la temperatura, salinidad y concentración de oxígeno disuelto que influyen en la transformación del alimento a biomasa. De la misma manera existen condiciones intraespecíficas de las especies que provocan efectos sobre la potencialidad del alimento (Lima, 2014). Con base a lo anterior podemos decir que existen muchas razones con las que se puede argumentar porque cada especie acuícola requiere de una dieta específica para su adecuado crecimiento bajo un esquema de cultivo, en el caso de *M. tenellum* sus requerimientos implican que su dieta contemple insumos que puedan favorecer tanto su crecimiento como su supervivencia. Entre estos insumos se encuentra la quitina que es el principal material que forma parte de su exoesqueleto (Proespraiwong *et al.*, 2010) y con frecuencia no es considerada al momento de elaborar su alimento, pues se desconocen las cantidades adecuadas de este ingrediente para que el organismo pueda producir con éxito su exoesqueleto y mudar. Esta información es de vital importancia, pues la muda es el proceso a través del cual los crustáceos crecen de manera escalonada y continua (Vega-Villasante *et al*., 2011 y Yamasaki-Granados *et al*., 2012).

La quitina en un polímero con una estructura de β-1,4-*N-acetil*-d-glucosamina, uno de los polisacáridos más comunes en los ecosistemas y principal elemento en el exoesqueleto de insectos y crustáceos, aunque también lo encontramos en gran medida en la pared celular de los hongos (Zhu *et al*., 2010). La quitina en crustáceos es degradada por las enzimas endógenas, conocidas como quitinasas (Swiontek-Brzezinska y Lalke-Porczyk, 2007). Powell y Rowley (2006) mencionan que en vida libre algunos crustáceos comen su caparazón después de la ecdisis, suponiendo beneficio en la dieta. Shiau y Yu (1998) citan que el 5% de quitina en la dieta de camarones promueve la eficiencia alimenticia, la relación de eficiencia proteica y el crecimiento.

Sobre el efecto de la inclusión de quitina en la dieta de crustáceos, Shiau y Yu (1998) y Jones *et al.* (1996) han evaluado el crecimiento de crustáceos y por otro lado Chang *et al*. (1988), Terwilliger (1999) y Chang *et al*. (2001) han evaluado el efecto de la quitina en relación al proceso de muda.

Stevenson (1985) y Casillas-Hernández *et al.* (2002) establecen que durante la ecdisis el organismo utiliza las reservas orgánicas que guardan y son transportadas para ser usadas en este proceso de formación del nuevo exoesqueleto e iniciar el proceso de mineralización.

Por otro lado, son diversos los factores ambientales que hay que considerarse para favorecer el efecto de una dieta en los langostinos de agua dulce, desafortunadamente no son muchos los trabajos que hacen referencia a ésta relación y son aún menores para los langostinos nativos de México. Entre los diferentes trabajos que abordan cuestiones ambientales vinculadas al crecimiento o desarrollo de los langostinos de agua dulce, tenemos a los realizados por Tidwell *et al.* (2001), en donde evalúan el crecimiento del langostino malayo con respecto a la cantidad y calidad de sustrato, disminuyendo el contacto directo de la luz. Yasharian *et al.* (2005) evaluó el efecto de la coloración de tanques de cultivo sobre su comportamiento. De Araujo y Valenti (2011) y Gardner y Maguire (1998) estudiaron el efecto algunos parámetros, como la temperatura, la intensidad luminosa y fotoperiodo sobre el desarrollo de langostino. Con respecto a estos últimos Aiken y Waddy (1992) indican que la temperatura y el fotoperiodo son considerados los principales factores en la regulación de los ciclos de muda y por consecuencia del crecimiento en crustáceos.

La temperatura es la variable que más afecta a todas las funciones metabólicas y, por tanto, determina el crecimiento (Hoang *et al*., 2003, Lagerspetz y Vainio, 2006), debido a esto, la definición de la temperatura óptima es esencial para la producción eficaz, evaluación y gestión de las especies bajo cualquier técnica de la acuicultura (Deering y Fielder, 1995). En los decápodos, el manejo adecuado de la temperatura provoca un crecimiento rápido y el uso correcto de nutrientes (Chen *et al.,* 1996; O'Brien, 1994; Vijayan y Diwan, 1995). La temperatura afecta la capacidad de procesar alimentos asi como la absorción de nutrientes, en donde si la temperatura disminuye (menos de 20°C) los organismos pueden dejar de alimentarse, por ejemplo, en *Procambarus clarkii* y *P. zonangulus*, expuestos a cambios de temperatura, tienen un marcado efecto en la capacidad de obtener nutrientes del medio ambiente y, por tanto, en su tasa de crecimiento (Croll y Watts, 2004). En *Macrobrachium rosembergii* Stephenson y Knight (1980) mencionan que los cambios bruscos de temperatura pueden afectar a la movilidad y la actividad en las post-larvas. En langostinos del Pacifico mexicano Hernández *et al*. (1995), Vega-Villasante *el al*. (2011) y Flores *et al.* (2012) indican que la preferencia térmica de *M. tenellum* está entre 27 y 30°C, ya que en este intervalo los procesos biológicos se presentan a un nivel óptimo, como la reproducción, el metabolismo, la conversión alimenticia y el crecimiento.

En cuanto a la luz y el fotoperiodo han recibido poca consideración a pesar de que son variables que afectan el crecimiento de los langostinos. Para camarones y langostinos tanto la intensidad como la duración del fotoperíodo actúan modificando la frecuencia de la ecdisis y afectando el ritmo del crecimiento (Dalley, 1980; Chamberlain y Lawrence, 1981). Nakamura (1988) no encontró diferencias en el intervalo de muda de camarones peneidos mantenidos con variaciones en los fotoperiodos de día largo y día corto. Aunque otros autores como Vijayan y Diwan (1995) informaron también para camarones peneidos que los fotoperiodos no influyeron significativamente en el intervalo de muda y crecimiento. Wang *et al.* (2004) encontraron que el crecimiento de los crustáceos presenta diferencias bajo diferentes fotoperiodos, indicando que también existe efecto de este parámetro sobre la actividad enzimática, afectando por consecuencia la digestibilidad, asimilación y crecimiento.

Respecto a la actividad enzimática de los crustáceos, las variaciones que presentan se dan en función de su: ciclo de muda, ciclo circadiano, desarrollo (larvario, de crecimiento y de reproducción), tipo de alimento y factores ambientales; Ceccaldi (1989) indica que dentro de las enzimas de los crustáceos que presentan mayor participación durante su desarrollo se encuentran las tripsinasas dentro de las proteolíticas (la actividad de quimotripsinasas es muy bajas), seguidas por las lipasas y amilasas; indicando que también participan una serie de enzimas denominadas quitinasas. Éstas enzimas de encargan de la descomposición biológica de la quitina, la cual se lleva a cabo mediante un sistema de enzimas exocelulares que hidrolizan consecutivamente el polisacárido en un monómero de B-N-acetil-glucosamina (Jeuniaux, 1966). Hernández *et al.* (2002) establecieron la relación entre la actividad enzimática y los procesos de muda en *Litopenaeus*, también se han identificado en estos camarones algunas enzimas digestivas, como proteasas y glucógenasas que actúan sobre la quitina de langostilla (Vega-Villasante *et al*., 2006), promoviendo el crecimiento de los crustáceos, mediante el reemplazo de su esqueleto quitinoso. Por otro lado no se han encontrado en crustáceos y peces reservorios de quitina, por lo que se debe asumir que se degrada rápidamente y podría proporcionar una fuente de energía a través de la hidrólisis enzimática.

Finalmente otros parámetros que se considera afectan directamente la rentabilidad del cultivo de langostinos y que indirectamente son el resultado del efecto de los insumos de la dieta (dando énfasis a la quitina) y los parámetros del fotoperiodo y temperatura, es el desfase de crecimiento que es evidente a través de la variación de su tamaño, el cual es el reflejo de una estructura poblacional compleja, compuesta de tres morfotipos masculinos principales, que difieren en su morfología, fisiología, comportamiento e interacciones sociales entre los jóvenes y adultos (Karplus, 2005). Esta

variación de tamaño es debida en gran parte al crecimiento individual heterogéneo (HIG), causado por las interacciones agresivas entre los diferentes morfotipos, especialmente los varones (Daniels *et al.,* 1995) o también considerados como machos alfa (Espinosa-Chaurand *et al.,* 2011). Los morfotipos más grandes inhiben el crecimiento de los más pequeños, presentándose el efecto toro, en donde Karplus *et al*. (1992) realizaron una descripción del papel que tienen las jerarquías sociales en el crecimiento de langostino. Con base a lo anterior Ranjeet y Kurup (2002) indican que el cultivo exitoso de *M. rosenbergii* radica en la estandarización del tamaño de la población; Moraes y Valenti (2004) señalan para el langostino del amazonas que la intensificación de producción se basa en el uso de la energía externa alóctona, como los piensos alimenticios, una gestión muy rigurosa de la calidad del agua y un adecuado manejo de los tamaños y densidades de cultivo.

El Capítulo II de la presente tesis, muestra el efecto de diferentes cantidades de quitina (0%, 5%, 10%, 15%, 20% y 25%), adicionados al alimento del langostino *M. tenellum*, sobre la cantidad y velocidad de biomasa asimilada, su sobrevivencia y la actividad enzimática (en especial las quitinasas).

En el Capítulo III se retoma la dieta con el nivel de quitina que presenta mayor crecimiento y actividad enzimática (20%), y los langostinos son expuestos a diferentes condiciones combinadas de temperatura (ambiental, 26ºC y 30ºC) y fotoperiodo (12HL-12HS, 14HL-10HS y 10HL-14HS; en donde: H=horas, L=luz y S=sombra), evaluando la cantidad y velocidad de biomasa asimilada, la sobrevivencia y la actividad enzimática.

En el Capítulo IV se evaluó el crecimiento individual heterogéneo y sobrevivencia en los langostinos, mediante el efecto que la presencia y número de machos dominantes ejercen sobre juveniles de *M. tenellum* y como el sustrato utilizado como refugio puede reducir el efecto de los machos dominantes.

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CAPITULO II
EFECTO DE LA CANTIDAD DE QUITINA EN LA DIETA

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Effect of dietary chitin on digestive enzyme activity, growth, and survival of *Macrobrachium tenellum* juvenile prawns

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ABSTRACT. *Macrobrachium tenellum* is a freshwater prawn native from Mexico to Peru, with potential for cultivation. Currently, it is extensively exploited by local fisheries. To understand its chitin requirements, a formulated diet with chitin added (5, 10 15, 20, and 25%) was given to juveniles for 60 days. Growth, survival, and enzyme activity (trypsin, chymotrypsin, lipase, amylase, and chitinase) were measured every 15 days. Highest final weight and specific growth rate resulted from diets with 20% chitin. Poorest performance resulted from diets with 5 and 10% chitin. Chitin in the diet did not have a significant effect on survival. Statistically significant differences in enzyme activity were not found in any treatment.

Keywords: Chitin, enzyme activity, *Macrobrachium tenellum*, nutrition.

Efecto de la quitina incluida en la dieta sobre la actividad enzimática digestiva, el crecimiento y la supervivencia de juveniles del langostino *Macrobrachium tenellum*.

RESUMEN. *Macrobrachium tenellum* es un langostino de agua dulce nativo de México a Perú, con potencial para el cultivo. Actualmente, es explotado ampliamente por las pesquerías locales. Para entender sus requisitos de quitina, una dieta formulada con quitina añadida (5, 10 15, 20, y 25%) fue proporcionada a los juveniles durante 60 días. El crecimiento, la supervivencia, y la actividad enzimática (tripsina, quimotripsina, lipasa, amilasa, y quitinasa) se midieron cada 15 días. El mayor peso final y la tasa de crecimiento específico fueron el resultado de la dietas con 20% de quitina. Los rendimientos más pobres fueron el resultado de las dietas con 5 y el 10% de quitina. La quitina en la dieta no tuvo un efecto significativo sobre la supervivencia. Estadísticamente no se encontraron diferencias significativas en la actividad de la enzima en algún tratamiento.

Palabras clave: Quitina, actividad enzimática, *Macrobrachium tenellum*, nutrición

INTRODUCTION

Within the American species of the genus *Macrobrachium* (Bate, 1868) only few are economically important. Some species have potential for cultivation: *M. carcinus* (Linnaeus, 1758), *M. americanum* (Bate, 1868), *M. digueti* (Bouvier, 1895), *M. acanthurus* (Wiegmann, 1836), *M. amazonicus* (Heller, 1962), and *M. tenellum* (Smith, 1871). *M. tenellum*, the long arm river prawn, has been considered a candidate for cultivation because it grows quickly, tolerates high densities and wide fluctuations in water conditions, and adapts well to captivity (Ponce-Palafox *et al*. 2002; Espinosa-Chaurand *et al*. 2011; García-Guerrero *et al*. 2013). This species is still common in its natural environment, which is necessary for recruitment.

Currently, there are few works focused on *M. tenellum* cultivation, particularly feeding and nutrition. Previous studies include García-Ulloa *et al*. (2004) who determined the effects of protein diets on growth, replacing fish meal with soybean meal and Espinosa-Chaurand *et al*. (2012) who determined the effect of levels of protein in diets on growth and survival of juveniles. Additional studies of feeding and nutrition are required to establish the type and balance of feed ingredients to

produce good results under cultivation. Espinosa-Chaurand (2013) describes enzyme activity in this species. However, enzymes that act on chitin are not well studied yet. This information is required because *Macrobrachium* prawns consume crustacean exoskeletons, which provide ingredients for new exoskeletons after molting (Kumar *et al*. 2006; Zhang *et al*. 2014). Chitin digestive enzyme in this prawn has not been studied (García-Guerrero *et al*. 2013).

Most of the works on the effect of chitin in the diet of crustaceans have been developed with penaeid species (Shiau & Yu 1998; Chih-hui & Chen-Chun 2012; Chang *et al*. 1988; Terwilliger 1999; Chang *et al*. 2001; Casillas-Hernández *et al*. 2006). For *Macrobrachium* prawns, Kumár *et al*. (2006) compared the effect of natural chitin and purified chitin on the growth of *Macrobrachium rosenbergii* (De Man, 1879).

Chitin in diets of prawns advance growth (Kumar *et al*. 2006), promotes antimicrobial activity in diets (Somjit *et al*. 2005), and supports molting (Zhang *et al.* 2014). In our study, we measured the effect of different levels of chitin in the diet for *Macrobrachium tenellum* juveniles on growth and digestive enzyme activity, with special emphasis on chitinases.

MATERIALS AND METHODS

Experimental design

A batch of *Macrobrachium tenellum* juveniles were collected in the *Chacahua* lagoon (15°58′20″N, 97°32′28″W) in Oaxaca, Mexico. They were transported in a 250 L tank with permanent aeration to laboratory facilities and then, acclimated during two weeks at 25°C. During this time, they were fed with marine shrimp commercial pelletized food (40% crude protein, Camaronina 40® Agribrands Purina, Mexico).

In order to evaluate chitin effect on growth, a completely randomized experimental design of five treatments was executed. Every treatment had a different amount of chitin, included in a isoproteic-isolipidic diet. Different chitin amounts were 5, 10, 15, 20 and 25% of the food. An experimental control (no chitin) was also included. The experimental diet was prepared in agreement with Cortes-Jacinto *et al.* (2003) and Espinosa-Charaund *et al.* (2012) with the following ingredients: *Cyprinodontid* fish meal (50.05%), soybean meal in bulk (25.03%), Maseca® commercial corn flour

(17.8%), and San Antonio® commercial wheat flour (7.12%) and vitamin and mineral supplements (Fervinac with selenium®, Laboratorios Brovel, Mexico). All ingredients were blended and mixed with an Oster® blender. Chitin was prepared by pulverizing dried shrimp carcasses (*Litopenaeus sp.*). The carcasses were cleaned by rinse and then, dried at sunlight. When completely dry, they were pulverized with the blender (Oster®) and added to the mixture. Then, the mixture was sieved through a 500 µm mesh and mixed again with the same blender. At this step, cornstarch were added as agglutinant. The resultant mixture was passed through a meat grinder in order to obtain 2 mm diameter pellets. Those pellets were dried in a Felisa® convection oven (Fabricantes Feligneos SA de CV; Mexico) at 30°C during 12 hours. Table 1 shows the proximate analysis of the diet, to establish the values for gross energy, the conversion factors suggested by Anh *et al*. (2009) were applied: 4.2 for carbohydrates; 5.56 for proteins; and 9.54 for lipids.

Table 1. Proximate analysis of tested diet. Analytical method: [1]Differences in moist and dry weight, [2]AOAC (micro-Kjendahl method), [3]AOAC (Soxhlet method), [4]AOAC (Weende method), [5]AOAC (oven at 550°C).

	g 100 g^{-1} of dry food Average value	SD
Humidity (%)[1]	4.783	± 0.37
Crude protein[2]	44.137	± 0.19
Total lipids[3]	6.517	± 0.20
Crude fiber[4]	1.557	± 0.09
Ash[5]	9.700	± 0.38
Nitrogen-free extract	38.090	± 0.77
Gross Energy (kcal g^{-1})	4.741	

Every treatment had six replicates and every replicate consisted on a dark non-translucid plastic container (50L). All replicas of the same treatment were placed together at different levels. In the container at the lowest level no specimens were placed. A water pump was placed with a hose that throw water to the highest tray. Every tray had an outcome flowing to its lower next. This way, a cascade is produced and water is constantly recirculating through all containers of the same treatment. In every treatment, water temperature was maintained always at 28°C, with submersible 300 W heaters (RENA Aquatic Supply; US) installed in the lowest non-specimen tray of every treatment. Oxygen concentration [O_2] was daily measured with a Hanna® HI98186 oximeter (Hanna Instrumenta Inc; Italy) and maintained always above 5 mg [O_2/L]. The photoperiod was 12 h light - 12 h dark. In each container, a 0.5 m^2 of plastic mesh was placed inside the water to provide shade and shelter. Ten organisms (0.2 ± 0.04 g) were placed in every container. A week before the first

day of the experiment, total daily food was provided at 7% of the prawn biomass. After one week, the ration was adjusted to 10% of their body weight for all treatments. They were fed every day at 18:00 h. Survival was registered daily. The trial lasted 60 days. At days 0, 15, 30, 45, and 60, all prawns were individually weighed (std ±0.001 g).

The following parameters were obtained: Average individual weight gain (g): AIWG = final weight– initial weight; Gain weight per day (g day^{-1}): GWPD = (final weight-initial weight) t^{-1}); Gain weight in percentage (%): GW = 100 x (final weight – initial weight)/initial weight); and specific growth rate (%): SGR = [(Ln final weight – Ln initial weight) t^{-1}] ×100. Also the Feed conversion ratio was calculated as: FCR = supplied food (g)/weight gain (g); and Feed efficiency ratio: (FER) = weight gain (g)/supplied food (g). SGR and FCR were calculated in agreement with Cortes-Jacinto *et al.* (2003); Gitte and Indulkar (2005) were followed for GW and GWP; Vega-Villasante *et al.* (2011) for AIWG and SR; and Hasan *et al*. (2012) for FER.

Enzyme activity

For measuring enzymatic activity, two prawns from each replica (eight by treatment) were randomly selected on days 0, 30, and 60. These were weighed as fresh weight and kept at -20°C until analysis. Only the hepatopancreas and intestine were analyzed. By the size of juvenile prawns the anterior section of head, all appendages, and the exoskeleton were discarded during dissection. The remaining tissue after dissection were individually weighed and separately homogenized with cold distilled water (4°C) in a v/w proportion of four mL water for g fresh organ tissue. Raw extracts were separated by centrifugation at 14 000 rpm for 10 min at 4°C, and clarified crude extract was kept at -40°C until analyzed for soluble proteins and enzyme activity. All measurements were made in quadruplicate for all enzymatic analysis, a control sample (blank) was also included, where the enzyme reagent was added after the reaction ceased. Protein concentrations in the enzyme raw extracts were quantified by the Bradford method (1976). In glass tubes (100 mm × 15 mm), 8 µL of crude extract, 792 µL distilled water, and 200 µL Bradford reagent were mixed and gently vortexed. Absorbance was measured at 595 nm. Bovine serum albumin (05470, Sigma-Aldrich, St. Louis, MO) was used as the protein standard. Lipase activity was determined as described by Versaw *et al*. (1989), using β-naphthyl caprylate as the substrate. Lipase activity was expressed as lipase units/mg protein (one lipase unit was the quantity of enzymes required for an increase of 0.01 absorbance units at 540 nm min^{-1}). Amylase activity was measured, as described by Vega-Villasante *et al*. (1993), using 1% starch in 50 mM Tris-HCl at pH 7.5, as the substrate. This activity was expressed as amylase units/mg

protein (one amylase unit was defined as the quantity of enzyme to increase absorbance units by 0.01 at 550 nm min^{-1}). Trypsin activity was determined, using BAPNA as the substrate (García-Carreño & Haard, 1993), adapted to a 96-well microplate by adding 10 µL crude extract, 160 µL 60 mM Tris-HCl at pH 8.0, 10 µL 192 mM $CaCl_2$ at pH 8.0), and 10 µL 9.6 mM BAPNA dissolved in DMSO, in each well to start the reaction. Chymotrypsin activity was determined, using 9.6 mM SAAPNA dissolved in DMSO. For both enzymes, absorbance at 414 nm was recorded every 15 s for 30 min. At the end of the assay, a linear coefficient was calculated to determine the increase of absorbance per second. Enzyme activity was calculated using molar extinction coefficient of ρ-nitroaniline (8800). Chitinase activity was determined by mixing 20 µL of crude extract, 50 µL 60mM Tris-HCl at pH 8, and 530 µL substrate (C3020, Sigma-Aldrich). The reagent mixture was shaken with a vortex at 120 rpm (at 45º angle) for incubation during 2 h. The reaction was stopped by centrifugation after 5 min at 14,000 rpm for 10 min at 4°C. The supernatant was separated immediately and absorbance was measured at 570 nm (one chitinase unit was defined as the quantity of enzymes required for an increase of 0.001 absorbance units at 570 nm min^{-1}).

Statistical analysis

Survival rate (%), Average individual weight gain (g), Gain weight per day (g day^{-1}), Specific growth rate (%), Feed conversion ratio, Feed efficiency ratio and Enzyme activity (U/mg Prot Sol) were determined and then compared by one way ANOVA test. Normality tests were performed (Kolmogorov-Smirnov test, $\alpha = 0.05$). Significant differences between treatment were determined by the Duncan multiple range test ($P > 0.05$). Minitab 17 statistical package (Minitab, College Park, PA) was utilized for statistical analyses.

RESULTS

During the 60 day trials, the water in all experimental units were maintained at 28.3°C, pH 7.5, and 6.8 mg L^{-1} O_2. After 60 days, only the growth parameter of absolute weight was statistically significant (Fig. 1).

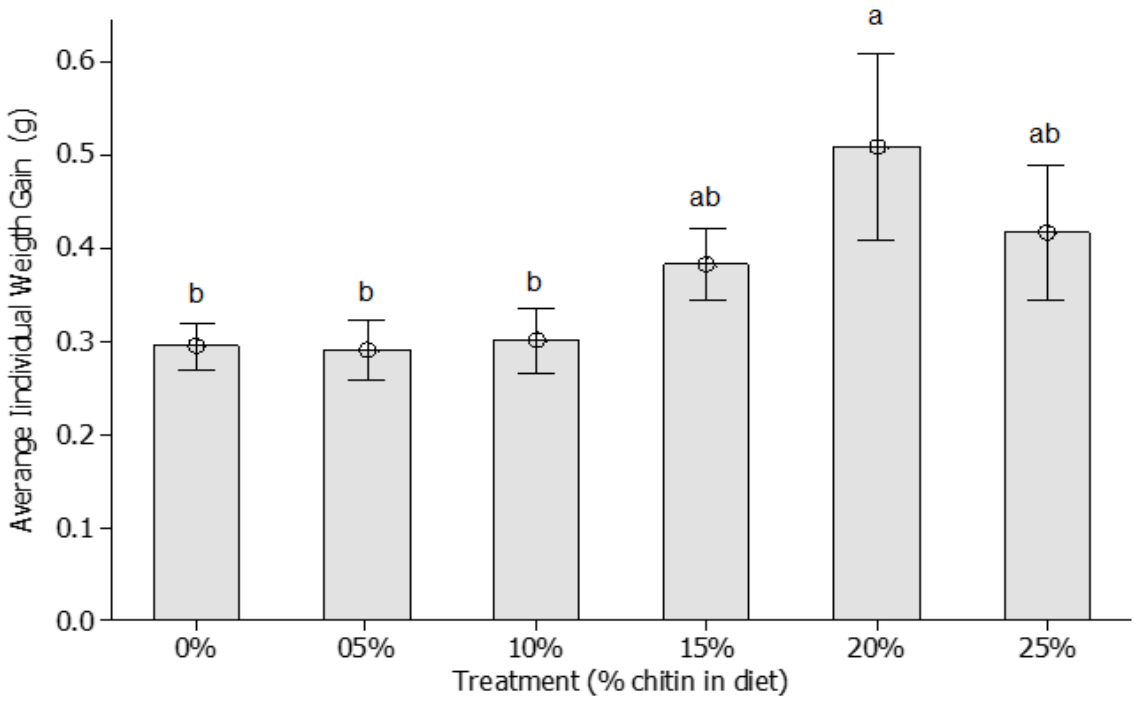

Figure 1. Average individual weight gain (g) of *Macrobrachium tenellum*: effect of chitin level in diet. Same letters represent no differences between treatments (P = 0.05). Bars represent amount of chitin in each treatment. Whisker lines are SE from the mean

The range of each growth parameter was: GWPD (0.0048 to 0.0085 g day^{-1}); GW (147.1 to 286.5%); SGR (1.42 to 1.88%); FCR (3.86 to 2.91); and FER (2.72 to 3.90). Also, Table 2 shows growth (weight) per treatment.

Table 2. Parameters of growth in weight for each treatment of chitin added to diet. AIWG = Average individual weight gain; GWPD= Gain weight per day; GW= Gain weight in percentage; SGR= Specific growth rate; FCR= Feed conversion ratio, and FER= feed efficiency ratio. Values with same letter do not show statistically significant differences (P= 0.05), using Duncan multiple range test.

Treatment	AIWG (g)	GWPD (g day^{-1})	GW (%)	SGR (%)	FCR	FER
0% chitin	0.294 ± 0.025**b**	0.0049 ± 0.00041**b**	151.3 ±15.1**a**	1.48 ± 0.09**a**	3.53 ± 0.31**a**	0.296 ± 0.029**a**
5% chitin	0.291 ± 0.033**b**	0.0048 ± 0.00055**b**	150.8 ±17.0**a**	1.45 ± 0.11**a**	3.77 ± 0.51**a**	0.295 ± 0.047**a**
10% chitin	0.301 ± 0.036**b**	0.0050 ± 0.00060**b**	147.1 ±17.4**a**	1.42 ± 0.11 **a**	3.86 ± 0.40**a**	0.272 ± 0.026**a**
15% chitin	0.383 ± 0.038**ab**	0.0064 ± 0.00063**ab**	201.7 ±23.2**a**	1.74 ± 0.12**a**	3.53 ± 0.30**a**	0.293 ± 0.022**a**
20% chitin	0.508 ± 0.101**a**	0.0085 ± 0.0017**a**	286.5 ±72.6**a**	1.88 ± 0.20**a**	2.91 ± 0.39**a**	0.390 ± 0.071**a**
25% chitin	0.417 ± 0.073**ab**	0.0070 ± 0.0012**ab**	271.9 ±66.2**a**	1.78 ± 0.22**a**	3.50 ± 0.55**a**	0.329 ± 0.060**a**

The inclusion of 20% in one diet produced the best average individual weight gain (0.508 ± 0.101 g) and the best gain weight per day (0.0085 ± 0.0017 g day^{-1}). Treatment with 15% and 25% chitin were less successful. The best growth rate occurred by day 45, afterward declining. The least growth occurred in the 10% and 5% chitin treatment and in the control treatment (Fig. 2). There was no statistically significant difference in survival between treatments, ranging from 72.2% (treatment 5) to 83.3% (treatments 1, 2, 3, and control). For FCR, the best treatments were the 20% chitin (2.91 ± 0.39) and 25% chitin (3.50 ± 0.55), with the highest feed conversions around day 45.

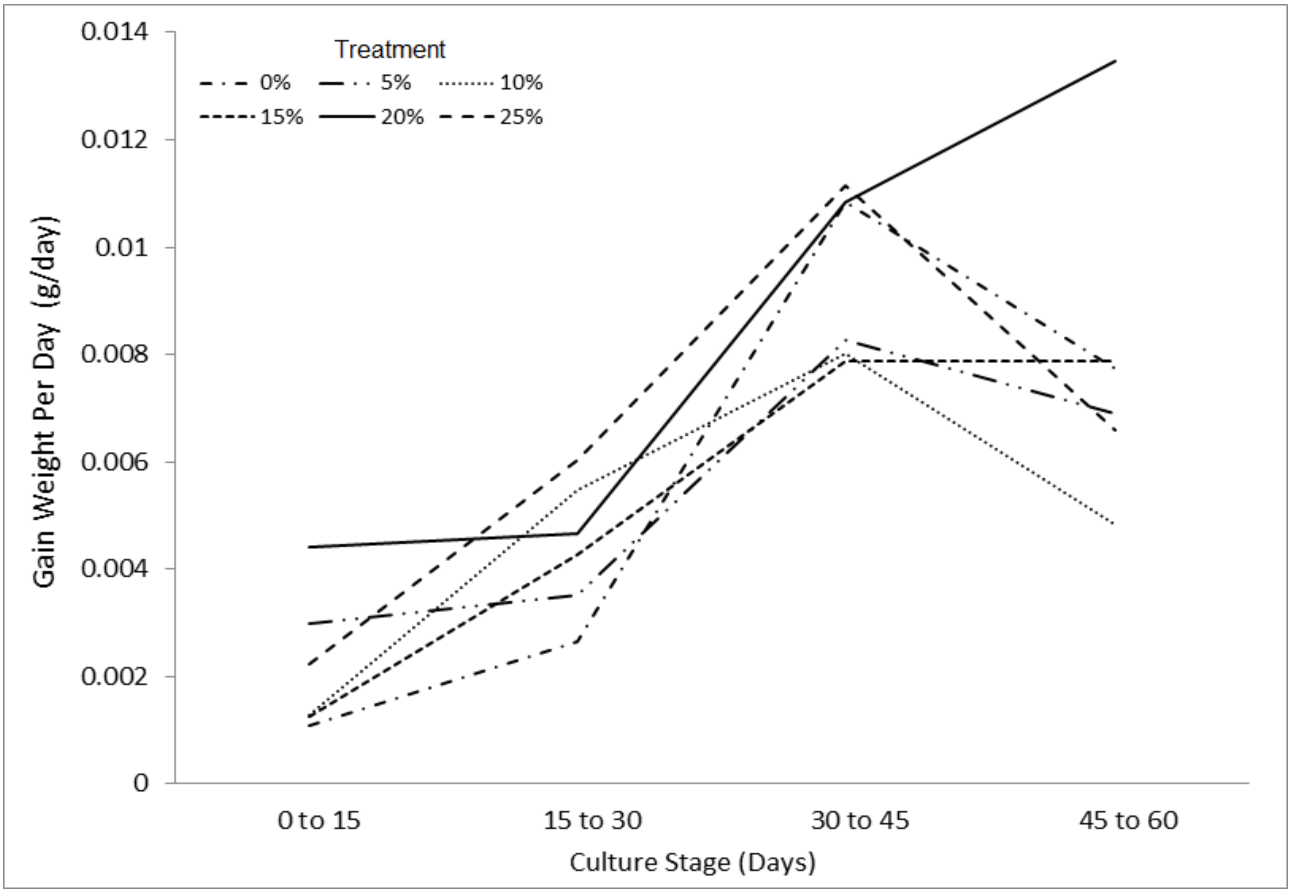

Figure 2. Gain weight per day (g day^{-1}) of *Macrobrachium tenellum*: effect of chitin amount in diet, at four intervals in trials. Chitin amount ranges from 0% to 25%.

For soluble protein concentrations, there were no statistically significant differences between treatments. Chitinase activity was the only enzymatic activity with statistically significant differences between treatments. Other enzymes had similar trends in the final phase of the culture (Fig. 3). Like specific growth data (see Fig. 2), chitinase activity was highest at day 45, declining thereafter to the end of the trials (Fig. 4), differing from lipase, amylase, trypsin, and chymotrypsin enzyme activity.

DISCUSSION

The water physicochemical conditions were always maintained as required by *Macrobrachium tenellum* in culture, as stated in previous studies (Espinosa-Chaurand *et al.* 2011; Vega-Villasante *et al*. 2011; and García-Ulloa *et al*. 2008. For this species, the best growth used to be observed at a water temperature range from 28-32°C in agreement with Espinosa-Chaurand *et al*. (2011). Schiff & Hendrickx (1997) and Vega-Villasante *et al*. (2011) report that *M. tenellum* in culture grows very well with 5.0 mg L^{-1} [O_2]. For pH, Hernández *et al*. (2007) and Vega-Villasante *et al.* (2011) suggest pH from 7.5 to 8.5 for *M. tenellum* cultivation conditions.

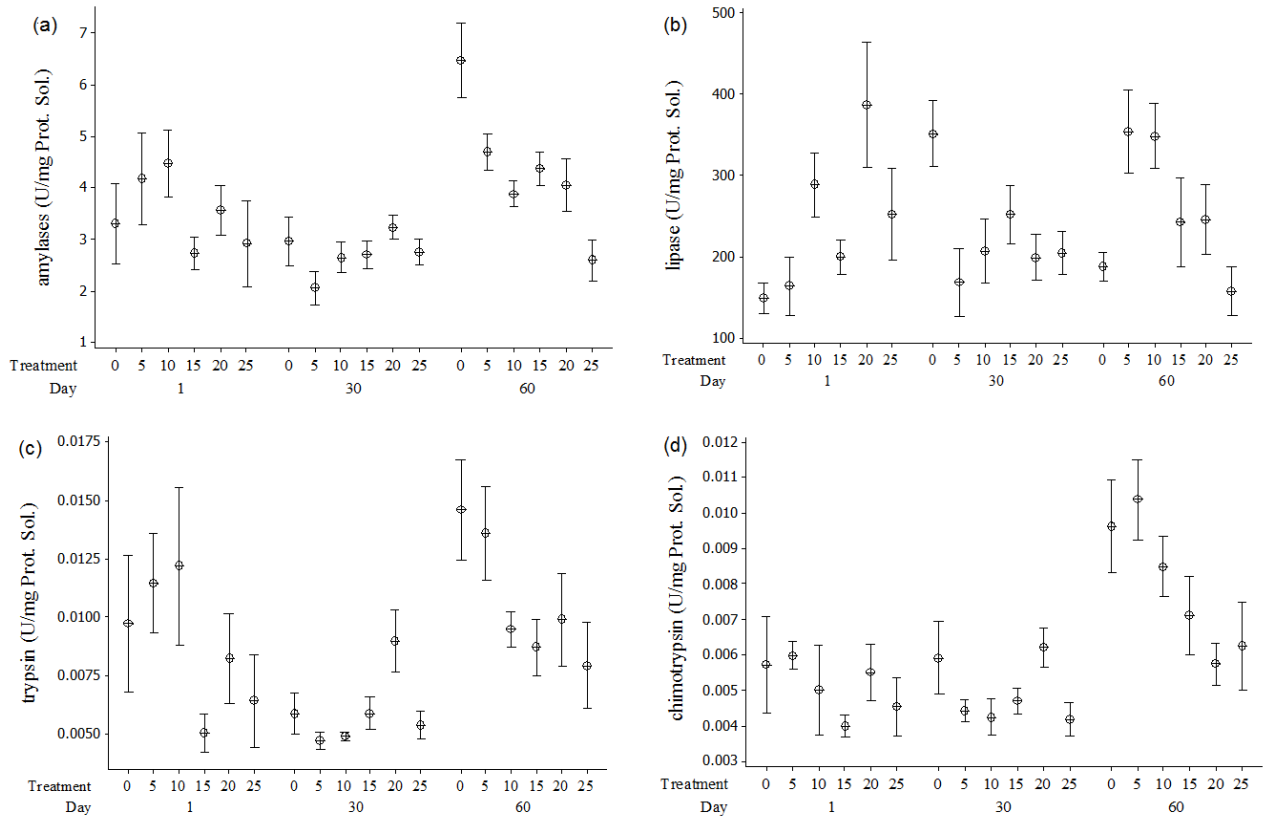

Figure 3. Effect amount of chitin in the diet on the digestive enzyme activity in *Macrobrachium tenellum*: a) Amylase, b) Lipase, c) Trypsin, and d) Chymotrypsin. Samples taken on days 1, 30, and 60. Treatments are chitin amounts in diet (0%, 5%, 10%, 15%, 20%, and 25%). Whisker lines are SE from the mean.

Two trends on survival rate were observed in present work. Prawns fed chitin at 0%, 5%, 10%, and 15% had an average survival of 83.3%. Prawns fed 20% and 25% chitin had lower survival (75% and 72.2%, respectively). Lower content of chitin apparently had no direct effect on survival,

what is in agreement with previous studies. Kumar *et al*. (2006), and Shiau & Yu (1998) studied chitin and shrimp head meal, respectively, on survival of *M. rosenbergii* and *Penaeus monodon* (Fabricius, 1798). Kumar *et al*. (2006) find a survival of 40–80% while Shiau & Yu (1998) reported 74–84%, without significant differences or correlation between the amount of chitin and survival. These results are similar to present work although a precise comparison cannot be executed

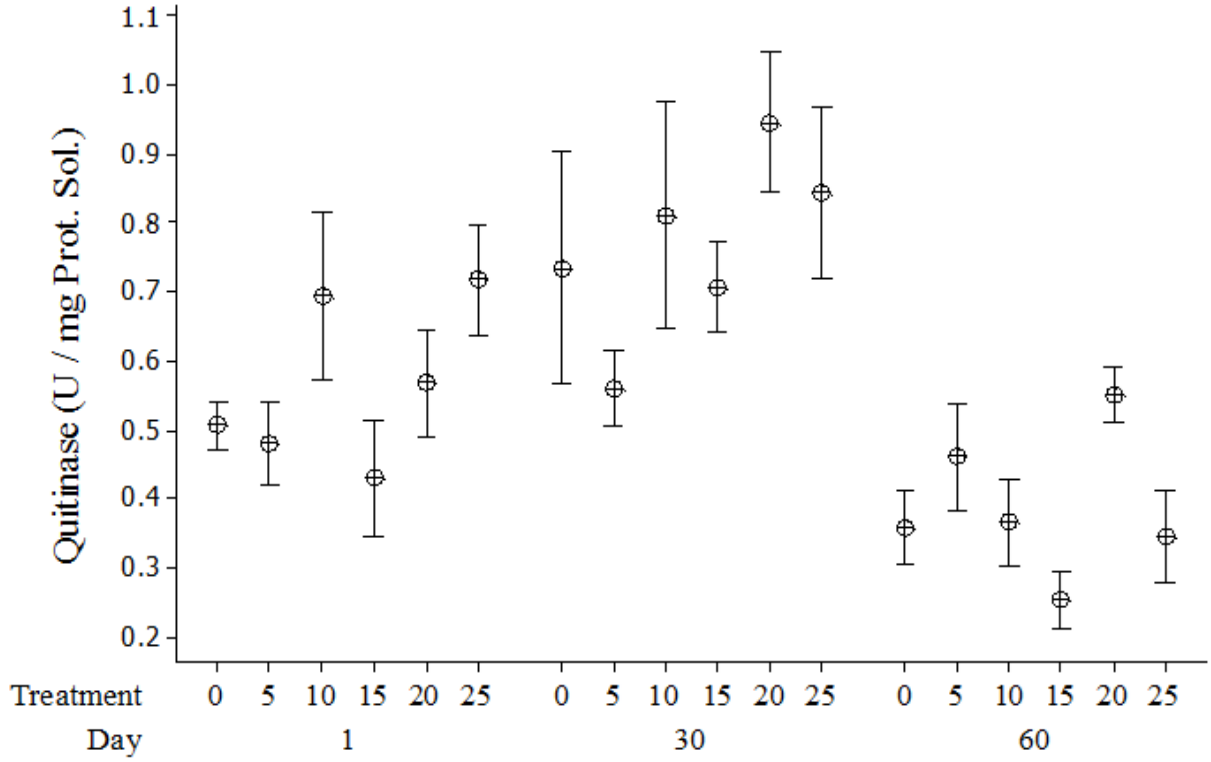

Figure 4. Chitinase activity in *Macrobrachium tenellum*: effect of the amount of chitin in the diet. Treatments are chitin amounts in diet (0%, 5%, 10%, 15%, 20%, and 25%). Sampling times: days 1, 30, and 60. Whisker lines are SE from the mean.

Previous studies with present species includes the works of Espinosa-Chaurand *et al*. (2012), who fed *M. tenellum* crude protein in diets (20, 25, 30, 35, and 40%) and maintained 95 to 100% survival, without statistical differences between treatments. In contrast, Ponce-Palafox *et al*. (2006) reported 50% survival after prawns were fed a diet containing 25% of protein. The most common cause of death during cultivating prawns is from cannibalism. Common causes for this behavior are lack of shelters or large variations in size among individuals in the same tank (García-Guerrero & Apun-Molina 2008; Nair *et al*. 1999) observe that adding chitin to the diet reduced cannibalism in *M. rosenbergii*. It is important to state that the only chitin sorce was form diet, since every time one specimen moult, the exuviae was inmediatly removed from the tank. Survival of 75 and 72% in diets

containing 20 and 25% chitin, respectively, had the lowest survival, despite having the highest growth. There were no significant differences in survival among treatments and it is suggested that different diets do not influence survival since chitin variation effect could be mostly on growth rate.

Present results suggest that the adding of chitin in the diet has a statistically significant effect on *M. tenellum* growth. The highest average individual weight gain (AIWG = g) was obtained with the 20% treatment. Chitin in the di*et al*so increases chitinase activity, which was evident at day 30, when the highest weight gain per day (GWPD = g day^{-1}) occurred. The GWPD are in agreement with Ceccaldi (1989) and Zhang *et al*. (2014) who state that chitinase is essential for digestion and molting. However, molt frequency were not determined in present study so its direct relation with growth could not be determined even considering that in present study, a decrease in chitinase activity occurred when growth rate decreased. As Lemos *et al*. (2000) find, different growth stages engenders different feeding behavior and different digestion tract functional structures, thus, different digestion capabilities. This contributes to differences in enzyme activity, as observed in shrimp by Chang *et al*. (1988). Casillas-Hernández *et al*. (2002) observe that enzymatic, hormonal, and environmental issues are involved with molting, causing an indirect effect on growth rate. Chitin at 15% and 25% in the diet contribute to molting, as reported by Chang (1995). In present study, treatments containing 20%, 25%, and 15% chitin produced better weight gain in prawns. Prawns fed diets containing 10%, 5%, and 0% chitin had poorer weight gain. Statistical differences ($P = 0.046$) suggest that small variations in the amount of chitin may not affect weight gain in a measurable way, consequently, no differences in specific growth rates, increase in weight; feed conversion, and feed conversion efficiency were found. Kumar *et al*. (2006) report a similar trend in growth rate, using diets with different chitin content for *M. rosenbergii*. They report that diets containing 5% natural chitin (22% shrimp head meal), 10% natural chitin (44% shrimp head meal), and 5% purified chitin produce good weight gain and low feed conversion ratio. Shiau & Yu (1998) report a high weight gain in giant tiger shrimp (*Penaeus monodon*) fed a diet containing 22% chitin (equivalent to 5% purified chitin). They state that chitin in diets that is >22%, reduces weight gain. Fox (1993) mentions that natural chitin from shrimp head meal has high nutritional value for *P. monodon*, compared to purified chitin. In his study, there were not significant differences in growth where diets contained 0%, 4%, 8%, 12%, or 16% natural chitin. It seems that similar percentages of chitin can produce different results in different species. Prawns may take better advantage of dietary chitin compared to shrimp. Ceccaldi (1989) states that there is no difference in the use of chitin or other carbohydrates between shrimp and prawn digestive tracts; however, there are differences in tissue and biochemical activity (Cruz-Suarez 2002). More research is required before conclusions on this topic.

In *M. tenellum*, Espinosa-Chaurand (2013) states that different concentrations of digestive enzymes in each phase of the life cycle are related to protein amount in diets and with circadian rhythm, since alterations of these variables affects proteolytic activity of chymotrypsin, trypsin, lipase, and amylase. Since we used identical protein diets, enzyme activity did not differ significantly between treatments, however, lowest activity was observed for all enzymes during the middle of the trial (day 30). Lower physiological activity was observed by Espinosa-Chaurand (2013). This depends on fitness, metabolic rate, and nutrient requirements of specific stages (Vega-Villasante *et al*., 1999). Casillas-Hernández *et al*. (2006) report a relationship between feeding and enzyme activity, caused by demands of each developmental stage and production of biomass. Alexandre *et al*. (2014) and Cordova-Murrueta *et al*. (2003) also consider variations in enzyme activity as responses to growth stage, molting, digestion, and nutritional condition. These factors, together, determine enzyme activity independent of dietary ingredients. Other factors also influence enzyme activity. Since enzyme activity was not significantly different between treatments, growth also depends on other factors, such as availability and expenditure of energy, as in the case of *Penaeus monodon* (Chen & Lin 1989).

It was also found an inverse relationship between enzymatic activity and digestion of proteins, lipids, and carbohydrates and the amount of chitin in the diet. Kumar *et al*. (2006) observe a decrease in digestibility with an increase in dietary chitin. They used purified chitin, which counteracts hydrolytic activity of other enzymes, probably because chitin was present in excess. Chitin content at 20% increases chitinase, compared to diets with less chitin ($P < 0.01$). Similar to their findings, we observed significant differences in enzyme activity between different stages ($P < 0.01$), chitinase activity peaked at day 30 and lower at the beginning and end of the trial. We also observed that from days 30 to 60, when chitinase activity increases, activity of all other digestive enzymes decreased. Additionally, when chitinase activity declines, lipase and amylase activity increased, supporting the hypothesis of an inverse relation between chitinase and other digestive enzymes.

Another relationship was a decline in growth rate at high chitin content in the diet. The likely cause is an inability to absorb or metabolize big amounts of glucosamine if all dietary chitin was digested. This was observed in *M. rosengergii* (Kumar *et al.* 2006) and *P. monodon* (Fox 1993). These authors suggest that this response could be ameliorated with a diet rich in lipids, proteins, and minerals. In fish diets, containing chitin, Lindsay (1984) and Danulat (1986) report that chitinase activity could be increased with dietary protein. However, in crustaceans, this did not occur with diets

containing purified chitin, but this chitinase activity has occurred in the basal region of the peritrophic membrane in the gut (Ceccaldi 1989; Alexandre *et al*. 2014). It is likely that the same mechanism occurs in *M. tenellum* because chitin is re-absorbed when chitinase dissolves the old exoskeleton into a more soluble form in the integument, which is common in decapods (Dall *et al*. 1990; Chang, 1995; Shechter *et al*. 2007; Stillman *et al*. 2008).

In addition to chitin level in the treatments, variations in chitinase activity may be influenced by prawn physiological reactions to water conditions, such as temperature and dissolved oxygen, as reported by Alexandre *et al*. (2014) concerning enzyme activity in the whiteleg shrimp *Litopenaeus vannamei*. In *Macrobrachium nipponense* (De Haan 1849), Zhang *et al*. (2014) found variations in chitinase activity by diet induced, but not from size, developmental stage, hierarchical position, molting stage or acclimation time.

In summary, diets containing 20% natural chitin produced the best growth of *M. tenellum*. Further research should focus in the study of how some variables such as temperature or photoperiod in the culture may affect chitin digestion causing differences in enzymatic activity, particularly chitinases. In addition, further research is needed in the relationship between the molting cycle and chitin levels in the diet to determine the impact of the chitin amount and quality on the molt cycle and growth.

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ANEXO 1 - 1

Colecta de juveniles de *Macrobrachium tenellum*, en la boca barra de Cerro Hermosos, San Pedro Tututepec, Oaxaca. Método de colecta manual mediante arrastre.

Foto: M. García G., 2013
CIIDIR Oaxaca

ANEXO 1 - 2

Unidades experimentales para el trabajo experimental con juveniles de *Macrobrachium tenellum*, Unidad experimental acuícola del CIIDIR unidad Oaxaca.

Foto: M. García G., 2013
CIIDIR Oaxaca

ANEXO 1 - 3

Elaboración de alimento

Insumos para preparar el alimento de los langostinos, basado en el trabajo realizado para la misma especie por Espinosa-Chaurand *et al*. (2012)

	CANTIDAD	FACTOR	% PROTEINA
H pescado	50.05	0.6	30.03
H soya	25.03	0.49	12.26
H trigo	17.80	0.12	2.14
H maíz	7.12	0.08	0.57
			45

Foto: RdelosSR, 2013
CIIDIR Oaxaca

ANEXO 1 - 4

CURVA ESTANDAR PARA PROTEINAS SOLUBLES

Objetivo: determinar la curva estándar adecuada, para realizar las pruebas de proteínas solubles

Se preparó una solución Stock de albumina de 1.0 mg/ml aprox =.001 g/ml. Con la solución stock se prepararon las siguientes concentraciones.

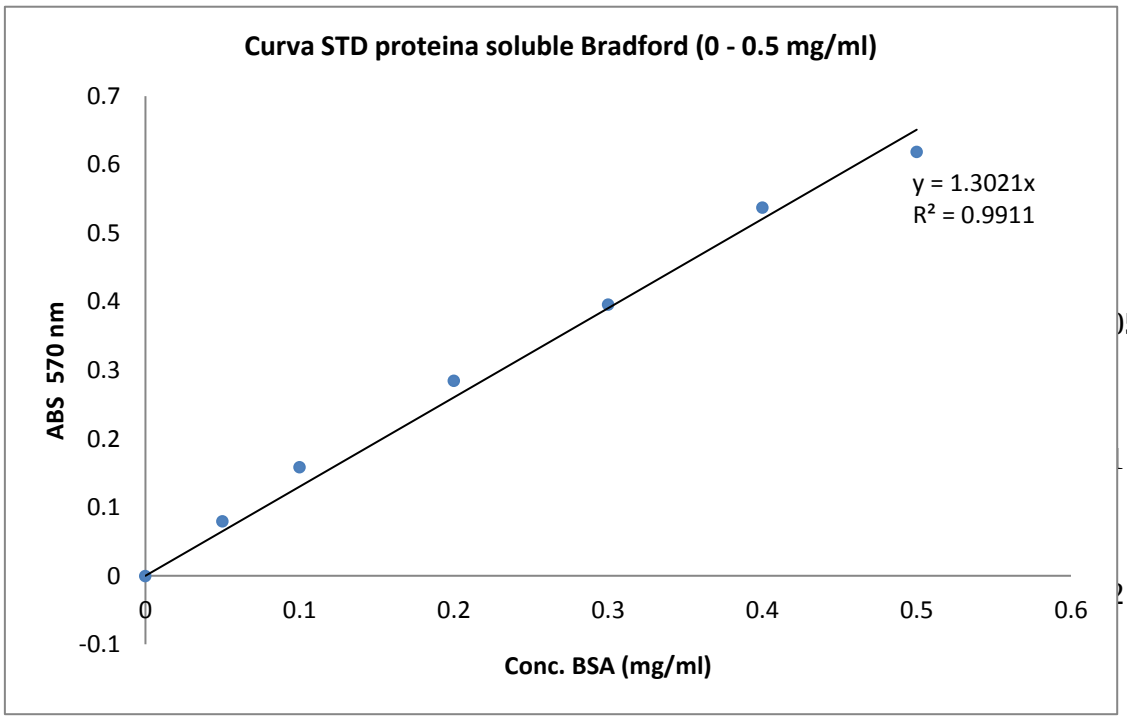
Conc BSA mg/ml	Vol. Stock	Vol. agua
0	0	100
0.05	5	95
0.1	10	90
0.2	20	80
0.3	30	70
0.4	40	60
0.5	50	50

El volumen de la solución stock y del agua es en µl

Se utilizaron las anteriores concentraciones para generar la curva estándar. Cada concentración se preparó por cuadruplicado. Además de utilizar como blanco la concentración 0.0

Cada pozo de la placa terminó con la siguiente mezcla:
10 µl de EC (agua para el blanco)
200 µl de Solución Bradford (1:5)

La placa se agitó en 8 y se dejó incubar durante 5 minutos
Se realizó la lectura de las absorbancias de la placa a 570 nm



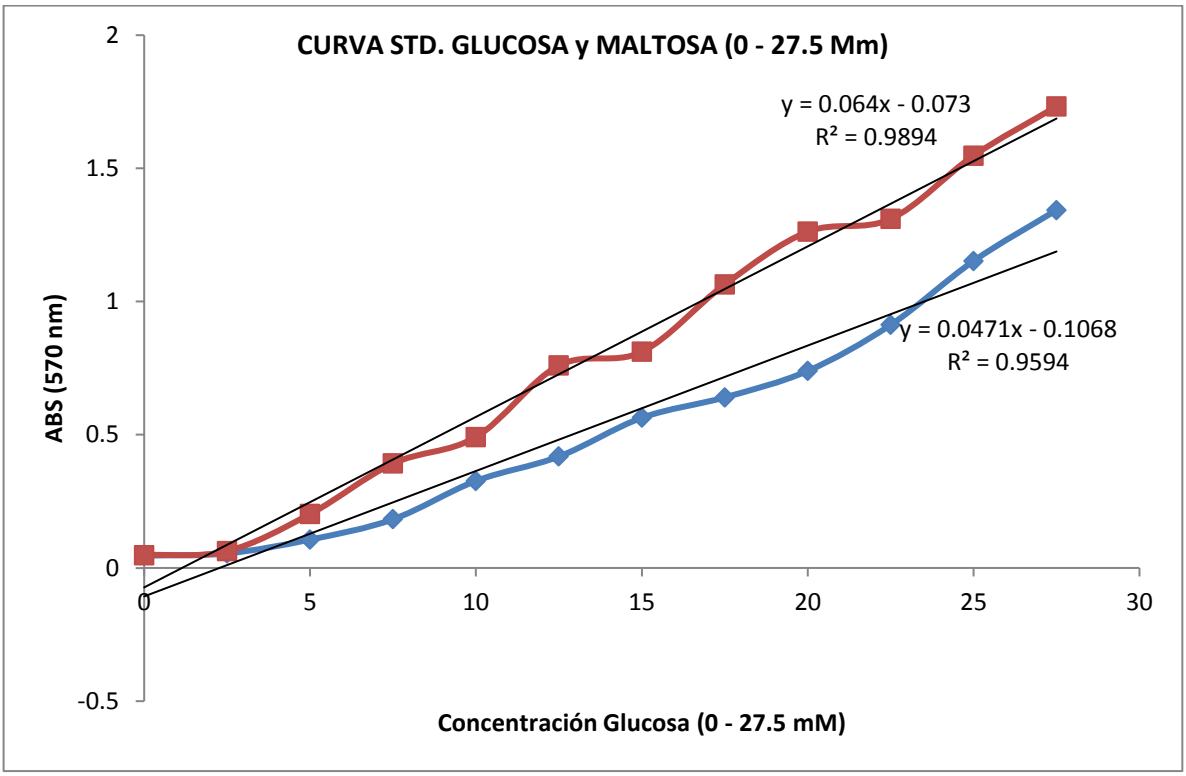
ANEXO 1 - 5

CURVA ESTANDAR PARA AMILASAS

Preparación de las soluciones de glucosa y maltosa 30 mM

	0 mM	2.5 mM	5 mM	7.5 mM	10 mM	12.5 mM	15 mM	17.5 mM	20 mM	22.5 mM	25 mM	27.5 mM
µl de Glucosa o Maltosa 30 mM	0	250	500	750	1000	1250	1500	1750	2000	2250	2500	2750
µl de agua destilada	3000	2750	2500	2250	2000	1750	1500	1250	1000	750	500	250
µl de volumen final	3000	3000	3000	3000	3000	3000	3000	3000	3000	3000	3000	3000

Los EC de las muestras fueron utilizadas sin diluir. Se agrego a cada pozo
10 µl de EC
25 µl de TRIS (100 mM) pH 8.0
20 µl del Almidon 1% en TRIS (100 mM)
20 µl de Carbonato de Calcio 1.0 M
100 µl de solución DNS
La lectura de la ABS se realizó a 540 nm



ANEXO 1 - 6

CURVA ESTANDAR PARA LIPASAS

*Preparación de β-Naftol 50mM (Pesar 0.0072g de naftol y agregar 1000µL de DMSO)

Diluciones:
*2500µM (25µL de 50mM + 475µL de DMSO)
*2000µM (320µL de 2500µM + 80µL de DMSO)
*1500µM (120µL de 2500µM + 80µL de DMSO)
*1000µM (200µL de 2000µM + 200µL de DMSO)
*800µM (160µL de 1000µM + 40µL de DMSO)
*600µM (120µL de 1000µM + 80µL de DMSO)
*400µM (80µL de 800µM + 80µL de DMSO)
*200µM (40µL de 2000µM + 360µL de DMSO)
*100µM (100µL de 200µM + 100µL de DMSO)
*50µM (100µL de 100µM + 100µL de DMSO)
*25µM (100µL de 50µM + 100µL de DMSO)
*0µM (Agua destilada)

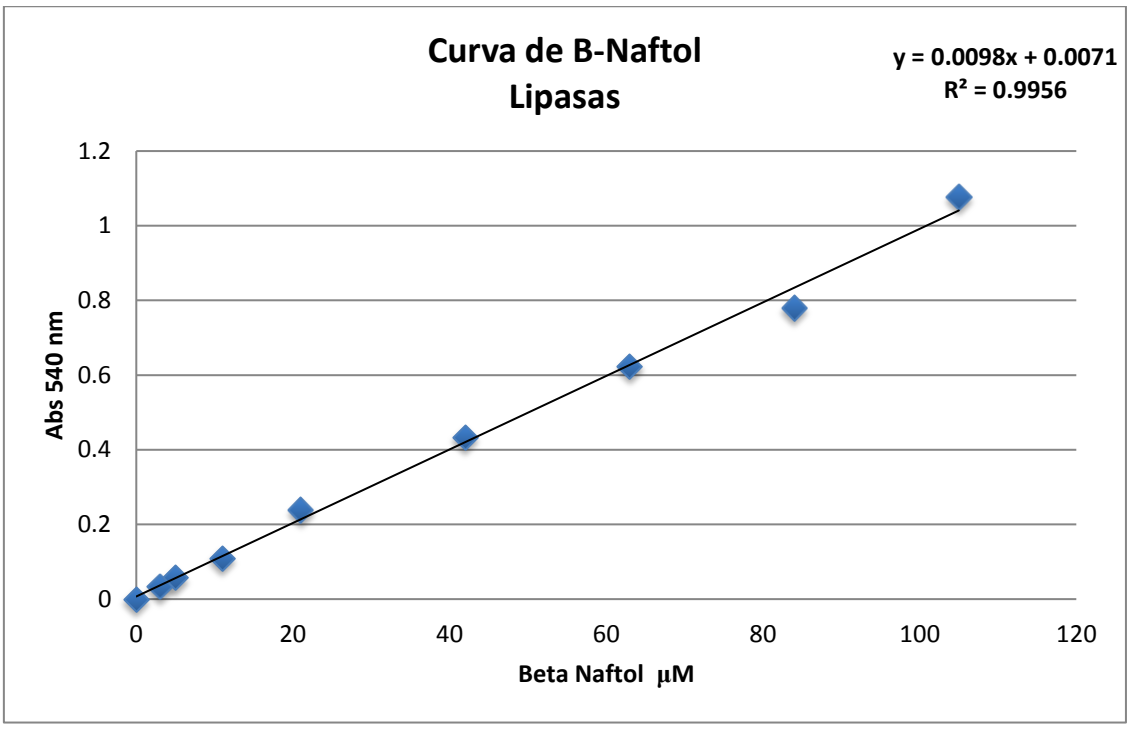
Stock, µL	Cantidad, µL	Vol. Total, µL	Concentracion de reaccion, µM
2500	20	190	263
2000	20	190	211
1500	20	190	158
1000	20	190	105
800	20	190	84
600	20	190	63
400	20	190	42
200	20	190	21
100	20	190	11
50	20	190	5
25	20	190	3
0	20	190	0

Se agregó a cada pozo las soluciones y cantidades indicadas abajo:

Reactivo	µL / pozo
T-Na (9.6 mM)	10
Tris, 200mM, pH=8.0	40
Extracto crudo	20
Incubación 9.5 min	
FBBB (19.2 mM) en DMSO	10
SDS (12.5%)-TCA 2%	110
Volumen total	**190**

**Para la curva, se agregaron las diferentes soluciones de naftol en lugar de extracto crudo.
Agitación en "8" sobre la mesa
Reposo de 5 minutos
Agitación en el equipo por 3 minutos a 900 rpm
Lectura a 540nm en espectofotómetro

CONC µM	0	3	5	11	21	42	63	84	105
ABS 540 nm	0	0.03325	0.057	0.10933	0.23875	0.433	0.624	0.7795	1.0773



ANEXO 1 – 7

PROCEDIMIENTO PARA DETERMINAR QUIMOTRIPSINA

Los EC de las muestras NO fueron diluidos
Se agrego a cada pozo
10 µl de EC sin dilución
160 µl de TRIS -HCl (60 mM) pH 8.0
10 µl del $CaCl_2$ en TRIS-HCl pH 8.0
10 µl de BAPNA (9.6 mM)
Se agita por 30 s a 1500 rpm
La lectura de la ABS se realizó a 414 nm durante 30 minutos con lecturas cada 15 segundos

A los resultados de la ABS de las muestras se les resto el valor de la ABS del blanco.
El valor de la ABS del blanco se obtiene con el uso agua destilada en lugar de EC

Se realizó el cálculo inicial de la actividad de la quimotripsina de las muestras (U/ml)

$$\text{Unidades Actividad} = \frac{A(410)/10\text{min}) \times (1000) \times (\text{vol de la MR})}{8800 \times \text{mg proteina}}$$

Utilizando para esto el valor del CEM de la **ρ-nitroanilina** el cual es de 8800
Se multiplico por 2 para ajustar la altura de la cubeta

ANEXO 1 – 8

PROCEDIMIENTO PARA DETERMINAR TRIPSINA

Los EC de las muestras NO fueron diluidos
Se agrego a cada pozo
10 µl de EC sin dilución
160 µl de TRIS -HCl (60 mM) pH 8.0
10 µl del $CaCl_2$ en TRIS-HCl pH 8.0
10 µl de SA APNA (9.6 mM)
Se agita por 30 s a 1500 rpm
La lectura de la ABS se realizó a 414 nm durante 30 minutos con lecturas cada 15 segundos

A los resultados de la ABS de las muestras se les resto el valor de la ABS del blanco.
El valor de la ABS del blanco se obtiene con el uso agua destilada en lugar de EC

Se realizó el cálculo inicial de la actividad de la tripsina de las muestras (U/ml)

$$\text{Unidades Actividad} = \frac{A(410)/10\text{min}) \times (1000) \times (\text{vol de la MR})}{8800 \times \text{mg proteina}}$$

Utilizando para esto el valor del Coeficiente de Extinción Molar (CEM) de la **ρ-nitroanilina** el cual es de 8800
Se multiplico por 2 para ajustar la altura de la cubeta

ANEXO 1 – 9

ANOVA DE LA ACTIVIDAD ENZIMÁTICA

One-way ANOVA: lipases (U/mg Prot. Sol.) versus Treatment

Source	DF	SS	MS	F	P
Treatment	5	83414	16683	1.20	0.314
Error	102	1417293	13895		
Total	107	1500707			

S = 117.9 R-Sq = 5.56% R-Sq(adj) = 0.93%

One-way ANOVA: lipases (U/mg Prot. Sol.) versus Day

Source	DF	SS	MS	F	P
Day	2	11979	5990	0.42	0.657
Error	105	1488728	14178		
Total	107	1500707			

S = 119.1 R-Sq = 0.80% R-Sq(adj) = 0.00%

One-way ANOVA: amylases (U/mg Prot. Sol.) versus Treatment

Source	DF	SS	MS	F	P
Treatment	5	22.17	4.43	1.95	0.093
Error	102	231.93	2.27		
Total	107	254.10			

S = 1.508 R-Sq = 8.73% R-Sq(adj) = 4.25%

One-way ANOVA: amylases (U/mg Prot. Sol.) versus Day

Source	DF	SS	MS	F	P
Day	2	46.75	23.38	11.84	0.000
Error	105	207.35	1.97		
Total	107	254.10			

S = 1.405 R-Sq = 18.40% R-Sq(adj) = 16.85%

One-way ANOVA: chimotrypsins (U/mg Prot. Sol.) versus Treatment

Source	DF	SS	MS	F	P
Treatment	5	0.0000657	0.0000131	1.91	0.099
Error	102	0.0007017	0.0000069		
Total	107	0.0007674			

S = 0.002623 R-Sq = 8.56% R-Sq(adj) = 4.08%

One-way ANOVA: chimotrypsins (U/mg Prot. Sol.) versus Day

Source	DF	SS	MS	F	P
Day	2	0.0002024	0.0001012	18.81	0.000
Error	105	0.0005650	0.0000054		
Total	107	0.0007674			

S = 0.002320 R-Sq = 26.38% R-Sq(adj) = 24.97%

One-way ANOVA: trypsins (U/mg Prot. Sol.) versus Treatment

Source	DF	SS	MS	F	P
Treatment	5	0.0002235	0.0000447	1.94	0.094
Error	102	0.0023514	0.0000231		
Total	107	0.0025749			

S = 0.004801 R-Sq = 8.68% R-Sq(adj) = 4.20%

One-way ANOVA: trypsins (U/mg Prot. Sol.) versus Day

Source	DF	SS	MS	F	P
Day	2	0.0004120	0.0002060	10.00	0.000
Error	105	0.0021629	0.0000206		
Total	107	0.0025749			

S = 0.004539 R-Sq = 16.00% R-Sq(adj) = 14.40%

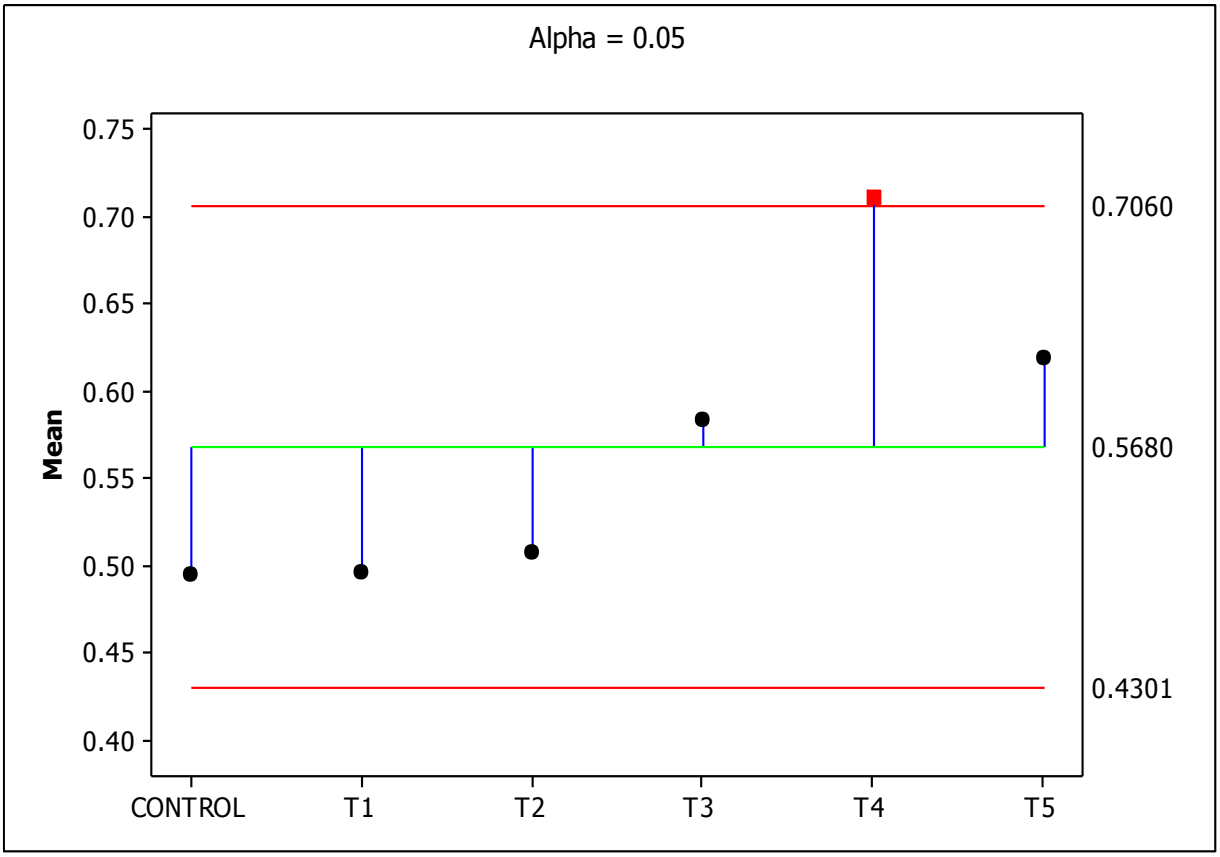
One-way ANOVA: Chitinases (U/ml) versus Treatment

Source	DF	SS	MS	F	P
Treatment	5	161.39	32.28	4.30	0.001
Error	102	765.10	7.50		
Total	107	926.49			

S = 2.739 R-Sq = 17.42% R-Sq(adj) = 13.37%

ANEXO 1 – 10

GRAFICA DE LOS EFECTOS PRINCIPALES QUE SE PRESENTARON ENEL CRECIMIENTO DE LOS JUVENILES DE LANGOSTINOS EXPUESTOS A UNA DIETA CON DIFERENTES CANTIDADES DE QUITINA



En el eje de las X:

Control = 0% de quitina en la dieta

T1 = 5% de quitina en la dieta

T2 = 10% de quitina en la dieta

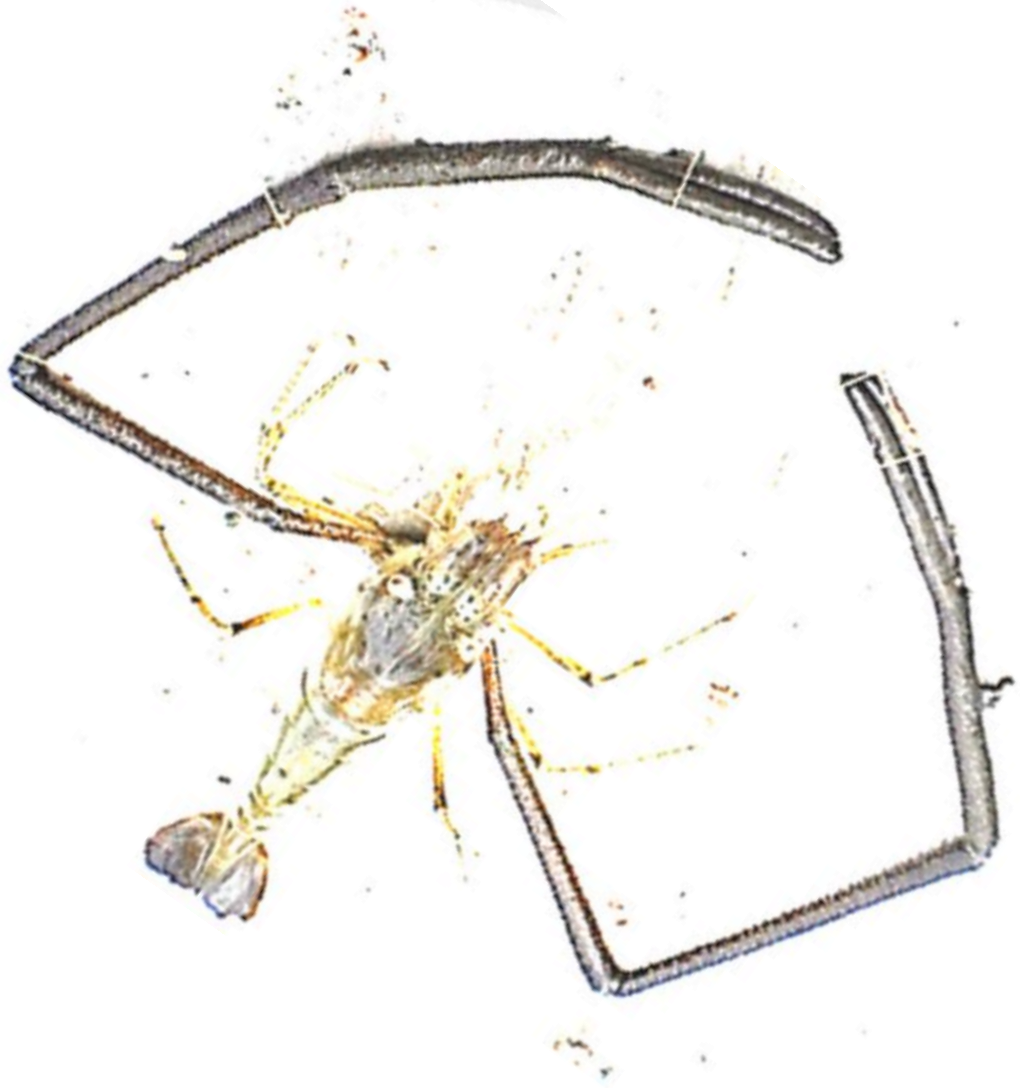
T3 = 15% de quitina en la dieta

T4 = 20% de quitina en la dieta

T5 = 25% de quitina en la dieta

En el eje de las Y: es la media de crecimiento en peso

CAPITULO III
EFECTO DEL FOTOPERIODO Y TEMPERATURA



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Effect of photoperiod and temperature on digestive enzyme activity, and growth of juvenile longarm river prawn (*Macrobrachium tenellum*).

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ABSTRACT. In Mexico *Macrobrachium tenellum* (Smith, 1871) is considered as a candidate for its exploitation and conservation because it grows fast, is still common in their natural environment, tolerates higher densities and wide fluctuations in water physico-chemical parameters. To understand how environmental conditions affect the production parameters of juveniles prawns *M. tenellum*, an experiment in which was combined the temperature (26 and 30) and photoperiod (14L/10D and 10L/14D) associated a chitin diet (20%) was developed for 60 days. Growth parameters, survival, and enzymatic activity (trypsin, chymotrypsin, lipase, amylase, and chitinase) were measured every 15 days. The higher final weight and specific growth rate were the result of the combination of photoperiod 14L/10D with temperatures 30 and 26°C showing significant differences from the results of a temperature 22°C in relation to a photoperiod 12L/12D. Treatments with 30 and 26°C temperature had no significant effect on survival. Statistically significant differences were found only on the enzyme activity of chitinase in the treatment 14L/10D at a temperature of 30°C, an high enzymatic activity (chitinase) occurred when temperatures low.

Running title: Effect of temperature and photoperiod on growth in prawn juvenile
Keywords: *Macrobrachium tenellum*, Chitin, enzyme activity, nutrition

RESUMEN. En México *Macrobrachium tenellum* (Smith, 1871) es considerado como candidato para su aprovechamiento y conservación, ya que crece rápido, es todavía común en su entorno natural, tolera altas densidades y amplias fluctuaciones de los parámetros físico-químicos del agua. Para entender como las condiciones ambientales afectan los parámetros de producción de juveniles del langostino M. tenellum, se desarrolló un experimento en el cual se combinó la temperatura (26 y 30ºC) y el fotoperiodo (14L/10D y 10L/14D) asociados a una dieta con quitina (20%) durante 60 días. Los parámetros de crecimiento, supervivencia, y la actividad enzimática (tripsina, quimotripsina, lipasa, amilasa, y quitinasa) se midieron cada 15 días. El mayor peso final y la tasa de crecimiento específico fueron el resultado de la combinación de un fotoperiodo de 14L/10D con las temperaturas 30 y 26ºC presentando diferencias significativas respecto a los resultados de una temperatura 22ºC con un fotoperiodo de 12L/12D. La temperatura 30 y 26ºC no tuvo un efecto significativo sobre la supervivencia. Estadísticamente se encontraron diferencias significativas solo en la actividad de la enzima de las quitinasa en el tratamiento 14L/10D a una temperatura de 30ºC, se presentó una mayor actividad enzimática (quitinasa) cuando las temperaturas descienden.

Titulo Corto: Efecto de la temperature y fotoperiodo sobre el crecimiento de juveniles de langostinos
Palabras clave: Temperatura, Fotoperiodo, Actividad Enzimática, *Macrobrachium tenellum*.

INTRODUCTION

Freshwater prawns are Decapoda included on the Caridea infraorder which is world wide distributed (Valencia and Campos, 2007; Hernández *et al.* 2007). In this infraorder, the Palaemonidae family are fond in marine, brackish or freshwater environments (De Grave *et al.*, 2008). Within this family, the genus *Macrobrachium* (Bate, 1868) is the most diverse and common. It includes freshwater prawns that live mostly in coastal areas across the tropical and subtropical regions of the world (Vega-Villasante et al., 2011, García-Guerrrero et al., 2013). Some are very common in the pacific continental slopes of Mexico as is the case of *Macrobrachium tenellum* (Smith, 1871). This specie has been considered as a good candidate for cultivation because it grows fast and may tolerate stressful conditions. The prawns of genus *Macrobrachium* have a particular life history wherein needs saltwater or freshwater depending on life stage, besides adequate temperature and photoperiod conditions; which has an impact on culture technique (Espinosa-Chaurand *et al*, 2011; Garcia-Guerrero et al, 2013). They can tolerate extreme conditions and adapts easy to captivity (Espinosa-

Charnaud *et al.*, 2011; García-Guerrero *et al*., 2013). Moreno-Reyes *et al*., 2015, report that to provide proper nutritional and water quality requirements in prawns are important to their successful culture in captivity. This also can help to improve small-scale aquaculture practices as an alternative way to recovering natural populations.

Some studies suggests that optimal growing conditions promotes a good survival as well as efficient use of the nutrients in food (Dall, 1990; Hoberman, 2000). Temperature and photoperiod are among major factors in regulating molt cycle in crustacean. Freshwater prawns inhabit estuaries, coastal lagoons and rivers, places in which temperature and daylight may change radically depending on season, location and life cycle (García-Guerrero *et al*., 2013). Several studies had evaluated the effect of temperature and/or photoperiod on crustacean development but separated, and in most cases, results are not conclusive or even contradictory. Temperature, acting either independently or simultaneously with other environmental factors, is one of the major physical factors affecting prawn life (Liu, *et al*., 2013). It is perhaps the most limiting factor for metabolic functions and thus, determines growth (Kumlu et al., 2000; Hoang et al., 2003; Lagerspetz and Vainio 2006). Because of this, to find the best temperature for every species is required to optimize growth and thus, best results on production (Deering and Fielder, 1995; Chen et al., 1996; O'Brien, 1994; Vijayan and Diwan, 1995). Temperature may highly affect the ability to digest food. For example, in *Procambarus clarkii* and *P. zonangulus*, temperature variations, causes fluctuations in rate of the intake and digestion of food (Croll and Watts, 2004). In *Macrobrachium rosenbergii* Stephenson and Knight (1980) mention that extreme changes in temperature may affect postlarvae locomotion. When temperature decreases to less than 20°C, organisms may stop feeding. Hernandez *et al.* (1995) indicate that the thermal preference of *M. tenellum* is from 27 to 30°C, so in this interval, reproduction, metabolism, feed conversion and growth should be optima.

Macrobrachium prawns pass through different life stages, living first in brackish water of estuaries and then in freshwater of rivers. These changes in habitat are accompanied with changes in feeding habits, expression of digestive enzyme activity, and food requirements. In the wild, prawns may feed on a wide variety of organisms and so have a greater opportunity to obtain essential nutrients, but an artificial diet not always grants the nutritional requirements of confined animals.

Other factors, may also affect food uptake and digestibility. Photoperiod and light intensity, for example, have received little attention as a variables that affects growth (Hoang *et al*., 2003). Photoperiod is among the factors that could control growth during early life stages of any aquatic

organism because its effect on feeding rhythms and the ability of food catching. Because of this, it is considered that growth could be improved by manipulating photoperiod. Taylor *et al*. (2006) stated that to change photoperiod might affect appetite, food intake and feed conversion ratio, improving or affecting growth. It is among the factors that most affect physiological activity, feeding behavior, and movement of decapods (Dalley 1980, Gardner and Maguire, 1998), and because of this may have a direct effect on growth and survival (Minagawa, 1994). Light intensity and photoperiod influences most physiological functions (Vega-Villasante, 2015), endocrine control (Eagles et al., 1986), enhances cannibalism (Hetch and Piennar, 1993). The effects of photoperiod on survival in early stages have been studied in different Decapoda such as shrimp, crab and lobsters (Dalley, 1980; Nakanishi, 1987; Bermudes and Ritar, 2008 and Matsuda et al, 2012). Those studies have shown that changes of feeding habits or digestive enzyme activity are associated with light intensity and day length. Marine shrimps, as *Litopenaeus schmitti*, show diurnal activity habits (Gonzalez et al., 1995), while *P. serratus, P. notalis* and *L. vannamei* show nocturnal activity (Van Wormhoudt, 1980; González et al., 1995; Nolasco-Soria and Vega -Villasante, 2000 and Hernandez-Cortes *et al.*, 1998; respectively). Many species depends on photoperiod variations as an external trigger that starts many biochemical and physiological processes, most of them related with digestion and growth such as composition of tissues and circadian variation of enzymes activity in hepatopancreas (Moreno-Reyes *et al.*, 2015; Casillas-Hernández *et al.*, 2006). For *M. tenellum* Espinosa-Charaund (2013) stated that digestive enzyme activity could be affected by natural photoperiod by changing enzyme levels depending on light intensity. In general terms, enzymatic activity is a good indicator of digestion capabilities.

To define the ranges in which the parameters of the water improves digestive physiological activity and consequently the increase in biomass of native prawns, the aim of this study is to analyze the effect of different temperature and photoperiod combinations on growth, survival and digestive enzyme activity on M. tenellum juveniles fed with a balanced diet containing chitin.

MATERIAL AND METHODS

Experimental design

A batch of *Macrobrachium tenellum* juveniles were collected in the *Chacahua* lagoon (15°58′20″N, 97°32′28″W) in Oaxaca, Mexico. They were transported in a 250 L tank with permanent aeration to laboratory facilities and then, acclimated during two weeks at 25°C. During this time, they

were fed with marine shrimp commercial pelletized food (40% crude protein, Camaronina 40® Agribrands Purina, Mexico).

In order to evaluate the photoperiod and temperature effect on growth, a completely randomized experimental design of five treatments was executed. Treatments consisted on the combination of two different temperatures (26ºC and 30ºC) and two different photoperiods (14 hours light-10 h dark and 10 h light-14 h dark). A control (environment temperature and photoperiod 12L-12D) was included. Every treatment had six replicates and every replicate consisted on a dark non-translucid plastic container (50L). All replicas of the same treatment were placed together at different levels. In the container at the lowest level no specimens were placed. A water pump was placed with a hose that throw water to the highest tray. Every tray had an outcome flowing to its lower next. This way, a cascade is produced and water is constantly recirculating through all containers of the same treatment. In every treatment, water temperature was maintained always at 26°C or 30°C, with submersible 300 W heaters (RENA Aquatic Supply; US) installed in the lowest non-specimen tray of every treatment. Oxygen concentration [O_2] was daily measured with a Hanna® HI98186 oximeter (Hanna Instrumenta Inc; Italy) and maintained always above 5 mg [O_2/L]. Photoperiod was controlled whit white light lamps or black plastic curtains depending on the desired regime. In each container, a 0.5 m^2 of plastic mesh was placed inside the water to provide shade and shelter. Ten organisms (0.2 ± 0.04 g) were placed in every container.

Prawns were fed with an enriched diet with 20% of chitin. The experimental diet was prepared in agreement with Cortes-Jacinto *et al.* (2003) and Espinosa-Charaund *et al.* (2012) with the following ingredients: *Cyprinodontid* fish meal (50.05%), soybean meal in bulk (25.03%), Maseca® commercial corn flour (17.8%), and San Antonio® commercial wheat flour (7.12%) and vitamin and mineral supplements (Fervinac with selenium®, Laboratorios Brovel, Mexico). All ingredients were blended and mixed with an Oster® blender. Chitin was prepared by pulverizing dried shrimp carcasses (*Litopenaeus sp.*). The carcasses were cleaned by rinse and then, dried at sunlight. When completely dry, they were pulverized with the blender (Oster®) and added to the mixture. Then, the mixture was sieved through a 500 µm mesh and mixed again with the same blender. At this step, cornstarch were added as agglutinant. The resultant mixture was passed through a meat grinder in order to obtain 2 mm diameter pellets. Those pellets were dried in a Felisa® convection oven (Fabricantes Feligneos SA de CV; Mexico) at 30°C during 12 hours. Table 1 shows the proximate analysis of the diet, to establish the values for gross energy, the conversion factors suggested by Anh *et al*. (2009) were applied: 4.2 for carbohydrates; 5.56 for proteins; and 9.54 for lipids.

Table 1. Proximate analysis of tested diet. Analytical method: [1]Differences in moist and dry weight, [2]AOAC (micro-Kjendahl method), [3]AOAC (Soxhlet method), [4]AOAC (Weende method), [5]AOAC (oven at 550°C).

	g 100 g^{-1} of dry food	
	Average value	SD
Humidity (%)[1]	4.783	± 0.37
Crude protein[2]	44.137	± 0.19
Total lipids[3]	6.517	± 0.20
Crude fiber[4]	1.557	± 0.09
Ash[5]	9.700	± 0.38
Nitrogen-free extract	38.090	± 0.77
Gross Energy (kcal g^{-1})	4.741	

A week before the first day of the experiment, total daily food was provided at 7% of the prawn biomass. After one week, the ration was adjusted to 10% of their body weight for all treatments. They were fed every day at 18:00 h. Survival was registered daily. The trial lasted 60 days. At days 0, 15, 30, 45, and 60, all prawns were individually weighed (std ±0.001 g).

The following parameters were obtained: Average individual weight gain (g): AIWG = final weight– initial weight; Gain weight per day (g day^{-1}): GWPD = (final weight-initial weight) t^{-1}); Gain weight in percentage (%): GW = 100 x (final weight – initial weight)/initial weight); and specific growth rate (%): SGR = [(Ln final weight – Ln initial weight) t^{-1}] ×100. Also the Feed conversion ratio was calculated as: FCR = supplied food (g)/weight gain (g); and Feed efficiency ratio: (FER) = weight gain (g)/supplied food (g). SGR and FCR were calculated in agreement with Cortes-Jacinto *et al.* (2003); Gitte and Indulkar (2005) were followed for GW and GWP; Vega-Villasante *et al*. (2011) for AIWG and SR; and Hasan *et al*. (2012) for FER.

Enzyme activity analysis

Two prawns each replica (eight per treatment), random samples were taken on days 0, 30 and 60. The fresh weight was registered and animals were stored at -20 ° C until analysis. To analysis of enzyme activity in juvenile prawns the previous section head, exoskeleton and all appendices discarded. The body portion surrounding the hepatopancreas and intestine was kept for small and fragile organs. The dissected bodies were individually weighed and homogenized separately with

distilled water (4 ° C) in a v / w ratio of 4 ml of water per g of tissue. Raw extracts were separated by centrifugation at 14000 rpm during 10 min at 4°C, and the crude extract was kept at -40°C until analyzed for soluble proteins and enzyme activity. All enzymatic analysis were made four times including a control sample or blank in where the enzyme reagent was added after the reaction had ceased. Protein were quantified by Bradford method (1976), then, absorbance was measured at 595 nm Bovine serum albumin (05470, Sigma-Aldrich, St. Louis, MO) was used as standard. Lipase activity was determined in agreement with Versaw et al. (1989), using β-naphthyl caprylate as the substrate. Lipase activity was expressed as lipase units/mg protein (one lipase unit was the quantity of enzymes required for an increase of 0.01 absorbance units at 540 nm/min). Amylase activity was measured, as described by Vega-Villasante et al. (1993), using 1% starch in 50 mmol Tris-HCl at pH 7.5 as substrate. This activity was expressed as amylase units/mg protein (one amylase unit was defined as the quantity of enzyme to increase absorbance units by 0.01 at 550 nm/min). Trypsin activity was determined, using BAPNA as the substrate (Garcia-Carreño & Haard 1993), adapted to a 96-well microplate by adding 10 µL crude extract, 160 µL 60 mmol Tris-HCl at pH 8.0, 10 µL 192 mmol $CaCl_2$ at pH 8.0), and 10 µL 9.6 mmol BAPNA dissolved in DMSO, in each well to start the reaction. Chymotrypsin activity was determined, using 9.6 mmol SAAPNA dissolved in DMSO. For both enzymes, absorbance at 414 nm was recorded every 15 s for 30 min. At the end of the assay, a linear coefficient was calculated to determine the increase of absorbance per second. Enzyme activity was calculated using molar extinction coefficient of ρ-nitroaniline (8800). Chitinase activity was determined by mixing 20 µL of crude extract, 50 µL 60 mmol Tris-HCl at pH 8, and 530 µL substrate (C3020, Sigma-Aldrich). The mixture was shaken with a vortex at 120 rpm placed at 45^{o} after that it was placed for incubation during 2 h. The reaction was stopped by centrifugation after 5 min at 14,000 rpm for 10 min at 4 °C. The supernatant was separated immediately and absorbance was measured at 570 nm (one chitinase unit was defined as the quantity of enzymes required for an increase of 0.001 absorbance units at 570 nm/min).

Statistical analysis.

Survival rate (%), Individual weight gain (g), Gain weight per day (g/day), Specific growth rate (%), Feed conversion ratio, Feed efficiency ratio and Enzyme activity (U/mg Prot Sol) were determined and then compared by one-way ANOVA test. Normality tests were performed (Kolmogorov-Smirnov test, $\alpha = 0.05$). Significant differences between treatments were determined by the Duncan multiple range test ($p > 0.05$). Data were processed with Minitab 17 statistical package (Minitab, College Park, PA).

RESULTS

The water quality parameters not considered as variable were maintained at an average of: pH 8.1; and dissolved oxygen concentration 6.7 mg l-1. The control treatment had an average water temperature of 22.8ºC.

After 60 days of trial, all the growth parameters of weight (g) had statistically significant differences (figure 1) in comparison to control treatment. The temperature of 30ºC combined with a photoperiod of 14: 10 L:D had the highest IWG (1.14 ± 0.2 g and 1.13 ± 0.11 g at 26ºC combined with a photoperiod of 14: 10 L:D respectively) and the highest SGR (0.019 ± 0.0033 g/day at 30ºC and 0.018 ± 0.002 g/day at 26ºC). The combination of a photoperiod of 10:14 L:D at 30ºC had the best growth and the same photoperiod at 26ºC were second best (table 1). The minimum and maximum growth intervals for the other parameters were as follows: RW (64.81 to 370.87 %); SRG (0.83 to 2.55) and FCR (16.7: 1 to 2.2: 1). In addition, in table 1, growth averages parameters per treatment are shown. In figure 2, the growth versus time indicating when statistics differences appear.

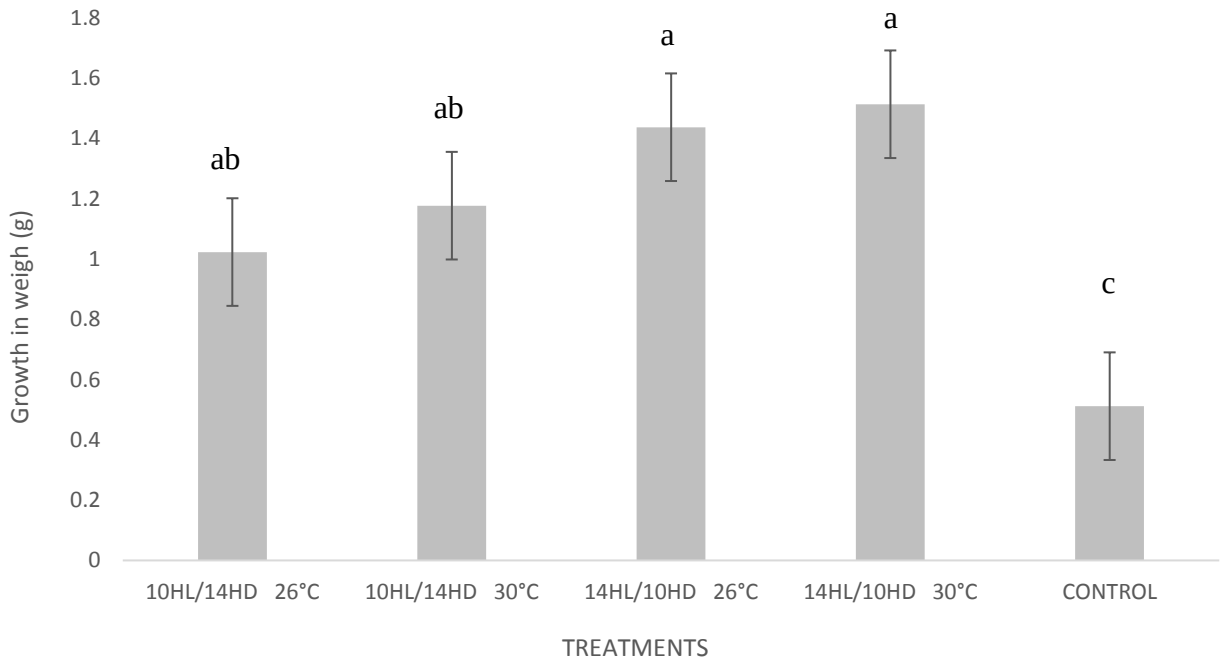

Figure 1. Photoperiod and temperature effect on growth of *Macrobrachium tenellum* juveniles maintained at different photoperiod and temperature regimes. 10HL/14HD = 10 hours light and 14 hours dark; 14HL/10HD = 14 hours light and 10 hours dark. Control = 12HL/12HD. The same letters show no differences between treatments (p = 0.05)

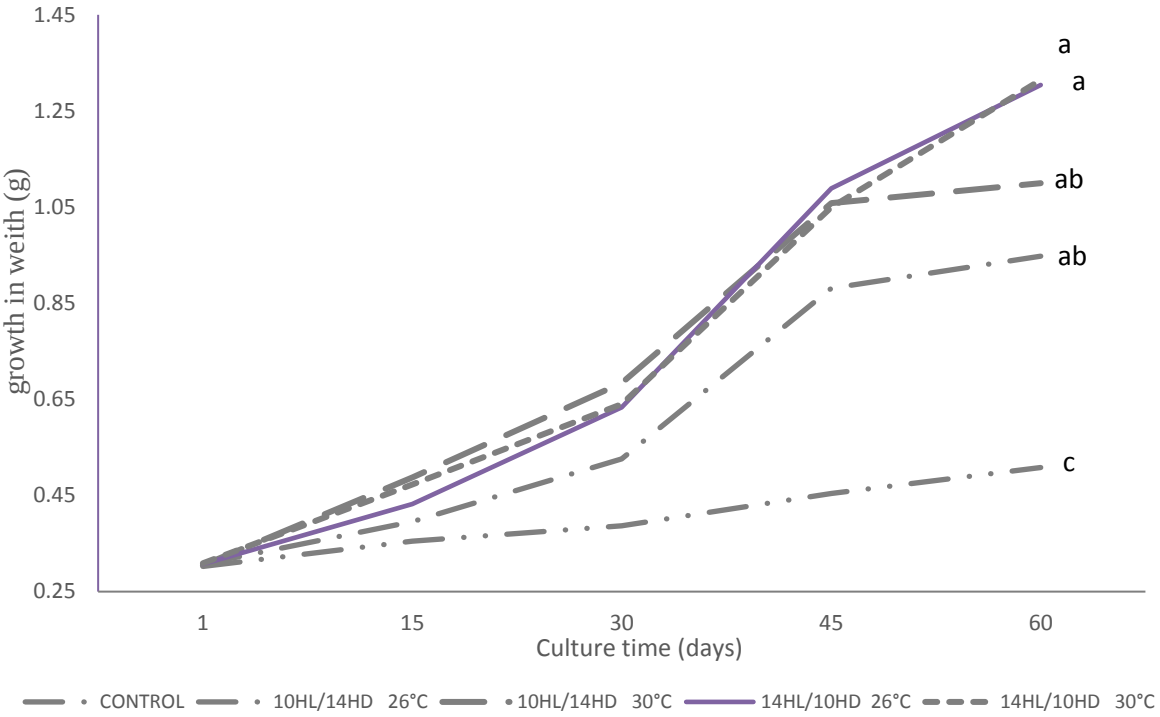

Figure 2. Photoperiod and temperature effect on growth versus time for *Macrobrachium tenellum* juveniles maintained at different photoperiod and temperature regimes. 10HL/14HD = 10 hours light and 14 hours dark; 14HL/10HD = 14 hours light and 10 hours dark. Control = 12HL/12HD. The same letters show no differences between treatments (p = 0.05)

Table 1. Grown parameters of *Macrobrachium tenellum* juveniles fed maintained at different photoperiod and temperature regimes. 10HL/14HD = 10 hours light and 14 hours dark; 14HL/10HD = 14 hours light and 10 hours dark. Control = 12HL/12HD.

TREATMENT		**S**	**±SE**	**IGW**	**±SE**	**GWPD**	**±SE**	**GWP**	**±SE**	**SRG**	**±SE**	**FCR**	**±SE**
10HL/14HD	26°C	75.0	± 3.6	0.758	±0.19	0.0126	±0.0032	248.50	±65.8	1.97	±0.30	4.71	±1.06
10HL/14HD	30°C	66.8	± 4.3	0.950	±0.11	0.0158	±0.0019	310.38	±38.7	2.33	±0.15	2.49	±0.27
14HL/10HD	26°C	66.8	± 6.0	1.130	±0.11	0.0188	±0.0018	368.56	±36.6	2.55	±0.14	2.22	±0.39
14HL/10HD	30°C	52.7	± 6.7	1.147	±0.20	0.0191	±0.0033	370.67	±63.9	2.51	±0.24	2.42	±0.34
CONTROL		83.2	± 4.3	0.205	±0.02	0.0034	±0.0002	64.81	±5.9	0.83	±0.06	16.75	±5.69

The moment in which the best growth rate (GWPD) was observed was at day 45 with a decrease towards the final days, apparently in all parameters (Figure 3). Figure 3 shows that the lowest growth was observed in the control. Organisms of this treatment did not increase in size after day 45.

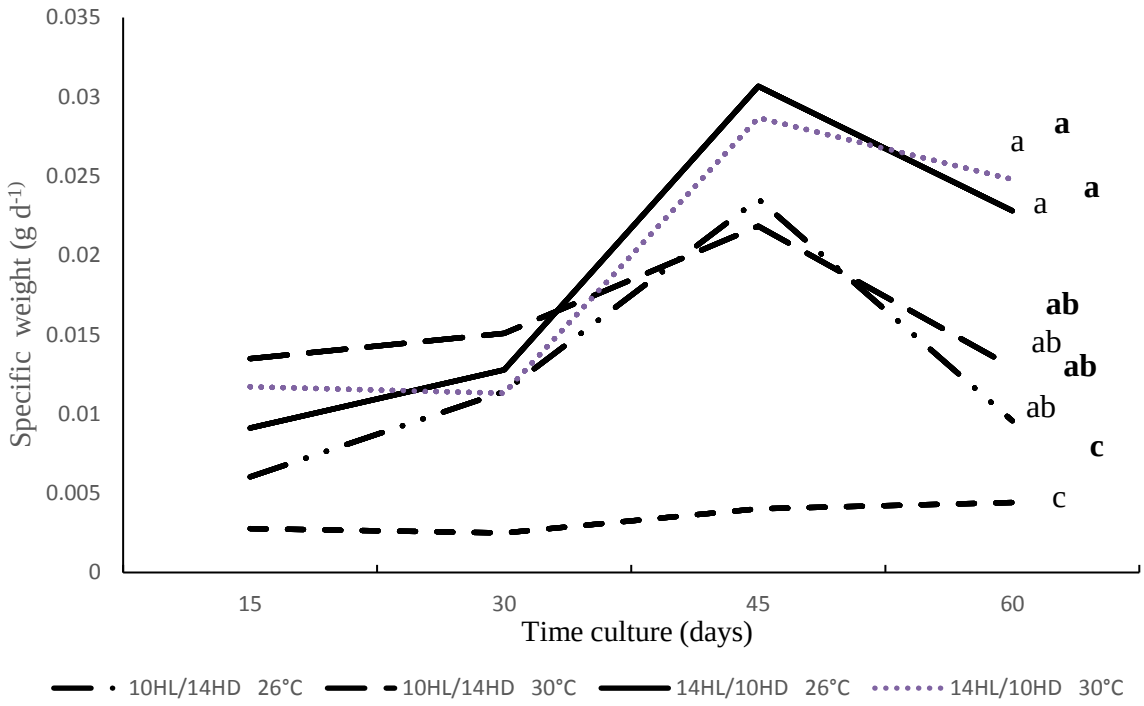

Figure 3. Photoperiod and temperature effect on specific growth rate of *Macrobrachium tenellum* juveniles maintained at different photoperiod and temperature regimes. 10HL/14HD = 10 hours light and 14 hours dark; 14HL/10HD = 14 hours light and 10 hours dark. Control = 12HL/12HD. The same letters show no differences between treatments (p = 0.05)

Survival rate was significantly affected only between all treatments and control with this last with the best survival (83.2% p = 0.05). The lowest survival was 52.7% (14 hL/10 hD to 30°C). The best survival had an inverse relationship with growth. As to FCR, the best treatment was the combination of 14 HL/10 HD with 26°C and 30°C with values of 2.22 and 2.42 respectively. There were no statistically significant differences on FCR between treatments (P=0.05).

In relation to soluble protein concentration differences among treatments, chitinase activity seems to be the only one with statistically significant differences (p = 0.05). Similarly, to what happened with specific growth, (Fig. 2), chitinase activity was the highest at day 45 and decrease after that day (Fig. 4). The other enzymes (lipase, amylase, trypsin and chymotrypsin) did not had that trend (Fig. 5 a-d) and show no statistically significant differences between treatments (P=0.05).

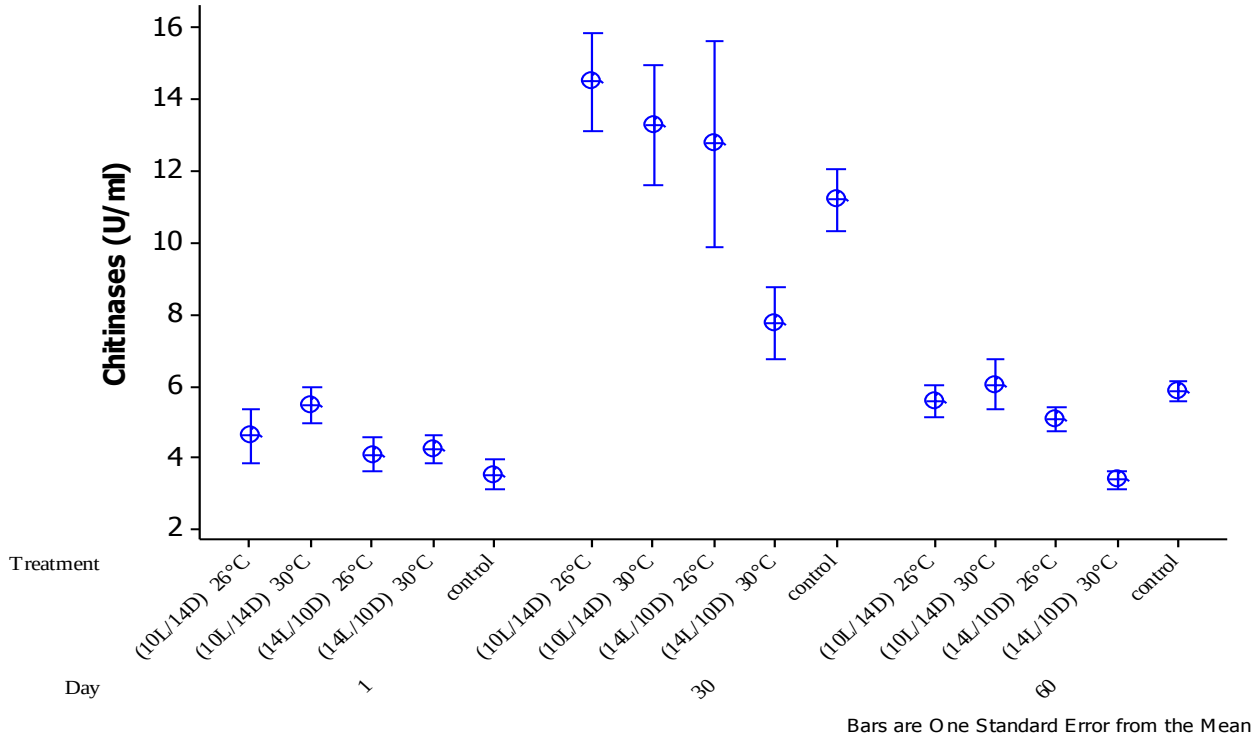

Figure 4. Photoperiod and temperature effect on chitinase activity of *Macrobrachium tenellum* juveniles maintained at different photoperiod and temperature combination

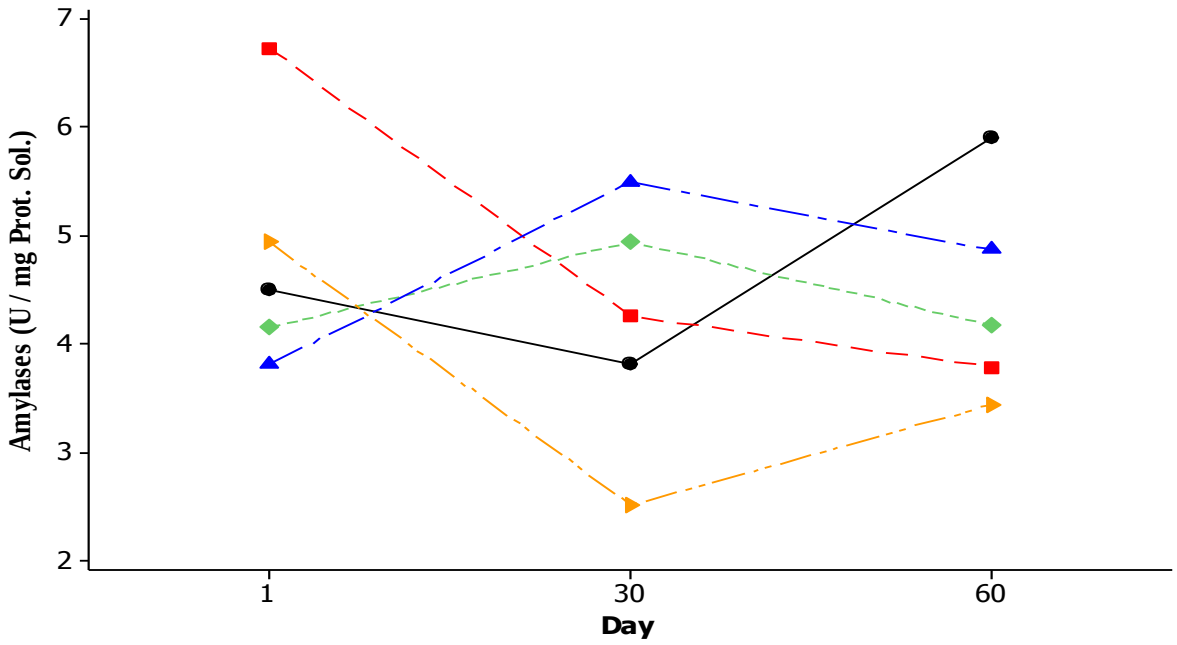

Figure 5a. Photoperiod and temperature effect on amylases activity of *Macrobrachium tenellum* juveniles maintained at different photoperiod and temperature combination

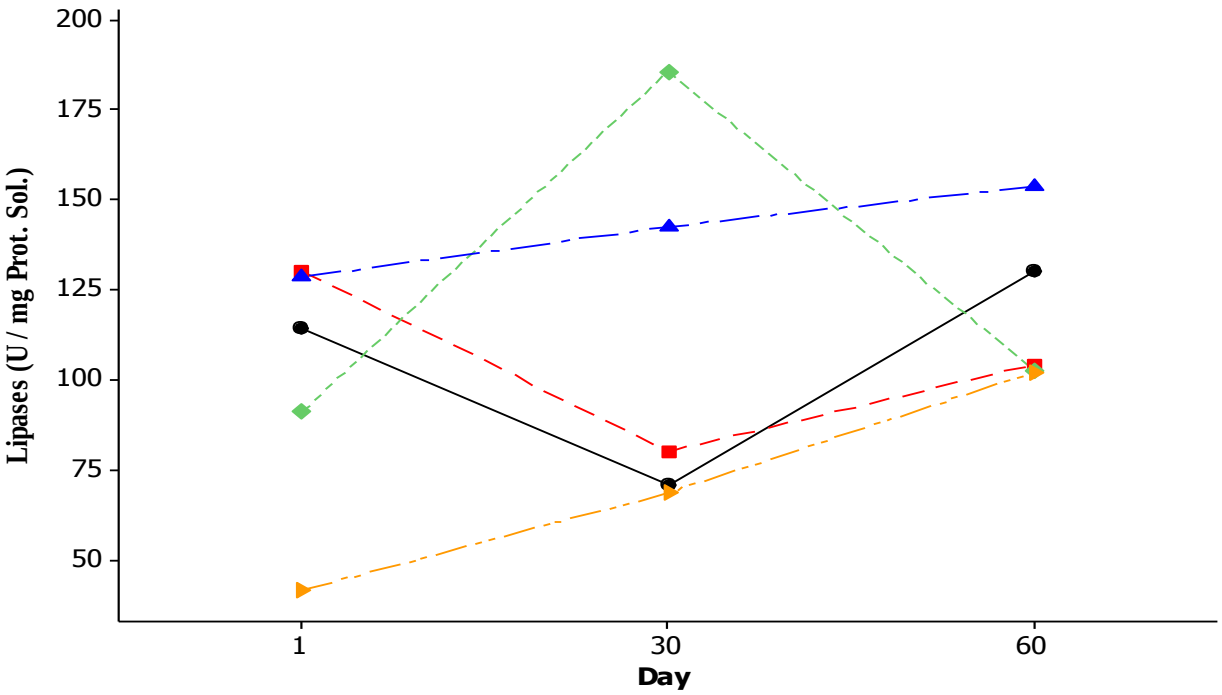

Figure 5b. Photoperiod and temperature effect on lipases activity of *Macrobrachium tenellum* juveniles maintained at different photoperiod and temperature combination

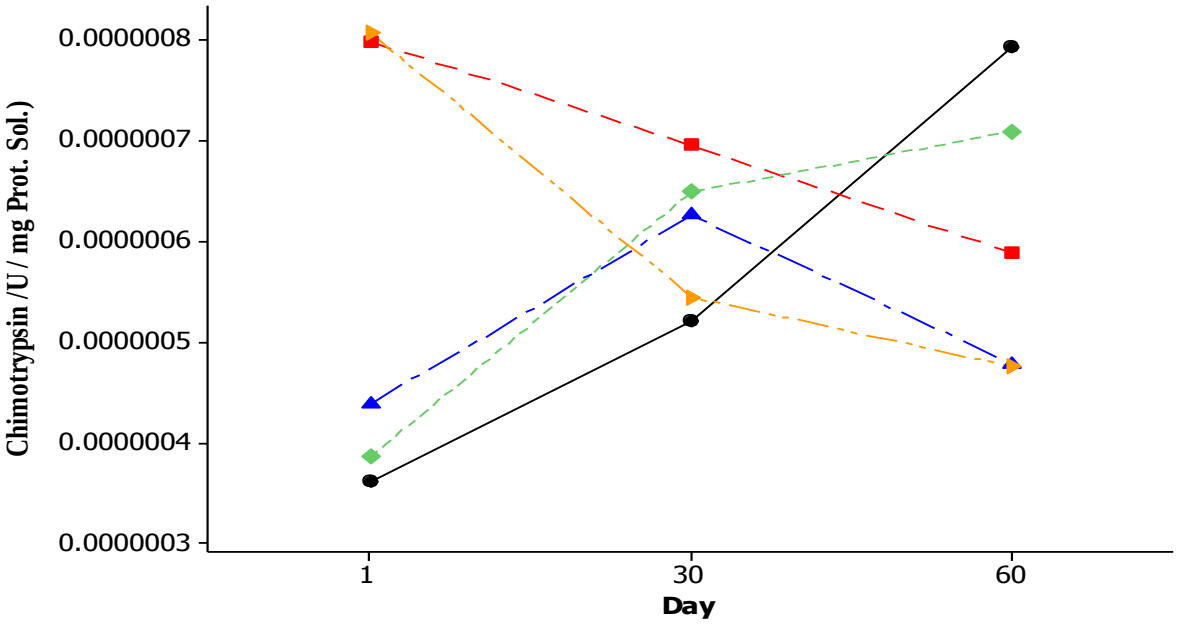

Figure 5c. Photoperiod and temperature effect on chymotrypsin activity of *Macrobrachium tenellum* juveniles maintained at different photoperiod and temperature combination

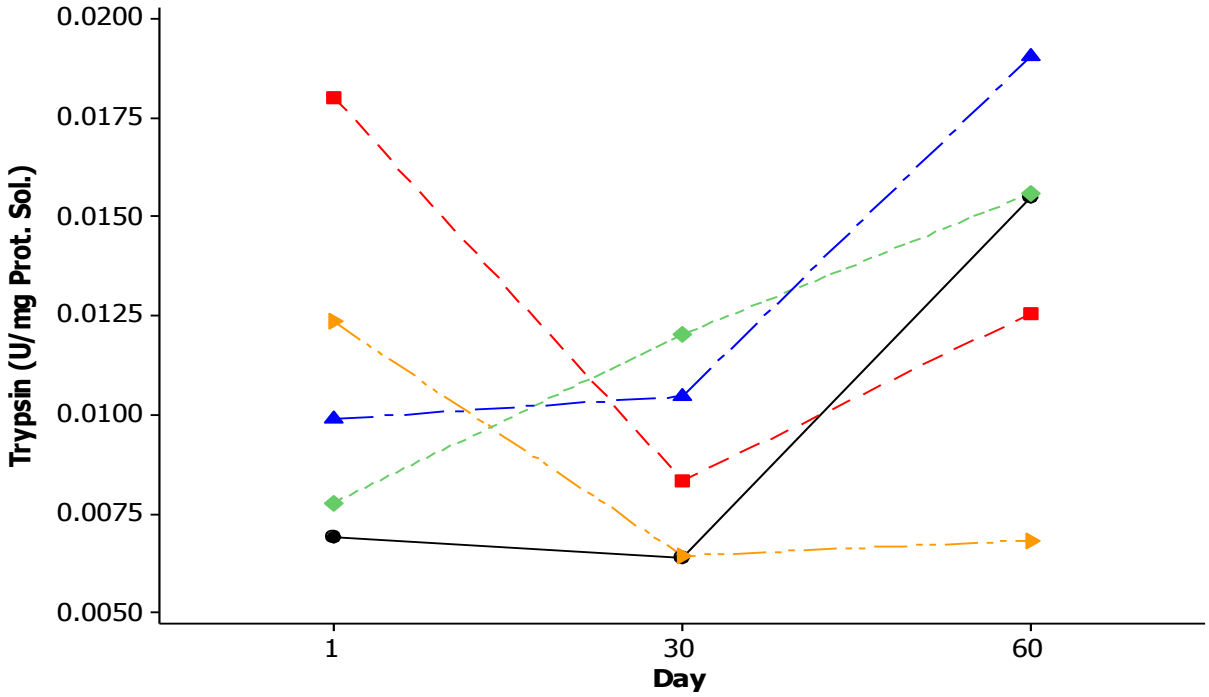

Figure 5d. Photoperiod and temperature effect on trypsin activity of *Macrobrachium tenellum* juveniles maintained at different photoperiod and temperature combination

DISCUSSION

The present study agrees with the work of Pervaiz (2015a) which suggest that molt and growth could be improved inside a limit, by manipulating both temperature and photoperiod. Temperature itself always have a huge effect on metabolism of prawns while variations in photoperiod may only cause significant differences in extreme intervals and its direct effect seems to be mostly in digestion. Aiken and Woody (1992) indicate that temperature and photoperiod are among the main factors in the regulation of moult cycle and therefore, growth. However, previous research analyze those effects on prawns separately and the effect of the interaction is not well understood. To study them together may provide information of how the manipulation of one variable may affect the other as in present study.

In present work, it seems that the interaction between high temperature and long photoperiod produces better growth. This is in agreement with the tropical origin of *Macrobrachium* prawns (Anger, 2013) since tropical places may have similar scenarios. New (1995) reported that freshwater prawns may tolerate wide ranges of temperature but proper ones would be close to tropical and temperate regimes. Madlen et al. (2010) report that the growth rate of *M. rosenbergii* increases in the interval from 24° C to 29° C. Trinidad et al. (2010) mentioned that *Macrobrachium jelskii* higher average sizes (1.72 g and 5.55 cm) were observed at 29.97 ± 0.81°C. All those works however, used large specimens and juveniles as those from present work, may respond differently. Are few studies that have assessed effect of temperature and relation with other variables on *Macrobrachium* juvenile's growth, so that few comparisons can be offered, but it is suggested that the photoperiod may complemented the effect of temperature.

All this suggest that high temperatures, within proper interval, increase the frequency of molting and growth in prawns (Staples and Heales, 1991; O'Brien, 1994; Vljayan and Diwan, 1995; and Parado-Estepa, 1998). However when temperature goes outside this interval growth decrease, as reported by Hernández-Sandoval (2008). This author observed that *M. occidentale* increases in growth with increasing temperature at 25°C to 28°C but beyond 31° C both growth and survival decreased. In the case of *M, rosenbergii*, Madlen et al. (2010) indicates that above 34°C growth declines. Other non *Macrobrachium* but also with tropical origin Decapoda may respond in similar way. Firkins and Holdich (1993) report that growth and survival of *Procambarus* decreases beyond 34°C. In such cases, organisms cannot cope with a very high metabolism rate that demands huge energy and oxygen amounts. This may occur in most tropical Decapoda on the influence of abiotic characteristics like.photoperiod on the rate of survival and developmental period of the larvae of prawn and shrimps (Mazlum et al. 2011).

In present experiment, although the best growth was observed at 30°C, it has significant differences in comparison only with control (22.8°C) in which growth rate was very low. Morrissy (1990) and Kartamulia and Rouse (1992) suggests that low temperatures may inhibits growth because a very low metabolism rate, while higher temperatures may cause extreme energy and oxygen exhaustion that may cause stress and mortality. It is also assumed that this response cannot be only to unsuitable temperature and photoperiod since the prawn responds as a whole to all stimulus from the environment and its physiological condition, age and size may also play an important role in this response. Wiban, Walsh and Godin (1995) suggest that the optimum temperature for growth is inversely related with size. Dall (1986) find in adults of *Penaeus* shrimps that temperatures above

27°C severely affect physiological processes and may cause death in adults. By contrast, in the early stages Indulkar (1999) found that *M. rosenbergii* postlarvae grow well from 23.0°C to 29.5° C. Similar trend Arana-Magallón and Ortega-Salas (2005) observed for the same species at temperatures of 20°C to 33°C. All this suggests that the early stages of this shrimp may grow better at higher temperatures but larger sizes may have different requirements. Crispin and White (2013) suggest that body size and temperature may independently affect metabolic rate in prawns acclimated to 25°C in comparison with those acclimated to 16° and 21°C.

Growth rate in present work was affected by water temperature and this was reflected in high FCR as observed in present work. Similar results with *M. rosenbergii* are comparable, Niu et al. (2003) mention that feeding may increases when temperatures increases from 23°C to 33°C and because of this body weight goes from 7 to 38 mg. In the case of present work best growth was at 30°C but organisms grew between 25°C or above 32°C. A lowering in growth rate when temperature is far de optimum should be due to a low efficiency in energy use or a due to a very low metabolism rate depending if temperature is unsuitable high or unsuitable low. Other works with *M. tenellum* (Rodriguez-Flores et al., 2012) suggest that juveniles may survive in a wide temperature interval but best growth may occur only close to 29°C. In the other hand, Vega Villasante et al (2011) and Garcia -Ulloa et al (2008) reported values for *M. tenellum* average weight increase of 0.015 g/day at 27°C temperatures similar to those presented in this work. All this is an evidence of its tropical requirements and it is consistent with those reported by Sánchez and Soto (1976), Ponce-Palafox et al. (2006) and Vega-Villasante et al. (2011), who stated that the best growth of the species may occur between 29°C - 32°C and stops below 20°C.

In relation to survival in present study, significant differences were observed only between the control and all treatments. This suggest that even growth rate is affected by treatments, none of them were beyond the limits that means risk of death. In fact in this work, the treatment at 22.8°C showed the best survival where suggest that low temperatures are less lethal for this species but growth was lower, the inverse relationship of survival with growth, where Jayachandran (2001) y Moraes-Riodades y Valenti (2004) indicate that growth besides food is affected by environmental factors as temperature. This is in contrast with García-Ulloa (2008), Vega-Villasante (2011) and Espinosa-Chaurand (2012) in where the highest survivals are at higher temperatures (29°C to 30°C). This suggest that survival is temperature dependent but always other factors may intervene varying the result depending on experimental conditions, among which feeding and physiological conditions are the most important ones as stated by Espina et al. (1993) and Gutierrez-Yurrita (2000). Lagerspetz and Vainio (2006)

mentions that the relationship of temperature on survival and growth is only positive within certain intervals and under certain circumstances. In agreement with Cheng and Chen (2000) temperature has a direct relationship with the immune system efficiency of *M. rosenbergii* and this is an additional possible for this response since immune system may work better at certain temperatures.

Photoperiod effect on the growth is not easy to investigate since its effect might be hidden by stronger factors such as diet, feeding and digestive ability. More light might not be traduced in better growth. However, results from present study indicates that 14 hL / 10HD photoperiod significantly improves growth, becoming important in molting cycle. In agreement with Shan et al., (2008) photoperiod significantly affected lipase activity, being significantly higher at 18L:6D and 24L:0D than 12L:12D. Also Shan et al., (2008) stated that photoperiod did not affect the activity of trypsin or amylase, suggesting that the effect of photoperiod on digestive enzyme activities are enzymatic specific. Hoang et al. (2003) stated that a altered regime of 7L:5D cause best growth in *Penaeus merguiensis*, than the ordinary regime (12L:12D). Hoang et al. (2003) reported that photoperiod significantly improved weight gain of *P. merguiensis*, although its effect on molting was not significant. In *M. rosenbergii*, growth rate on juveniles at a photoperiod of 24L:0D was better than any other (Lin 1997) suggesting that permanent light may improve growth of these prawns. However, Wang et al (2005) did not find a measurable effect of photoperiod on growth of juvenile *P.chinensis* when the light time were lower than 12h/day. Withyachumnarnkul *et a*l. (1990) stated that *M. rosenbergii* juvenile at L0:D24 were heavier than those maintained at any lower regime. Feng Yue et al., (2009) observed that *Procambarus digueti* reduced their locomotor activity at regimes with large day light (20L:4D), suggesting that there is less energetic expenditure, what allows the organism to channel more energy to growth. Contrary, Saez-Royuela *et al*. (1996) mentions that the photoperiod has a minimal effect on growth and Hoang et al. (2003) stated that the effect of photoperiod is not significantly important for molt. Several authors report that light period and intensity can affect growth because feeding behavior and circadian rhythm is affected by light as observed in *L. vannamei* (Guo et al., 2012), in where lower intensity (600 lux) delay growth. As observed in present work with the longest photoperiod (14hL / 10HD) more light produces good growth at 26°C. However, Jesaja and Wenno (2014) and Vijayan and Diwan (1995) observed that a photoperiod of (24L-0D), (12L-12D) and (0L-24D) had no influence on the growth of *P. indicus*. Similarly, Nakamura (1998) did not find difference in *P. japonicus* growth when cultured under a long photoperiod (14L-10D) and short days (1L-23D. This can be explained on the assumption that digestion, which process nutrients and promote growth, is closely linked to circadian rhythm, which is a photoperiod-dependent process. Species natural history and life stage may have a huge effect on this since even in very different

crustaceans such as *Homarus americanus* (Nelson et al., 1998) the same is observed. From all those assumptions, is observed that the effect of photoperiod on growth is not as clear as that of temperature and the information about the effect of light regime on prawn growth is species and age dependent, but also can have an indirect effect by altering its behavior.

In this work, low temperatures and fixed photoperiod produced slow growth, but higher survival. Hoang et al. (2003) reported that the survival of juvenile *P. pelagicus* with a photoperiod of 12L-12D had similar results, while *P. merguensis* not show significant differences in growth if maintained under photoperiods of 12L-12D and 14L and 10D ($P > 0.01$). Hoang et al. (2002) mentions that the effect of photoperiod on the growth of *P. merguensis* is not immediate and may decreases over time. This is possible when the body has the capability to adapt to low water temperatures (24°C). There are no evidence if in freshwater prawns, this kind of combination interfere with moult. However, short days (12h light) have no effect on the growth of *M. rosenbergii* (Just et al., 1991) and *P. japonicus* (Nakamura, 1988). Same author mention that growth of *P.s merguensis* is not affected by photoperiod if water temperature is below 24°C. Similar trends were observed for *P. monodon* early stages (Khalik et al, 1992) *Scylla serrata* (Quinitio et al, 2001) or *Palaemonetes argentinus* (Diaz et al., 2003)

Survival in present study had significant differences ($P<0,05$) between the control (22.8°C 12hL / 12hD) and the rest of treatments, so there is no a direct effect of photoperiod over survival as observed by Vega-Villasante *et al.* (2015) for the same species. For *M. rosenbergii* Chavez-Justo *et al.* (1991) stated that an increase in photoperiod (15L-9D) combined with low temperature (24°C) cause low survival (21%). In addition, Tidwell *et al.* (2001) report that survival in *M. rosenbergii* was significantly better at L24:D0 (with survival 72%), but in conditions of L12:D12 or L0:D24 with a survival of 59 and 58% respectively, not was significant. This supports the idea that variations in photoperiod have secondary effects on survival in comparison with temperature. In addition, Hecht and Piennar (1993) and, Garnerd and Maguire (1998) they mention that photoperiod could be related with cannibalism as observed in *Pseudocarcinus gigas* because their food requirements increases. Authors stated that this species at certain stages find the food visually, so more darkness hours may diminish the chance to find food. This may promote cannibalism or death if food is not find.

Enzyme activity is important for growth and survival since digestion depends on digestibility efficiency in the tract, what depends on the diet composition. In crustacean with a varied diet, flexibility in the digestive enzyme activity through different dietary regimes and stages is necessary

for a proper grow and survival in different conditions (Brito et al., 2001). This must help to obtain all energy and nutrients contained in the food. Elevated enzyme activity could maximize hydrolysis, so it occurs when a particular component is scarce in the diet (Hofer, 1982). So, enzyme activity is not necessarily high for those substrates most common in the diet. For example, an increase in amylase activity might be due to low levels of carbohydrates in the diet and the rise in tripsin activity could be due to low protein ingestion. This could be also related to amino acid deficiency in the crude protein of the diet. The presence of chitin in the diet may alter enzymatic activity because it has low digestibility (Brito et al., 2001) and several authors have suggested that enzyme activity could change as an adjustment mechanism to low nutrient, particularly protein, in the diet, because of poor digestibility or by the direct stimulation by some active component present in the food. However, in present work, no evidence was observed that shown that there is an effect from the combined interaction of temperature and photoperiod on prawn enzymatic activity since all enzymes quantified had no statistical differences in activity. Previous works on this field with *M. dayanum* (Pervaiz *et al*. 2015b) state that an activity rate of was slower at low temperatures than in warmer independently of photoperiod. This author also state that if animals are under continuous light or dark, this will also cause less food consumption because enzyme activity, particularly trypsin becomes lower. Other research mention that in *M. rosenbergii* molting process is affected by various environmental factors such as temperature and photoperiod (Chavez-Justo et al., 1991). A long daylight may cause an increase in daily enzymatic or hormonal activity under certain interval what depend on species, but also may cause a lower growth rate perhaps due to an increase in energy demand for frequent molts, as reported with *P. argentinus* (Diaz et al., 2003).

Wormhoudt and Ceccaldi (1976) and Wang et al. (2004) found that the growth of *Fenneropenaeus chinensis* and *Palemon serratus* is significantly different if exposed to different photoperiods. This suggests that there is a relation between day light duration and the intensity on enzyme activity, which will affect growth because of its direct effect on digestion. Cannibalism is another characteristic that is known to be influenced directly by light. *F. chinensis* had more intense enzymatic activity when light is more intense as observed by Guo et al. (2013) and Wang et al (2006). In preseent work and in relation with the effect of photoperiod and temperature on chitinase activity, some statistical differences were observed when the time of light exposure was lower (10h) combined with low temperatures (26 ° C). However, only Ceccaldi (1989) and Zhang et al. (2014) mention that chitinase activity is essential for digestion and molting, but does not relate the effect of temperature and photoperiod on the chitinase enzyme activity. So, light variations are also involved with most physiological functions in which enzyme activity has an effect, but the magnitude of that effect will

always depend in the combination of various direct and indirect factors. Enzyme activity detected in homogenates does not always represent activity in the digestive tract since that activity has been observed outside the guts (Lovett and Felder, 1990). An additional problem associated with the determination of enzyme activity could be the stage of molt cycle, season and sexual condition, so any results cannot be conclusive.

In present work, the combination of temperature 26°C and 14 hours of light seems to be the best results for growth and is consistent with the environmental conditions in the natural distribution of this species. It seems that this combination allows a proper enzyme activity, particularly chitinase but also the other enzymes that promote growth and survival by making easier nutrient digestion. The adaptability of *M. tenellum* to grow and develop in various combinations of temperature and light, should be continued studied. The activity of hormones that regulate growth are also strongly linked with temperature and light intensity, so the inclusion of such factor in further studies is recommended for a better understanding on light and temperature effect on prawn growth. Experiments on that topics, combined with field sampling, would help assess the importance of these variables on the distribution, abundance and survival of prawns, at all life stages may lead to a better understanding of how those factors affect prawn life cycle and populations.

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ANEXO 2 - 1

Unidades experimentales con control de fotoperiodo y temperatura.

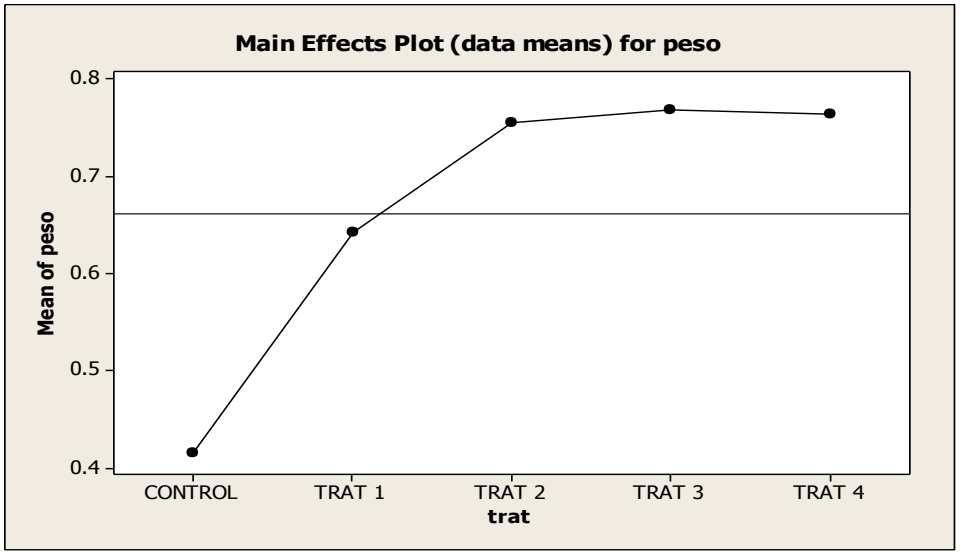
ANEXO 2 - 2

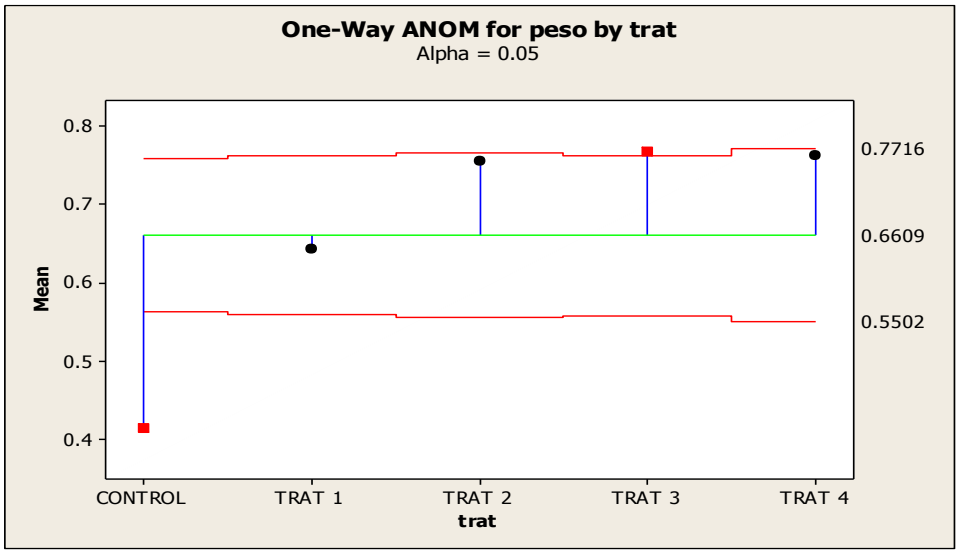
Efecto principales del fotoperiodo y la temperatura sobre el crecimiento (Control = temperatura ambiente y fotoperiodo 12L/12S; Tratamiento 1= temperatura 26ºC y fotoperiodo 10L/14S; Tratamiento 2= temperatura 30ºC y fotoperiodo 10L/14S; Tratamiento 3= temperatura 26ºC y fotoperiodo 14L/10S y Tratamiento 4= temperatura 30ºC y fotoperiodo 14L/10S.

One-way ANOVA: peso versus trat

Source	DF	SS	MS	F	P
trat	4	13.950	3.488	12.02	0.000
Error	717	208.056	0.290		
Total	721	222.006			

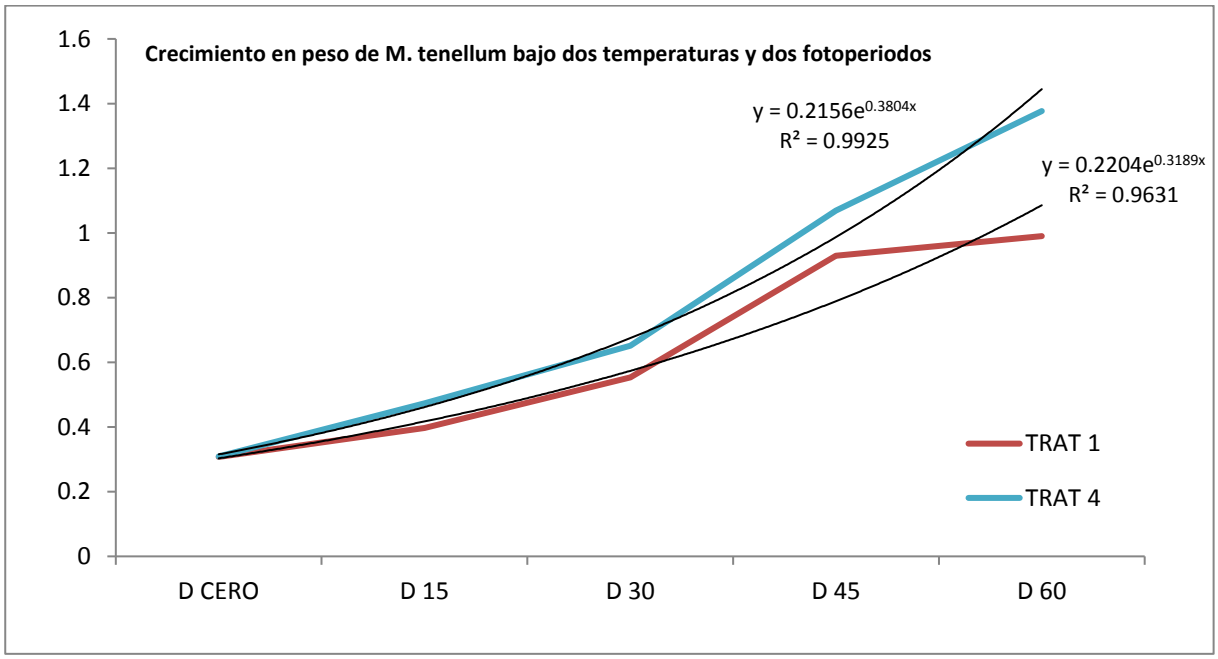
S = 0.5387 R-Sq = 6.28% R-Sq(adj) = 5.76%

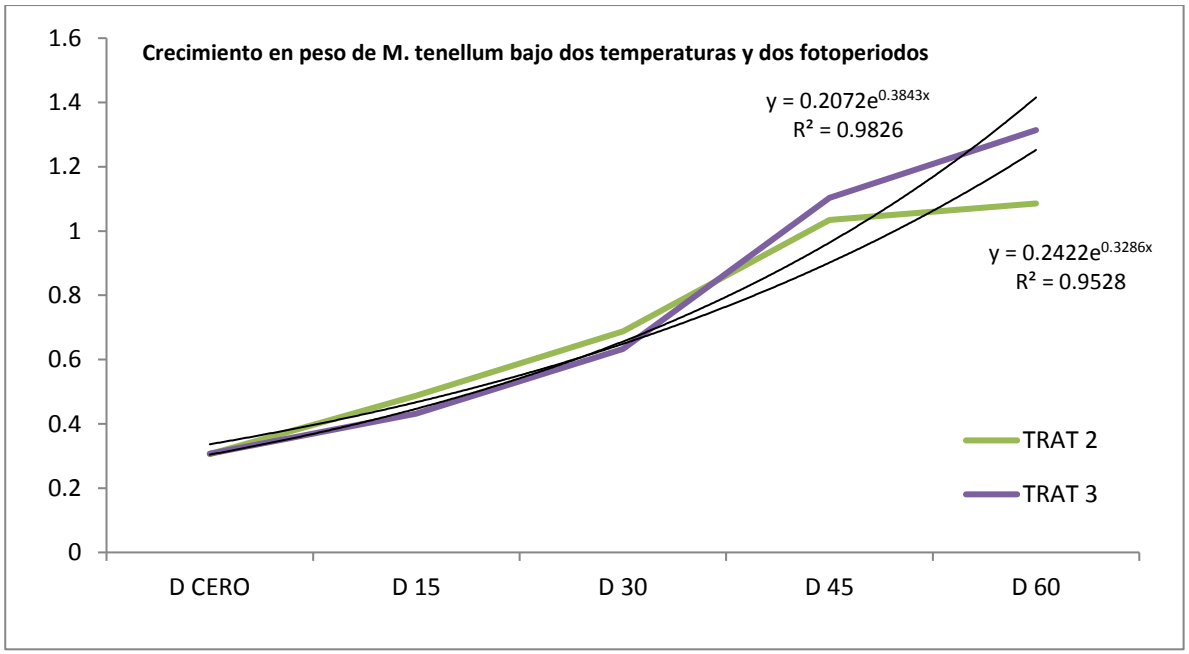




ANEXO 2 - 3

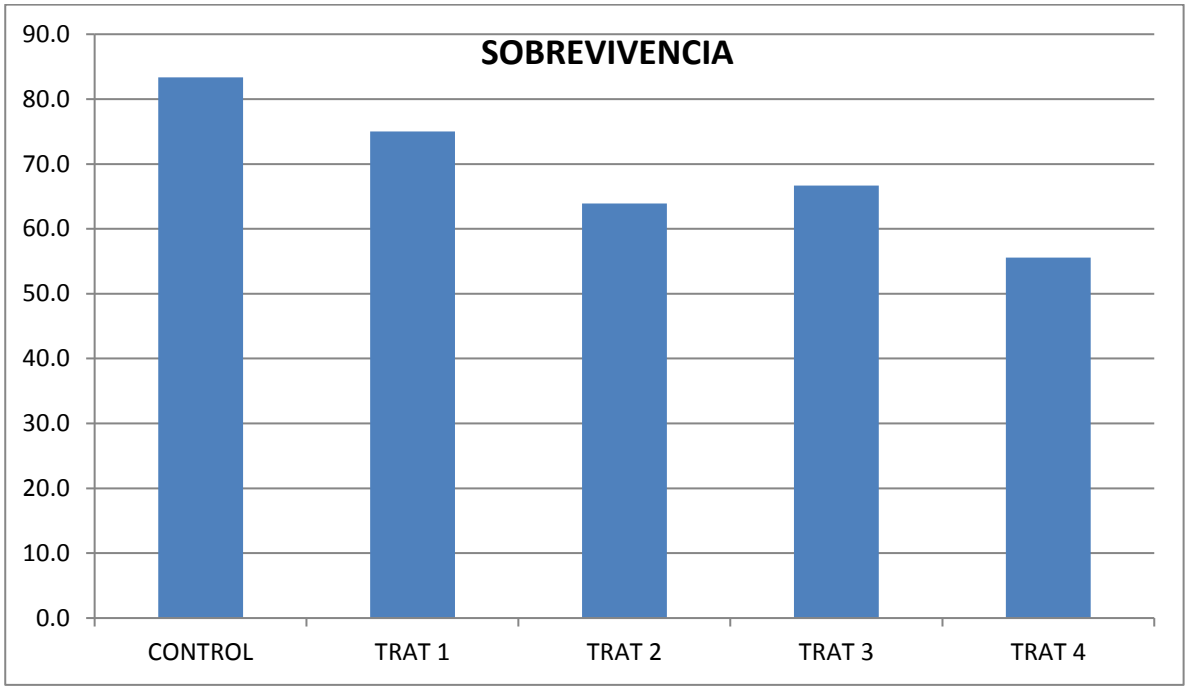
Comportamiento del crecimiento durante 60 días de experimento (Control = temperatura ambiente y fotoperiodo 12L/12S; Tratamiento 1= temperatura 26ºC y fotoperiodo 10L/14S; Tratamiento 2= temperatura 30ºC y fotoperiodo 10L/14S; Tratamiento 3= temperatura 26ºC y fotoperiodo 14L/10S y Tratamiento 4= temperatura 30ºC y fotoperiodo 14L/10S.





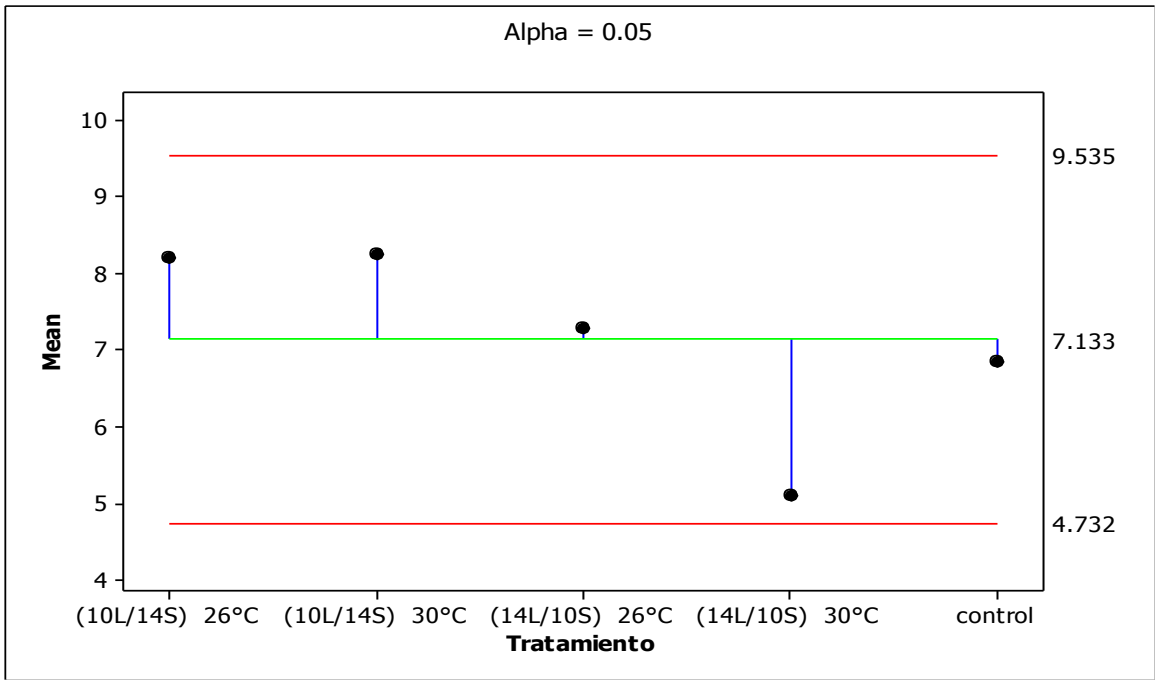
ANEXO 2 - 4

Sobrevivencia en los 60 días del experimento (Control = temperatura ambiente y fotoperiodo 12L/12S; Tratamiento 1= temperatura 26ºC y fotoperiodo 10L/14S; Tratamiento 2= temperatura 30ºC y fotoperiodo 10L/14S; Tratamiento 3= temperatura 26ºC y fotoperiodo 14L/10S y Tratamiento 4= temperatura 30ºC y fotoperiodo 14L/10S.

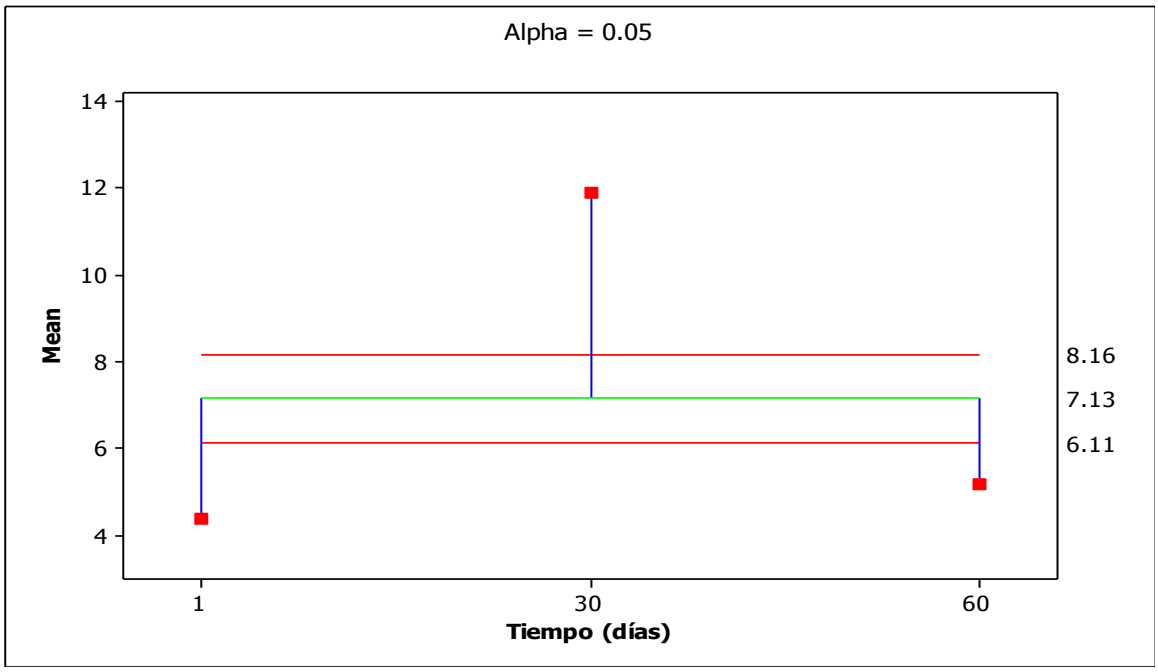


ANEXO 2 - 5

Efecto de la actividad de las quitinasas por tratamiento y por periodo de tiempo

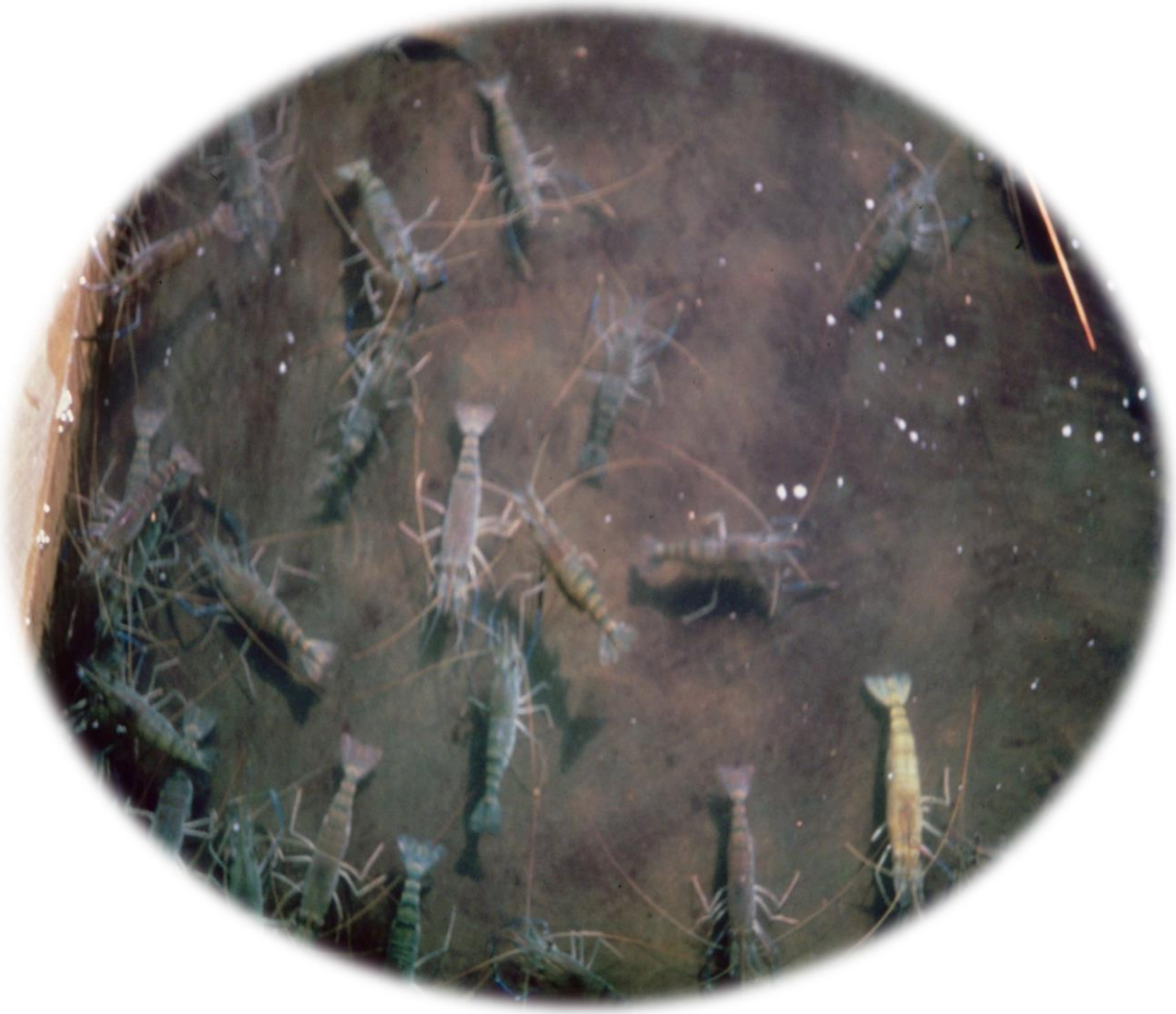


Efectos principales Una vía Quitinasas (U/ml) por Tratamiento



Efectos principales Una vía Quitinasas (U/ml) por Tiempo

CAPITULO IV
EFECTO DE LA JERARQUIA Y EL USO DE REFUGIOS



Fuente: FAO, 2002.

EFECTO DE LA PRESENCIA Y FRECUENCIA DE MACHOS DOMINANTES, Y EL USO DE REFUGIOS SOBRE EL CRECIMIENTO Y SOBREVIVENCIA DEL LANGOSTINO DE RÍO *Macrobrachium tenellum* (SMITH, 1871).

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RESUMEN

Los langostinos de agua dulce son organismos que tienen importancia ecológica y comercial, en donde su agresividad y territorialidad limita su conservación y manejo en cautiverio, tal es el caso del langostino del Pacifico mexicano *Macrobrachium tenellum*, en donde para entender como estos organismos responden a la presencia y número de machos dominantes en una población, se asocian a tres tipos de refugios con el propósito de reportar si se reduce la presencia de jerarquías sociales y aumentar la sobrevivencia. Los tratamientos experimentales que se establecieron para determinar el efecto sobre el crecimiento de diez juveniles fueron: la ausencia de macho dominante, la presencia de un macho dominante y la presencia de dos machos dominantes, cada uno de estos asociados a tres tipos de sustrato utilizados como refugios (tejas de barro, tubos PVC y malla mosquitera de plástico. De los resultado obtenidos se presentaron diferencias significativas ($P<0.01$) en cuanto al crecimiento de los langostinos en ausencia de machos dominantes, no se presentaron diferencias ($P>0.05$) para los diferentes sustratos utilizados como refugio, pero en la interacción entre el tipo de refugio utilizado y la relación jerárquica en la población solo se presentó diferencia ($P<0.05$) para el tratamiento ausencia de machos dominantes combinado con el uso de refugios de teja de barro. Se sugiere que los efectos sobre el crecimiento y sobrevivencia provocados por la jerarquía social de los langostinos y el uso de los refugios son parte de una mayor serie de interacciones que pueden ser de índole ecológico, etológico y fisiológico.

Palabras clave: *Macrobrachium tenellum*, jerarquía social, refugios, crecimiento

INTRODUCCIÓN

Los langostinos dulceacuícolas están incluidos en los decápodos suborden Caridea que tienen distribución mundial (Valencia y Campos, 2007; Hernández *et al*., 2007); en particular, la familia Palaemonidae es la más diverso y se puede encontrar en ambientes marinos, agua dulce o salobre (De Grave *et al*., 2008). Dentro de esta familia, el género *Macrobrachium* (Bate) incluye langostinos de agua dulce que viven principalmente en las zonas costeras en las regiones tropicales y subtropicales del mundo (Vega-Villasante *et al*., 2011, García-Guerrrero *et al*., 2013). Una de las especies más comunes en México es *Macrobrachium tenellum* (Smith, 1871), esta especie ha sido considerada como candidata para su aprovechamiento y conservación, ya que crece rápido, es todavía común en su entorno natural y puede tolerar altas densidades y amplias fluctuaciones de los parámetros físico-químicos del agua. También tiene un tiene buena adaptación al cautiverio (Ponce-Palafox *et al*., 2002, Espinosa-Chaurnad *et al*., 2011, y García-Guerrero *et al*., 2013).

Lograr lo anterior requiere que se cuente con información que permita establecer las condiciones de hábitat y ambiente para el desarrollo de los langostinos (Chaurnad-Espinosa *et al*., 2011). En este sentido Guzmán (1987); Román-Contreras (1991); Signoret y Bralovsky (2002); Espinosa-Chaurand *et al*., (2011); Vega-Villasante *et al*., (2011) han desarrollado estudios sobre las condiciones del hábitat para el desarrollo de los langostinos; Ruiz-Santos (1988); Vega-Villasante *et al*., (2011); Yamasaki-Granados *et al*., (2012) reportan el rol de las relaciones intra e interespecíficas de estas especies. En este sentido es donde aún no se cuenta con toda la información que permitan entender en comportamiento de estos organismos principalmente en cautiverio.

En general, los langostinos son agresivos y presentan marcada territorialidad (Holtschmit-Martínez, 1987; García-Guerrero y Apún-Molina, 2008), se ha demostrado la existencia de jerarquías sociales en la población de machos tanto en cultivo como en el medio natural, presentan efecto negativo en el del crecimiento por parte del macho alfa hacia los organismos subordinados, dicho fenómeno se le denomina efecto toro (New, 2002; Moraes-Riodades y Valenti, 2004; Thiel *et al*., 2010), tal efecto puede tener implicaciones en el crecimiento de los langostinos y debe de tomarse en cuenta para un adecuado manejo (Harán *et al*., 2004). Salmeron, 1985 y Jayachandran, 2001, mencionan que este dominio de los organismos más grandes tienen prioridad sobre el alimento o refugio, manifestándose por la presencia de un crecimiento desigual en organismos de la misma edad. Salmerón (1985) reporta que el macho alfa puede secretar alguna feromona al resto de la población, lo que inhibe el crecimiento.

A partir de lo anterior, el propósito del presente trabajo fue conocer el efecto que presentan las relaciones intraespecíficas de una población a través de la presencia de machos alfa y del tipo de refugio, sobre el crecimiento de *Macrobrachium tenellum,* bajo condiciones de cautiverio.

MATERIALES Y METODOS

Se colectaron adultos y juveniles prereclutas de langostino *Macrobrachium tenellum* en la bocabarra del río Colotepec (15°48'19''N y 97°01'09''O), en la región costa de Oaxaca, México. Para la captura de organismos se utilizaron redes de mano cuando se trató de juveniles escondidos en los pastos acuáticos y en el caso de los adultos se utilizó una red atarraya. Los organismos fueron transportados en contenedores de 250 litros con aireación continua y se aclimataron durante quince días en área de acuicultura experimental del CIIDIR-Oaxaca-IPN, en donde se alimentaron durante ese periodo con camaronina 40% de proteína.

Para evaluar el efecto que el macho alfa ejerce sobre juveniles de *M. tenellum* bajo diferentes tipos de refugios, se estableció un diseño experimental factorial 3x3 con nueve tratamientos tres variables experimentales: dos machos alfa, un macho alfa y sin machos alfa y tres factores que fueron los tipos de refugio (malla, pvc y tejas) cada uno de los tratamientos con 4 réplicas. Las 36 unidades experimentales consistieron en cajas de plástico (taras), con las siguientes medidas; 50 cm de largo y 35 cm de ancho y una altura de 30 cm, con capacidad de 50 L. Los refugios presentaron las siguientes características para todas las repeticiones: 10 tubos de PVC (policloruro de vinilo) de 1 pulgada de diámetro y 5 cm de longitud; malla de plástico tipo mosquitera verde $0.5m^2$ doblada en pliegues; y 2.5 tejas de barro de arcilla en pedazos pequeños para cubrir el fondo de la tara y formar cavidades. En cada unidad experimental se distribuyeron 10 juveniles de *M. tenellum* en cada replica. La distribución de los tratamientos quedo de la siguiente forma: T1 dos machos alfa y refugio de malla; T2 dos machos alfa y refugio de tubo pvc; T3 dos machos alfa y refugio de teja; T4 un macho alfa y refugio de malla; T5 un macho alfa y refugio de tubo pvc; T6 un macho alfa y refugio de teja; T7 sin macho alfa y refugio de malla; T8 sin macho alfa y refugio de tubo pvc; y T9 sin machos alfa y refugio de teja.

Para homogenizar las condiciones de los tratamientos y sus réplicas se utilizó un sistema de recirculación de agua en cascada, cada tratamiento conto con una bomba sumergible de 1200

litros/hora para garantizar el reflujo y el suministro de oxígeno para mantenerlo por encima de los 5 mg/l. Por otro lado para controlar la temperatura se colocó un calentador de termostato calibrado a una temperatura de 28°C para mantenerla constante en todos los tratamientos mediante el flujo continuo de agua y su paso por el calentador.

Después de 15 días de aclimatación se realizó la siembra de los organismos, los cuales presentaron un peso medio de 0.03g y una longitud media de 1.6 cm. Realizada la siembra cada unidad experimental de cubrió con malla de tela blanca para evitar el salto de los organismos fuera de las unidades. Dado las preferencias nocturnas del langostino, el suministro del alimento se realizó una vez al día durante la tarde entre las 17:00 y 18:00 horas con camaronina® con 35% de proteína. Para el mantenimiento de las unidades experimentales se realizó diariamente la remoción de las heces, alimento no consumido, animales muertos y mudas, realizando también cada dos días un recambio del 50 % del agua y cada 15 días un cambio total del agua para evitar la acumulación de desechos nitrogenados. Las biometrías en peso y longitud se realizaron cada 15 días durante los 90 días que duro el experimento. Para el registro de la longitud total de los langostinos se utilizó un vernier digital 0.1 cm y para el caso del registro de peso se utilizó una balanza digital analítica 0.001 g (Denver Instrument), la manipulación se realizó lo más rápido posible para disminuir el estrés.

Para determinar los parámetros del crecimiento se utilizaron los siguientes índices: Índice de sobrevivencia (%): S = 100 x (número inicial de organismos-número final de organismos/ número inicial de organismos); Incremento promedio de peso individual (g): IPPI= peso final - peso inicial; Incremento de peso por día (g/día): IPD = (peso final - peso inicial) / tiempo); Incremento de peso en porcentaje (%): IPP = 100 x (peso final - peso inicial) / peso inicial); y la tasa de crecimiento específico (%): TCE = 100 x [(ln peso final - peso inicial ln) / t]. TCE se calculó de acuerdo con Cortés-Jacinto *et al*. (2003). IPP e IPD se calcularon de acuerdo con Gitte y Indulkar (2005); S y IPPI se calcularon con lo propuesto por Vega-Villasante *et al*. (2011). Para obtener los estimadores de crecimiento en longitud, se utilizaron las mismas formulas en donde solo se sustituye por el valor del peso.

Para los análisis estadístico de los parámetros de cultivo como: sobrevivencia (S), crecimiento en peso y longitud (IPPI, IPD, IPP y TCE) se les aplicó el análisis de varianza de una vía para cada caso, previa prueba de normalidad (Kolmogorov-Smirnov, $\alpha=0.05$). Las diferencias significativas entre las medias de los tratamientos se determinaron por medio del método de Tukey ($p<0.05$).

RESULTADOS

El crecimiento en peso para los tratamientos sin las presencia de machos alfa independientemente del tipo de refugio utilizado presentaron diferencia estadísticamente significativas en cuanto al incremento en peso ($P<0.05$). Los refugios en los tratamientos sin considerar el efecto de la presencia-ausencia de machos alfa no presentaron diferencias estadísticamente significativas ($P>0.05$). Los tratamientos con ausencia de macho alfa relacionado con los refugios presentaron un incremento diario en peso de 0.008 g/d para los tratamientos con refugio de teja y pvc, y un menor incremento (0.004 g/d) para el tratamiento con refugio de malla. El tratamiento con dos machos alfa presento un incremento diario de peso de 0.005 g/d con refugio de teja, mientras que el menor incremento (0.003 g/d) se presentó en los tratamientos con refugio de malla y pvc. Los valores de crecimiento para el tratamiento con un macho alfa presentaron valores entre 0.004 g/d y 0.002 g/d; los demás parámetros de crecimiento para todos los tratamiento se observan en la tabla 1.

Tabla 1. Parámetros de crecimiento para el langostino *M. tenellum.*

Parámetro		**Presencia de 2 machos alfa**			**Presencia de 1 machos alfa**			**Ausencia machos alfa**		
		malla	**pvc**	**Teja**	**malla**	**pvc**	**teja**	**malla**	**pvc**	**Teja**
Peso inicial	(g)	0.02	0.02	0.03	0.03	0.03	0.02	0.04	0.04	0.04
Peso final	(g)	0.272	0.291	0.422	0.45	0.402	0.197	0.361	0.686	0.72
Incremento Promedio de peso Individual (IPPI)	(g)	0.252	0.271	0.392	0.42	0.372	0.177	0.321	0.649	0.68
Incremento en Peso por Día (IPD)	g/día	0.003	0.003	0.005	0.005	0.004	0.002	0.004	0.008	0.008
Incremento en Peso en Porcentaje (IPP)	%	1260	1355	1306.666	1400	1240	885	802.5	1615	1700
Tasa de Crecimiento Relativo (TCR)	%/día	16.8	18.066	17.422	18.666	16.533	11.8	10.7	21.533	22.666
Tasa de crecimiento especifico (TCE)	%/día	3.434	3.523	3.478	3.563	3.414	3.009	2.894	3.739	3.803

El incremento en peso absoluto para el tratamiento ausencia de macho alfa presentó diferencias significativas ($P= 0.046$) a los 45 días con respecto a la presencia de dos y un machos alfa, y altamente significativa (P 0.0002) a los 75 días (Figura 1).

La velocidad de crecimiento en peso (IPD) presentó el mayor incremento para el día 15 para todos los tratamientos, posteriormente se estabiliza el ritmo de crecimiento sin presentar diferencias

altamente significativas entre los tratamientos, hasta el día 75 en donde el tratamiento con ausencia de macho alfa y refugio de teja presento una velocidad de crecimiento con diferencias significativas.

La sobrevivencia entre los tratamientos no presento diferencias significativas (P<0.05) entre la presencia y la ausencia de machos alfa, pero si presento diferencias entre la presencia de dos machos alfa contra un macho alfa. La densidad inicial fue de 65 org/m^2 mientras que la densidad final promedio fue de 14 org/m^2, alcanzando sobrevivencias entre 17 y 30% en los tratamientos.

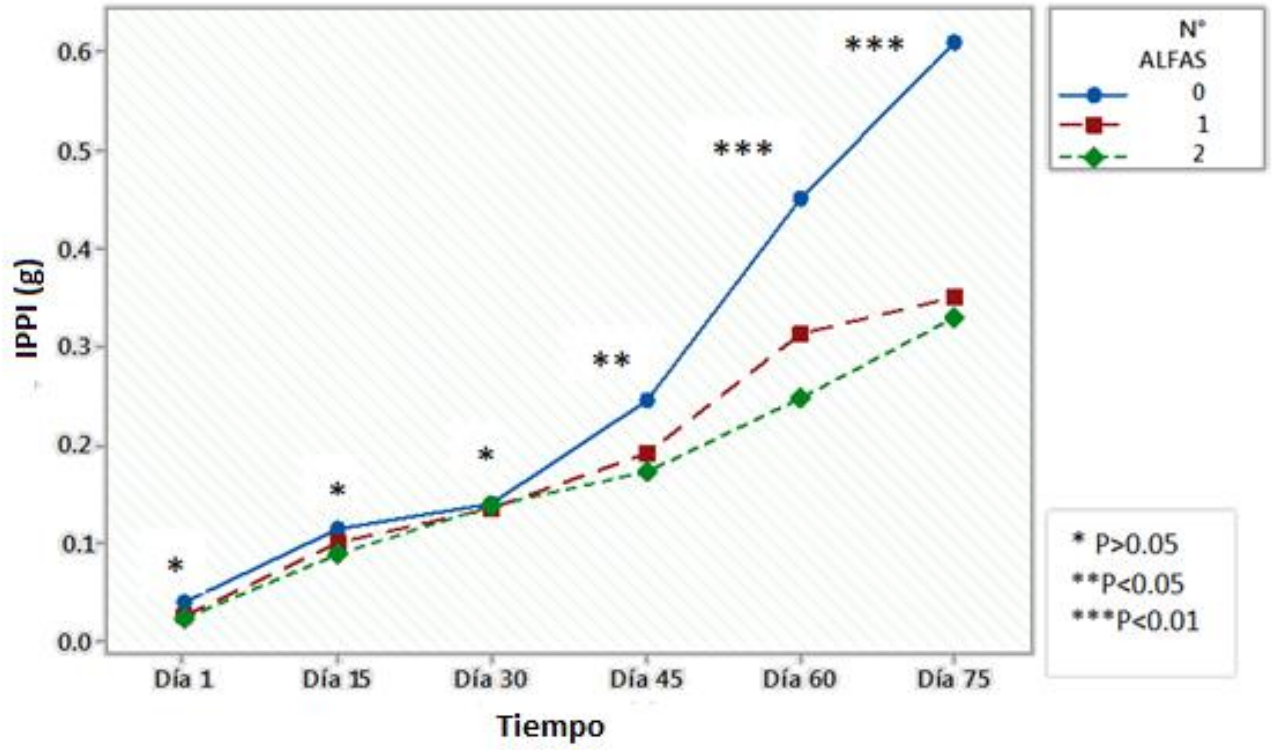

Figura 1. Incremento en peso en relación con la presencia y ausencia de machos alfa

En cuanto al coeficiente de asimetría la ausencia de macho alfa para los tres tipos de refugio presento un valor de coeficiente de regresión de b=3.245, para el tratamiento con un machos alfa de b=2.8348 y para el tratamiento con dos machos alfa presentó un valore de b=2.8318.

En la tabla 2 se observa los valores del efecto de la interacción entre la presencia de machos alfa y el tipo de refugio con respecto al crecimiento expresado en ganancia en peso, se observa que el tratamiento sin presencia de macho alfa con refugio teja obtuvo la mayor media mostrando diferencias significativas con un α=0.05.

DISCUSIONES

El efecto de las jerarquía social y de manera independiente el efecto de los refugios han sido ampliamente estudiados en la especie asiática *M. rosenbergii*, pero para el langostino de río nativo del Pacifico americano *M. tenellum* es escasa la información concerniente a la interacción de la jerarquía social y el tipo de refugio sobre su crecimiento y sobrevivencia.

Tabla 2. Promedios del peso de *M. tenellum* para los tratamientos en relación al número de machos dominantes y el refugio. Tukey ($\alpha = 0.05$)

Macho Dominante /refugio	Media	Agrupación Tukey		
0 / teja	4.067	a		
0 / pvc	3.950	a	b	
2 / teja	3.400	a	b	
1 / malla	3.400	a	b	
1 / pvc	3.313	a	b	c
0 / malla	3.186		b	c
2 / pvc	2.927			c
2/ malla	2.927			c
1 / teja	2.643			c

0 Ausencia de machos dominante; 1 presencia de un macho dominante; 2 Presencia de dos macho dominante

Jayachandran (2001) y Moraes-Riodades y Valenti (2004) mencionan que en los langostinos además de los factores genéticos y ambientales se presentan diferencias de crecimiento entre los organismos de una misma población, en donde un macho adulto sexualmente maduro limita el crecimiento de los machos jóvenes, dicho fenómeno que provoca la dominancia social en estos crustáceos es conocido como "efecto toro" Vega-Villasante *et al*. (2011). Para éste trabajo el crecimiento irregular reportado entre los tratamientos y las réplicas se ajusta a lo descrito por Casas-Sánchez *et al*. (1995) como crecimiento individual heterogéneo (CIH).

Karplus (2005) indica que la heterogeneidad en el tamaño de los machos en una población de langostinos Macrobrachium rosenbergii refleja una estructura compleja compuesta de tres morfotipos: los machos pequeños, machos con quela color naranja y machos con quela azul; los cuales difieren en su morfología, fisiología y comportamiento. Para el presente trabajo solo se reconocieron dos morfotipos: juveniles y machos adultos en donde no se presentaron jerarquías intermedias. En este sentido Barki *et al*. (1992), Karplus (2005) y Harrington y Hovel (2015) establecen que las interacciones sociales entre los machos sexualmente maduros y los jóvenes afectan el crecimiento de los últimos, sugiriendo que se afectan mecanismos sociales como: competencia directa por el alimento, alteración de la eficiencia de conversión de alimentos y aumento del gasto energético en la actividad motora; los anteriores mecanismos que de manera similar se presentan en éste trabajo son supuestos desarrollados a partir de la teoría de juegos de Maynard (1974).

Las diferencias que se presentaron en el presente trabajo, además del efecto provocado por el macho dominante puede deberse a que los juveniles también pueden presentar jerarquías, Fernandes-Silva y Arruda (2014) reportan que en juveniles *Macrobrachium rosenbergii* también pueden ser diferentes, pero sólo en el tamaño corporal, donde los juveniles presentan una jerarquía de dominancia relacionada con el tamaño y pueden afectar el perfil de comportamiento individual.

Dicho comportamiento jerárquico de los juveniles se reporta en el presente trabajo en donde la tasa de crecimiento presento los mayores valores en la fase inicial del experimento (día 15) para después estabilizarse, en donde Barki *et al.* (1991) y Barki *et al.* (1992) establecen que las jerarquías de dominio requieren cierto tiempo para establecerse dentro de los juveniles, aunque la posición del macho alfa se presenta desde el primer día, en donde la estabilización de la jerarquía fue evidente con la disminución en las frecuencias de interacción. Dicho crecimiento acelerado que presentan los juveniles en este trabajo sugiere que algunos organismos con crecimiento precoz y acceso rápido al alimento adquieren una posición dominante y son más propensos a convertirse en machos adultos dominantes (Fernandes-Silva y Arruda, 2015).

Además de las interacciones que presentan los juveniles dentro de la población subordinada, también se debe considerar la estructura dentro del grupo de langostinos que comparten un mismo espacio, lo anterior debido a que en etapas jóvenes no se separar las hembras de los machos, factor que puede provocar que se presenten jerarquías dentro del grupo juvenil, en este sentido Ranjeet y Kurup (2011) sugieren que las hembras de *Macrobrachium rosenbergii* presentan crecimiento

adecuados durante el cultivo, pero indican que en altas densidades la tasa de crecimiento de las hembras es lento en comparación con los homólogos masculinos y, por tanto, las posibilidades de que sean canibalizadas por los machos mayores, especialmente durante la fase temprana del cultivo.

El manejo de altas densidades resultan en un bajo porcentaje de supervivencia de las hembras de *Macrobrachium rosenbergii* en altas densidades, esto se debe principalmente a la competencia por el alimento y el espacio que enfrentan ante los langostinos adultos de sexo masculino, de la misma manera en altas densidades la proporción de machos pequeños fue relativamente alta, mientras que el porcentaje de adultos grandes y sus etapas de transición mostraron reducción (Ranjeet y Kurup, 2011; Vega-Villasante *et al.* 2011), lo anterior puede sustentar los bajos valores de sobrevivencia de *M. tenellum* en el presente trabajo de un 30 a un 17% con una densidad de 64 organismos m2, con la misma especie López-Uriostegui *et al.* (2014) alcanzaron valores de sobrevivencia de 47% con una densidad de 24 organismos m2. Estos valores se pueden incrementar bajo la propuesta de estandarizar la densidad de población en diferentes etapas del cultivo y la estructura de la población, realizándose desde la siembra (Malecha *et al*., 1981; Ranjeet y Kurup, 2002).

Debido a la agresividad y conducta caníbal de los langostinos ocasionado por sus jerarquías sociales o el CIH (García-Guerrero *et al*., 2015), el uso de sustratos como refugios permite incrementar la sobrevivencia de la población: En el presente trabajo el tipo de refugio por si solos no presentaron diferencias estadísticas ($P<0.05$), aunque si cuando se combina con la presencia o ausencia de un macho dominante ($P>0.01$). En el primer caso la falta de efecto posiblemente se debe a que cada especie presenta diferente afinidad al refugio utilizado en cuanto a su tamaño, material y densidad. Para denotar lo anterior Vásquez *et al.* (2000) menciona que *Macrobrachium brasiliense* prefiere la presencia de raíces, hojarasca y palos, en donde en su ausencia la aparición de excavaciones. Para *M. rosenbergii* esta especie prefiere los materiales gruesos como piedras, en comparación a los materiales finos como la arena y lodo (Murthy *et al.,* 2012) dichos sustratos presentaron mayor crecimiento en comparación al uso de tubos PVC o malla plástica como refugios para la misma especie (Tidwell *et al.,* 1998 y Tidwell *et al.,* 2000). Los datos en el presente trabajo en cuanto a ganancia en peso y TCE se ajustan a las ideas previas en donde los refugios con teja de barro presentaron mayor crecimiento sobre los tubos PVC y la malla plástica subsecuentemente. La tendencia anterior también se observa en juveniles *Cherax cuadricarinatus* criados en pequeñas piedras donde la masa corporal fue estadísticamente significativa respecto al grupo control.

Por otro lado para reducir el estrés y conducta agresiva de los langostinos es importante considerar el número y área de los refugios, en donde la vegetación marginal de los cuerpos de agua juegan un papel importante en la protección de éstos organismos (Soares *et al*, 2015), en donde materiales que simulan la vegetación marginal son idóneos para la protección. Tayasu *et al*. (1996) indica que la vegetación acuática forman una compleja y densa red de escondites que aumento de la calidad del refugio durante la interacción jerárquica, donde se demostró que los langostinos responden a estímulos de detección y movimiento de la especie. En el presente estudio el uso de malla plástica como sustituto de la vegetación acuática no presento ventajas sobre otros materiales a pesar de que la densidad y área del refugio fue mayor, donde para decápodos estas características reducen la locomoción y evita las jerarquías reduciendo la mortandad (Marshall *et al.,* 2005).

En cuanto a la interacción entre los refugios y las jerarquías dentro de los langostinos, para el langostino de río *M. tenellum* solo Villasante-Vega *et al.* (2011) evidencia como la jerarquía social asociada a un determinado tipo de refugio provocan diferencias en su crecimiento, pero sin detallar como afecta la relación intraespecífica de la población.

Los resultados en el presente trabajo con relación al crecimiento y sobrevivencia de los langostinos, puede deberse a una serie de interacciones entre la presencia y numero de machos dominantes, así como la forma en la que los grupos jerárquicos y subordinados usan los refugios, en los datos presentados en este trabajo la presencia de dos machos dominantes provoca competencia entre ellos ignorando a los juveniles, en donde se alcanzó el mayor crecimiento, Lammers *et al*. (2009) mencionan que los langostinos dominantes y subordinados responden a los mismos estímulos, los machos dominantes son más activos con la presencia de refugio y aumenta la actividad de los subordinados que presentan mayor actividad fuera de los refugios.

Dentro de los resultados observados entre los tratamientos del presente trabajo, las variaciones en el crecimiento también pueden ser atribuidas a aspectos fisiológicos como los hormonales, donde Vásquez-Acevedo *et al.* (2009) menciona que existen neuropéptidos en *M. rosenbergii* que estimulan su agresividad y otros neuropéptidos que modulan el comportamiento del langostino, donde el uso de ciertos péptidos reducen su agresividad y por consecuencia evita que se establezcan relaciones jerárquicas.

Los resultados que este trabajo evidencian como el efecto de la presencia y el número de machos dominantes afectan el crecimiento y sobrevivencia de una población de juveniles de *M. tenellum*, y

como estos organismos prefieren determinados tipos de sustrato utilizados como refugios para reducir el comportamiento agresivo de los machos dominantes, a pesar de lo anterior es necesario realizar más estudios de tipo ecológico, etológico y fisiológico que permita conocer los mecanismos biológicos que disminuyan la agresividad de los langostinos y que eviten la aparición de grupos jerárquicos, así como estudios de materiales durante el confinamientos que sustituyan aquellos que se encuentran en ambientes naturales y que también reduzcan la agresividad de los langostinos.

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CAPITULO V.

PRODUCTOS GENERADOS DURANTE EL DOCTORADO

PONENCIAS EN EVENTOS NACIONALES E INTERNACIONALES

Distribución de los langostinos nativos de importancia acuícola en Oaxaca. Decimo Simposio Internacional sobre estudios Oaxaqueños Mayo del 2013.

Los microcrustáceos una opción en la alimentación de especies de acuícolas en Oaxaca. Decimo Simposio Internacional sobre estudios Oaxaqueños. Mayo del 2013.

Evaluación de dietas isoproteicas en el cultivo del langostino *Macrobrachium tenellum* para el sureste mexicano. VIII Congreso Nacional de Investigación Politécnica. Octubre 2013.

El uso de microcrustáceos en la alimentación de especies acuícolas en el Estado de Oaxaca. VIII Congreso Nacional de Investigación Politécnica. Octubre 2013.

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Efecto de la presencia y frecuencia de machos dominantes, y el uso de refugios sobre el crecimiento y sobrevivencia del langostino de río *Macrobrachium tenellum* (smith, 1871*).* Segundo Congreso Internacional "Macrobrachium". Puerto Vallarta, Jalisco, UdeG-CUCosta, Mayo, 2014

PUBLICACIONES

Effect of dietary chitin on digestive enzyme activity, growth, and survival of *Macrobrachium tenellum* juvenile prawns
De los Santos Romero Rodolfo Benigno, García Guerrero Marcelo, Vega Villasante Fernando and Nolasco Soria Héctor.
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Effect of photoperiod and temperature on digestive enzyme activity, and growth of juvenile longarm river prawn (*Macrobrachium tenellum*).
De los Santos Romero Rodolfo Benigno, García Guerrero Marcelo, Vega Villasante Fernando, Cortes Jacinto Edilmar and Nolasco Soria Héctor.
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Efecto de la presencia y frecuencia de machos dominantes, y el uso de refugios sobre el crecimiento y sobrevivencia del langostino de río *macrobrachium tenellum* (smith, 1871).
De los Santos-Romero, R.B. y García-Guerrero, M.U.
Concluido en traducción para Someter

Conservation and aquaculture of native freshwater prawns: the case of the cauque river prawn *Macrobrachium americanum* (Bate, 1868)
Marcelo García-Guerrero, Rodolfo de los Santos Romero, Fernando Vega-Villasante & Edilmar Cortes-Jacinto
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