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Panulirus argus postlarva performance fed with fresh squid

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ABSTRACT

There is a great interest in the growout of spiny lobster postlarva captured in the wild. This activity has been developed rapidly in Asia and especially in Vietnam. However, lack of appropriated formulated diets remains the main constrain to the sustainable expansion of this activity worldwide. Until now, best growth rates have been obtained with the use of high inclusion of krill meal and/or krill hydrolysates which are expensive. However, squid is today avoided as food ingredient due to low digestibility reported. Also, fresh squid has been demonstrated to produce poor growth performance in temperate lobsters, but there is no report for *P. argus* fed exclusive on fresh squid. This work examines the effect of fresh squid on growth of *P. argus* (initial weight=1.8 ± 0.46g, initial cephalotorax length=10.62 ± 1.576 mm) during 60 days. Lobsters fed with fresh squid attained a final weight 4 times higher that the lobsters fed on fish dry pellets. Weight and size increments, as well as specific growth rates were higher for lobster fed with squid, and resulted similar to the better growth rates previously reported for *P. argus* and other lobsters. Results indicate that in spite of previous negative reports for spiny lobsters, fresh squid is an appropriated food for *P. argus*. It is suggested that processing technology should be improved for obtaining more soluble and digestible squid meals for lobsters.

Key words: Diet; Growth rate; *Panulirus argus*; Spiny lobster; Squid.

RESUMEN

En la actualidad existe gran interés en la engorda de langostas espinosas a partir de postlarvas capturadas en el medio natural. Esta actividad acuícola se ha desarrollado vertiginosamente en Asia y sustenta una industria millonaria sobre todo en Vietnam. El principal impedimento para la expansión sostenible de este tipo de acuicultura a otras regiones es la ausencia de dietas formuladas adecuadas que proporcionen altas tasas de crecimiento. Los mejores crecimientos con piensos hasta la fecha se han logrado con la inclusión de grandes cantidades de harina de krill y/o hidrolizados de krill, ingredientes de elevado costo. Sin embargo, actualmente se evita el uso de harina de calamar en dietas experimentales para langostas debido a reportes de baja digestibilidad. También se ha demostrado que el calamar fresco produce bajos crecimientos en algunas langostas de aguas templadas, pero no existen reportes de crecimiento de *P. argus* alimentadas exclusivamente con calamar. En el presente trabajo se estudió el efecto del calamar fresco sobre el crecimiento de postlarvas de *P. argus* (peso inicial=1.8 ± 0.46 g, largo de cefalotórax inicial=10.62±1.576 mm). El experimento tuvo una duración de 60 días. Las postlarvas alimentadas con calamar alcanzaron pesos finales 4 veces superiores a las que fueron alimentadas con pienso de pescado. Los incrementos de peso, talla y la tasa de crecimiento específica también resultaron superiores con calamar fresco y fueron similares a los mejores crecimientos reportados en la literatura para *P. argus* y otras especies. Los resultados indican que a diferencia de lo reportado anteriormente para langostas espinosas, el calamar fresco es un alimento apropiado para *P. argus*. Se sugiere que se debe mejorar los procedimientos de obtención de las harinas de calamar, con el objetivo de mejorar su digestibilidad para langostas espinosas.

Palabras clave: Calamar; Crecimiento; Dieta; Langosta Espinosa; *Panulirus argus*.

INTRODUCTION

There is a great interest in the development of spiny lobster aquaculture based on the growout of wild-caught postlarva. Seed availability and natural mortality rates of this stage are high (Phillips *et al.*, 2003, Cruz *et al.*, 2006) and therefore, minimum impact of this activity on fisheries has been predicted (Phillips *et al.*, 2003). Also, important advances have been reported recently on the obtaining of lobster from hatcheries. For example, the Australian company “Lobster Harvest” cultured several cohorts of second generation (F2) *P. ornatus* with survivals of 5-10% (Barnard *et al.*, 2011). Modest, but very positive results have been obtained USA with *P. argus* (Goldstein and Nelson, 2011).

Tropical species like *Panulirus argus* and *Panulirus ornatus* have been proposed as best candidates due to high growth rates (Jeffs and David, 2002). The growout of spiny lobsters (mostly *P. ornatus*) in Vietnam accounted for around USD 40 millons in 2004 (Thuy, 2004) and USD 100 millions in 2006 (Jones and Williams, 2007) based on fresh feeding. However, lack of appropriated formulated diets remains the main constrain to the sustainable expansion of this activity worldwide.

Nutritional requirements for some spiny lobsters have been assessed (Glencross *et al.*, 2001, Crear *et al.*, 2002, Smith *et al.*, 2003, Ward *et al.*, 2003, Johnston *et al.*, 2003, Smith *et al.*, 2005) but growth rates on formulated diets are still suboptimal for most species, even when lobsters fed on pellets of similar composition to natural preys (Simon and Jeffs, 2008). In spite of more than 20 years of research on diet development, efforts to develop high performance pelleted dry feeds for spiny lobster growout have not met with a lot of success until recently (Williams, 2007, and references therein). However, Smith *et al.* (2005) and Barclay *et al.* (2006) developed a high-protein pellet diet for *P. ornatus* with growth results better than mussels. This diet was the first pellet to achieve superior growth to fresh food, thus representing an inflection point in the long path of diet development for spiny lobsters. Results had been attributed to high feeding frequency, but also to the ingredient make-up of the formulation because it contains high amounts of krill meal. This component was also present at high level in a diet for *P. argus* recently reported to produce good growth rates (Cox and Davis, 2009).

Unfortunately, few studies on nutrients digestibility have been conducted, being available for proteins only two *in vivo* (Ward *et al.*, 2003a, Irvin and Williams, 2007) and one *in vitro* (Perera *et al.*, 2010) studies. These studies yielded surprising poor result for squid meal digestibility, being only 7% in the temperate lobster *Jasus edwardsii* (Ward *et al.*, 2003a) and 59% in the tropical lobster *P. ornatus* (Irvin and Williams, 2007). The insufficient nutriational value to the temperate lobster *J. edwardsii* was further corroborated, with poor growth and reduced survival through successive molts (Radford *et al.*, 2007) when lobster fed exclusively on squid. However, there is no report on the growth of *P. argus* (and any other tropical lobster) fed solely with fresh squid.

Squid or squid meal have been long recognized as desirable ingredients of fish and crustacean diets due to proved or suspected benefits. For example: high protein digestibility (Akiyama *et al.*, 1992, Lemos *et al.*, 2009), enhancing effects on the absorption of nutrients (Cruz-Ricque *et al.*, 1989), stimulating effect on digestive enzymes (Córdova-Murrueta and García-Carreño, 2002, Perera *et al.*, 2005), beneficial effects on gonadal development, egg quality and larval survival (Fernández-Palacios *et al.*, 1997, Watanabe and Vassallo-Agius, 2003, Hoa *et al.*, 2009) and growth rates (Åsgård, 1987, Cruz-Súarez *et al.*, 1992; Forster *et al.*, 2010). Taking into account the poor squid digestibility reported (Ward *et al.*, 2003; Irvin and Williams, 2007; Perera *et al.*, 2010), the advantage of using this component in lobster diets is no as obvious as for other crustaceans like shrimps. Actually, current information on squid digestibility would preclude its use in future experimental diets for *P. argus* or other tropical lobsters. If poor digestibility of squid meal is a result of the processing of raw material, then fresh squid should allow good growth rates.

Herein, we present the results of a growout trial of *P. argus* postlarva fed exclusively with fresh squid. In order to easy recognize any possibly negative effect of squid feeding, a commercial diet designed for cobia fish (*Rachycentron canadum*) was included as a negative control. This kind of diet (fish meal as main ingredient) has been regularly reported as inappropriate for lobsters. Results are discussed in terms of squid proteins utilization in comparison with data in literature for tropical and temperate lobsters.

MATERIALS AND METHODS

P. argus postlarvae were collected by floating sandwich collectors in Punta Francés, SE of Isla de la Juventud, Cuba. They were transported to the laboratory in tanks with algae as substrate and with the fauna in the collectors as food. Animals were acclimated to lab conditions for one week: salinity 35.1 ± 0.39, temperature 26.2 ± 0.60ºC, photoperiod 12h:12h, ammonia 0.04 ± 0.023 mg/L, nitrite 0.232 ± 0.0035 mg/L and phosphate 6.88 ± 0.197 mg/L. During this week postlarvae were fed *at libitum* with frozen adult *Artemia*. After this acclimation period, weight (W) and cephalothorax length (CL) were recorded, and animals were randomly distributed to 100 L experimental tanks connected to a recirculated sea water system. Mean initial weight (Wi) was 1.8 ± 0.462 g and mean initial cephalothorax length (CLi) was 10.62 ± 1.576 mm.

The trial lasted for 60 days and treatments were two diets: fresh squid (*Loligo sp.*) and fish pellet, each containing two replicated tanks of ten lobsters. Rations were adjusted in order to lobsters ingest 60-70% of pellet and 80-95% of squid offered. For achieving these ingestion rates, rations were 10% for pellet and 120% for squid (wet biomass basis). Siphoning of tanks was performed prior each feeding to collect uneaten food. Food wastes were rinsed with distilled water and dried at 60ºC until constant weight. Apparent feed intake was calculated by subtracting the dry weight of uneaten feed from the dry weight of food offered, taking into account the feed lost into the water. Food lost into the water was calculated by immersing samples into 2 replicate tanks overnight with aeration as in experimental tanks. Lobsters were weighed and measured every one week. Growth results were statistically compared by t-Student test (α= 0.05) with Statistica software v.7.0.

As a measure of attractibility of food the time to grasp food (in seconds, first individual responding) and the number of individuals responding (no. grasps) were recorded.

The following variables and indexes were recorded or calculated:

1. Initial weight (Wi) and final weight (Wf) (g).
2. Weight increment (g)= Wf-Wi.
3. Percent of weight increment (%)= ((Wf - Wi)/Wf)*100.
4. Initial cephalotorax length (CLi) and final cephalotorax length (CLf) (mm).
5. Size increment (CL, mm)= CLf-CLi.
6. Percent of size increment (CL, %)= ((CLf - CLi)/CLf)*100.
7. Ingestion rate (g food/g lobster/day)= Dry weight food offered - (dry weight food not ingested + dry weight loosed)/g lobster/day.
8. Number of molts.
9. Weight increment per molt (%)= (weight before molt/weight after molt)*100.
10. Size increment per molt (CL, %)= (CL before molt/CL after molt)*100.
11. Specific growth rate (SGR)= ((ln Wf – ln Wi)*100)/days of experiment.
12. Specific size increment = ((ln CLf – ln CLi)*100)/ days of experiment.

RESULTS AND DISCUSSION

During the two month of experiment, there were neither shell disease detected nor aggressive interaction during feeding, and only one dead at molt was observed in the pellet treatment (based on fish meal). The last has been long recognized as a result of nutritional unbalance (Kittaka and Booth, 2000). Cox and Davis (2009) also reported this phenomena in lobster fed formulated diets with more than 50% of fish meal in its composition. This observation indicates us that feeding lobsters exclusively with squid do not produce nutritional unbalance affecting survival of *P. argus* in two months. As mentioned above, this result is different to those obtained by Radford *et al.* (2007) in the lobster *J. edwardsii.*

The time for first individual to grasp food offered was the same for the two treatments but the number of individuals responding was higher for squid, being this variable a more reliable measured of atractibility. Beside squid to be more attractive, ingestion rate of squid was greater (dry weight basis).

In general, specific growth rate for tropical lobsters have been higher than for temperate lobsters (Table 1). The exception is the work of Glencross *et al.* (2001) which report very high specific growth rates (2.78%/day) for *Panulirus cygnus* fed with pellets. The growth on pellets in Glencross *et al.* (2001) is probably due to a right protein to lipid ratio, though growth of *P. cygnus* on mussels was also unusually high (5.58%/day) in this work (see specific growth rates in (Table 1).

Final weight of *P. argus* postlarva fed fresh squid was 4 times higher than in lobsters fed the fish pellet (t= 5.07, p≤ 0.05), being the weight increment of lobsters fed squid two times higher than for those ingesting the pelleted diet (Table 1). Similar results were obtained when cephalothorax length is analyzed (t= 4.92, p≤ 0.05). Percent of size increment (CL) observed was similar to those found for others in the same specie (Lellis and Russell, 1990, Díaz-Iglesias *et al.*, 1991; Fraga, 1996; Cox and Davis, 2009). However, except the work of Cox and Davis (2009), all other studies reported weight increments higher than the ones we found (Table 1). This could be a result of the higher temperatures these authors used: 29°C and 30°C (Lellis and Russell, 1990) and 28°C (Díaz-Iglesias *et al.,* 1991). In our experiment we led the temperature at ambient, 26°C, due to current trend to grow lobster in sea cages without control of temperature.

Lobsters grow faster with fresh squid by both increasing molts number (8 to 12) and weight increments at each molt. Weight increment per molt similar to those obtained in the present experiment were obtained before for postlarva of the same specie (Lellis, 1992; Lellis and Russell, 1990) and for *J. edwardsii* (Johnston *et al.,* 2003; Thomas *et al.,* 2003), both with formulated diets and fresh food (Table 1).

Specific growth rate in this work with the fish pellet is the smallest obtained for this specie, confirming this diet is not suitable for lobsters. The specific growth rate obtained for lobsters fed fresh squid, however, is similar and even superior to those previously reported for *P. argus* (Table 1). This means that squid protein for growth is at least similar to those for other sea food and support the idea that available digestibility data for squid in lobster are just applicable to meals. The exception is the work of Cox and Davis (2009), because they reported, to our knowledge, the highest specific growth rates so far reported for this specie (Table 1). The former work is, however, difficult to evaluate since it lacks any measure of variability in growth data. Probably, high dispersion of primary data account for the fact that they did not found differences between specific growth rate for the formulation 1 (3.49%/day $^{-1}$) and the frozen sea food (2.46%/day $^{-1}$). However, it is worthy to realize that in our experiment, the specific growth rate at 28 days (duration of Cox and Davis's experiment) was 2.26%/day^{-1}; this is only slightly smaller than the one obtained for Cox and Davis (2009) with a combination of different frozen sea food (squid, fish, shrimp, clam).

Table 1. Growth of *P. argus* postlarva under laboratory conditions fed fresh squid and a fish pellet. Results for other spiny lobsters are shown for comparisons only when the same indexes were calculated. Unless a range is presented, only the best results of the different studies are shown. For references in the table see foot note.

	Fish pellet[1]	Squid[2]	Tropical *(P. argus, P. ornatos, P. homarus)*	Temperate *(J. edwardsii , P. cygnus)*
Initial weight (g)	1.80 ± 0.162	1.79 ± 0.462	Only studies using lobsters of similar initial weight are cited herein.	
Final weight (g)	2.75 ± 0.862	5.92 ± 0.191		
Weight increment (g)	0.95 ± 0.70	4.13 ± 0.271		
Weight increment (%)	32.04 ± 15.405	69.92 ± 6.841	54-113 (pellets) (11) 135-300 (worm, gastropods)(12) 62.3 (formulation)(18) 49.8(frozen sea food)(18)	58-122 (pellets) (9) 186 (mussel) (9) 336 (mussel) (10) 189 (pellets) (10)
Initial CL (mm)	11.23 ± 0.130	10.62 ± 1.576		
Final CL (mm)	13.61± 0.968	17.30 ± 0.424		
Size increment (mm)	2.03 ± 0.597	6.68 ± 1.152		15.2 (pellets)16 21 (mussel)16
Size increment (%)	14.77 ± 3.337	38.68 ± 7.610	12-21 (pellets) (11) 35-47(alive gastropods) (12) 22.4(formulation 1)18 18.4(frozen sea food)18	
Ingestion rate (g/g/día)	0.07 ± 0 .01	0.17 ± 0.023		
No. molts	8	12		
Weight increment /molt (%)	20.24 ± 6.546	60.78 ± 3.337	57-59 (Artemia) (1 y 2) 20-27(Pellets) (1)	17-28(Pellets) (6) 27-58 (Pellets) (7) 52 (Pellets) (8)
Size increment/molt (%)	10.33 ± 0.274	19.23 ± 1.797	13-14 (Artem.) (1 y 2) 5-8 (Pellets) (1)	
Specific growth rate (% weight /day)	0.66 ± 0.381	2.02 ± 0.382	1.6 (rotational) (4) 2 (pellets) (5) 1.38 (pellets)17 0.80 (mussel)17 3.49 (formulation 1)18 2.46 (frozen sea food)18 1.63 (pellets)18 0.4-1.64 (fresh sea food)3	0.82 (pellets) (7 and 8) 0.4 - 0.8 (pellets) (9) 0.95 (pellets) (10) 2.78 (pellets)15 0.78 (pellets)16 0.97 (mussel) (7) 0.92 (mussel) (9) 1.3 (mussel) (10) 1.25 (rotational) 13 0.76 (squid) 14 1.65 (mussel)14 5.58 (mussel)15 0.96 (mussel)16 1.39 (pellet)19
Specific growth rate (% CL /day)	0.27 ± 0.065	0.82 ± 0.207	0.8 (frozen sea food) (3) 0.90 (formulation 1)18 0.73(frozen sea food)18 0.42 (pellets)18	

(1)Lellis, 1992, *P. argus,* (2)Lellis and Russell, 1990, *P. argus,* (3)Vijayakumaran *et al.*, 2009. *Panulirus homarus,* (4)Jones *et al.*, 2001. *P. ornatus,* (5)Smith *et al.*, 2003, *P .ornatus,* (6)Johnston *et al.*, 2003. *J. edwardsii,* (7)Thomas *et al.*, 2003. *J. edwardsii,* (8)Tolomei *et al.*, 2002. *J. edwardsii,* (9)Ward *et al.*, 2003, *J. edwardsii,* (10)Crear *et al.*, 2000, *J. edwardsii,* (11)Fraga, 1996, *P. argus,* (12)Díaz-Iglesias *et al.,* 1991, *P. argus,* (13)Johnston *et al.*, 2006. *P. cygnus,* (14)Radford *et al.,* 2007. *J. edwardsii,* (15)Glencross *et al.*, 2001. *P. cygnus,* (16)Simon and James, 2007. *J. edwardsii,* (17)Smith *et al.*, 2005, *P. ornatus,* (18)Cox and Davis, 2009. *P. argus,* (19)Johnston *et al.*, 2008, *P. cygnus.*

From the comparison of our results with those obtained before for *P. argus*, it can be postulated that growth rates obtained with a wide variety of food, both fresh and pelleted are not superior to those the *P. argus* postlarva can achieve when feeding solely fresh squid (Table 1).

Results indicate that squid protein are readily digested and assimilated by lobsters. Poor protein digestibility of squid meal (Ward *et al.*, 2003, Irvin and Williams, 2007) could be the results of poor quality meals. The aggregation of proteins of the squid mantel even at moderate temperatures (35°C), with a reduction of protein solubility, has been reported before (Gómez-Guillén *et al.*, 1998). Also, strong differences in free amino acid content and digestibility between squid muscle and squid meal proteins have been reported (Perera *et al.*, 2010). The growth information herein presented, coupled with previous reports on growth enhanced effects of squid on other crustaceans, indicate that squid would be an ingredient of choice for *P. argus*, and suggest that processing technology should be improved for obtaining more soluble and digestible meals.

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