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Feeding Responses of Cuttlefish (*Sepia pharaonis*) at Early Hatchling Stages

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ABSTRACT

The cuttlefish *Sepia pharaonis* is an important fishery resource in tropical archipelagic environments. This species is highly sought in both local and international market trades. Therefore, intensive efforts are currently undertaken to develop practical larval rearing protocols to produce juveniles for stock enhancement and for aquaculture purposes. However, the lack of basic information regarding the larvae prey preference, basic digestive physiology and nutritional requirements are considered major bottlenecks, slowing the progress in the development of a hatchery production system for this species. To fill these gaps, the present study was conducted following a factorial design to evaluate the overall feeding response of cuttlefish at different hatchling stages (1, 3 and 5 days after hatching) given with different prey types (*Artemia* larvae, mysids and tilapia fry) at varying densities (low, medium and high). Results indicate that regardless of age after hatching, *S. pharaonis* paralarvae had a strong feed preference for *Artemia* rather than mysids or fish fry. At all larval stages tested, a strong interaction of prey type and density was noted with *Artemia* as the preferred prey. Feeding ingestion of *Artemia* larvae as prey organism increased with increasing density. Lowest ingestion of prey was observed in the group given with tilapia fry at a low density. Collectively, the present findings suggest that *Artemia* larvae at a density of 15 *Artemia*/100 mL is suitable as a live prey for *S. pharaonis* at early hatchling stages.

Key words: *Sepia pharaonis*, cuttlefish, feed consumption, preference, paralarvae

INTRODUCTION

Cephalopod particularly the genus *Sepia* is an important fishery resource since the global trend in the market demand of these organisms as food is currently rising (Sykes *et al*., 2006). Generally, the high demand for this organism could be attributed to its high protein content, well-balanced fatty acid content and a highly acceptable meat texture (Sinanoglou and Miniadis-Meimaroglou, 2000). The global declining trend of catch in marine capture fishery and the rising demands and consumption of cephalopods could be a driving factor to the development of aquaculture technology for this species. Cuttlefishes possess desirable biological characteristics as potential species for mariculture, stock enhancement and sea ranching activities.

Among the cephalopod species with high fishery value, *Sepia pharaonis* is one of the biggest, fastest growing and considered to possess desirable traits for aquaculture production.

However, basic biological information about this species is lacking. Earlier reports about rearing and laboratory production of cephalopods were focused on temperate species specifically *Sepia officinalis* (Boletzky, 2003; Franco-Santos and Vidal, 2014). Like any other cultured marine species, larval rearing associated problems are considered major hindrance in the development of culture technologies for *Sepia* species. Earlier reports indicate that rearing of the cuttlefish paralarvae is the most challenging phase due to the occurrence of high mortalities in the first week after egg hatching (Iglesias *et al*., 2007; Uchikawa *et al*., 2009; Domingues *et al*., 2001). Further, the period subsequent to 1-3 Days After Hatching (DAH), is considered the most critical phase for cephalopod larval survival wherein yolk supply are fully consumed but prey capture mechanisms are not yet fully developed (Wakabayashi *et al*., 2005; Shigeno *et al*., 2001; Vidal *et al*., 2002).

These earlier reports suggest the importance of prey in the success of larvae to survive therefore suitable live prey items should be provided during this critical phase. Since, survival of hatchlings is dependent on first feeding during the first week after hatching but little is known about the feeding ability of larval *S. pharaonis*. The present study was conducted to evaluate the prey preference and influence of prey density on the feeding activity of *S. pharaonis* paralarvae. To our knowledge and intensive literature review, this is the first time that a study on *S. pharaonis* larval prey preference was conducted. This study is intended to improve cuttlefish post hatch production using the traditionally and locally available live prey items such as *Artemia* nauplii, mysids and tilapia fry. The feeding response of 1, 3 and 5 DAH cuttlefish hatchlings given different live prey types at varying densities is determined in this study.

MATERIALS and METHODS

Egg procurement and hatchling production: Newly spawned *S. pharaonis* eggs were collected from a cage pot fishery in the eastern coasts of Zamboanga City, Philippines. Eggs were incubated in a 40 L glass aquarium under ambient water condition (35 ppt, 28°C) with 60% daily water changes (Deepak and Patterson, 2002). All water parameters were monitored and maintained at the optimum until hatching. The newly hatched larvae were either directly used for the succeeding experiments or reared in a flow-through water system using rectangular white plastic culture tanks until 5 DAH.

Hatchlings were fed with mysids (*Monopogopsis orientalis*), tilapia fry (*Oreochromis mossambicus*) and 5-7 days old *Artemia salina* nauplii in ad libitum. Wild mysids and tilapia fry were sourced from the nearby fishpond adjacent to mangrove vegetation. *Artemia* cysts were hatched and incubated following the procedures recommended by Ikhwanuddin *et al*. (2005). During the culture period, *S. pharaonis* hatchlings were maintained at 28±1°C. Only healthy *S. pharaonis* that are hatched on the same day to ensure no age differences were used in the study.

Experimental design: The experiments were conducted at Zamboanga State College of Marine Sciences and Technology (ZSCMST), Multispecies Hatchery and Wet Laboratory, Fort Pilar, Zamboanga City. The experiment was conducted following a two factor in completely randomized design. Prey types including *Artemia* larvae, juvenile mysids and tilapia fry constituted the first factor and the prey density comprised the second factor. Three prey densities for each of the prey species were tested in the study (Low, medium and high densities). In *Artemia,* the 5 *Artemia* 100/mL was designated as low density while 10 *Artemia*/100 mL was designated as medium and 15 *Artemia*/100 mL was designated as high density. For mysids as prey, 2.5 mysids/100 mL, 5 mysids/100 mL and 7.5 mysids/100 mL constitute the low, medium and high density treatments, respectively. In treatments, receiving the tilapia fry as prey, the low density has 1 tilapia fry/100 mL, 2 tilapia fry/100 mL in the medium density treatment and 3 tilapia larvae/100 mL was used as the high density treatment. The sizes of the individual prey used were as follows: *Artemia* nauplii was about 409.73±1.31 µm, mysids ranged within 7.83±0.81 mm and tilapia fry had a length of 9.21±0.05 mm. The number of individuals per prey types was varied in order to normalize the total prey biomass given among the treatments.

In the assessment of the feeding response of the newly hatched larvae (day 1 after hatching), a total of 45 individuals were randomly distributed into 1 L white plastic containers containing aerated seawater at a density of one individual per container. Each container represents a single replicate and there are about 5 replicates per experimental treatment. The container with larvae were provided with gentle aeration, set-up in a water bath to prevent drastic temperature change and covered with plastic screen to limit the intrusion of dirt and flying insects.

Similar experimental design were followed in the subsequent trials using the larvae that were grown for 3 days (3 DAH) and for 5 days (5 DAH) as shown in Table 1.

Feeding response of *S. pharaonis* hatchlings at different stages given with different prey types and densities were quantified in terms of prey ingestion. Known numbers of live prey were added to the culture containers with individual *S. pharaonis*. After 10 h of host-prey exposure, the number of live prey remaining in the experimental tanks was counted individually. Damaged *Artemia* nauplii or mysids (missing antenna or abdomen) and tilapia fry (missing parts) were considered as ingested. The feeding responses of hatchlings were assessed through the total number of prey ingested after 10 h total feeding time.

Statistical analysis: Data was presented as Mean±Standard Error of the mean. All results were transformed using arcsine transformation and subjected to two-analysis of variance (ANOVA) through IBM SPSS version 21.0 at 0.05 significance level. If two-factor ANOVA results indicate no factor interaction main factor analysis were conducted and compared. However, if interaction among the factors are detected differences between treatment means were analyzed using Tukey Test.

RESULTS

Feeding activity of 1 DAH *S. pharaonis* paralarvae indicate that density is a factor affecting prey ingestion. Regardless of prey type, ingestion increases as the density increases. In terms of prey type as a factor in prey ingestion, the present data suggest that *S. pharaonis* paralarvae has a high preference for smaller crustacean as prey which is the *Artemia*. Further, these two factors, prey type and density, show an interactive effect indicating that *Artemia* at high density could result to high prey consumption of the larvae (Table 2). Similar pattern of prey consumption could also be observed in older larvae at 3 DAH and 5 DAH (Table 3-4).

At these larval stages, prey density was found to interact with prey types. Although artemia has a lower protein and lipid content but the present results suggest that *S. pharaonis* larvae prefer *Artemia* at high density as food prey (Table 5). Results also indicate the strong influence of

Table 1: Stages and size of larval *Sepia pharaonis* used in the experiment

	Larvae size	
Days after hatching	Length (mm)	Weight (mg)
1	7.83±0.25	100±10.00
3	8.03±0.16	130±20.00
5	8.34±0.44	150±10.00

Table 2: Interactive influence of prey types and densities on the feeding response (percentage of ingested prey) of 1 day old (1DAH) cuttlefish larvae

Prey types and density	Feeding response (ingested prey %)
Artemia	
Low	32.80±1.91[b]
Medium	60.80±3.79[c]
High	88.80±8.06[d]
Mysids	
Low	11.00±1.76[a]
Medium	12.40±1.21[a]
High	13.80±3.57[a]
Tilapia fry	
Low	0.00±0.00[a]
Medium	0.60±0.24[a]
High	1.20±0.49[a]
Two-way ANOVA, p-value	
Prey type, <0.001	
Prey density, <0.001	
Prey type×prey density, <0.001	

Table 3: Interactive influence of prey types and densities on the feeding response (percentage of ingested prey) of 3 day old cuttlefish larvae

Prey type and density	Feeding response (ingested prey %)
Artemia	
Low	28.80±0.86[c]
Medium	41.60±0.75[d]
High	52.20±0.97[e]
Mysids	
Low	12.20±1.16[b]
Medium	18.00±1.61[bc]
High	23.60±3.26[c]
Tilapia fry	
Low	0.00±0.00[a]
Medium	0.40±0.24[a]
High	0.80±0.37[a]
Two-way ANOVA, p-value	
Prey type, <0.001	
Prey density, <0.001	
Prey type×prey density, <0.001	

Table 4: Interactive influence of prey types and densities on the feeding response (percentage of ingested prey) of 5 day old cuttlefish larvae

Prey type and prey density	Feeding response (ingested prey %)
Artemia	
Low	39.60±0.93[de]
Medium	42.40±1.40[e]
High	48.80±5.27[e]
Mysids	
Low	17.60±1.21[b]
Medium	24.80±0.73[bc]
High	31.60±1.63[cd]
Tilapia fry	
Low	1.00±0.45[a]
Medium	1.40±0.24[a]
High	1.60±0.40[a]
Two-way ANOVA, p-value	
Prey type, <0.001,	
Prey density, <0.001	
Prey type×prey density, <0.029	

Table 5: Proximate composition of prey organisms used in the study

	Proximate composition (g/100 g)		
Prey types	Protein	Lipid	Moisture
Artemia	4.16±0.05	1.01±0.07	93.04±0.05
Mysid	13.94±0.04	2.45±0.01	93.42±0.01
Tilapia larvae	13.94±0.04	2.65±0.16	92.26±0.09

prey density on feeding activity. Regardless of prey type and larval age feeding activity was found to increase at increasing prey densities. The lowest consumption was observed in treatments given with the low density tilapia fry.

DISCUSSION

The ability of larvae at first feeding to capture and ingest prey is considered vital and a determining factor in larval survival. Environmental factors, prey density, physiological adaptation of the larvae and prey composition are factors known to influence feeding behavior of a larvae (Slembrouck *et al*., 2009). In marine aquatic larvae, first feeding is considered essential to provide energy and necessary nutrients to fuel growth and to provide energy for predator avoidance. In most marine aquatic larvae specifically crustaceans and fish, it has been suggested that type of prey and density is a critical factor for the successful capture and ingestion of prey organism. However, aspects regarding the predatory behavior, prey preference and prey ingestion of larval cephalopod specifically *S. pharaonis* has not been investigated until the present.

To our knowledge, information generated on this present work is the first time to be reported in *S. pharaonis* larvae. The findings of the present study suggest the strong interaction of prey types with prey density and this interactions manifest in the three larval stages used in the study. In all stages, it appears that live prey at higher density increases the prey capture and consumption rate of larval *S. pharaonis*. *Artemia* larvae given at a high density is the best treatment that promoted the highest prey capture and consumption of the cephalopod larvae in the present study. Although *Artemia* is not a common prey of *S. pharaonis* in the natural environment but the physical structure and the behavior of this prey item may resemble copepods, a natural prey of cephalopod larvae. Moreover, earlier works corroborate with our findings suggesting that *Artemia* is highly preferred as prey items of other species of cephalopods studied (Franco-Santos *et al*., 2014). The significantly higher consumption of *Artemia* nauplii by cuttlefish hatchlings implies that this type of prey is preferable compared to mysids and fish fry. *Artemia* has been known as instant live prey commonly used for the larvae of cultured organisms (Ikhwanuddin *et al*., 2012a, b; Soundarapandian *et al*., 2007). The sluggish swimming behavior, easily detected, contain highly digestible nutrients and high lipid contents are noted characteristics of *Artemia* making it highly preferred by most carnivorous larvae (Van der Meeren *et al*., 2008). Further, the preference of cephalopods on crustaceans as live prey has also been documented by Sartor and Belcari (2009) and Osman *et al.* (2014). The work of Boletzky (2003) elucidates the digestive adaptation of young cephalopod larvae to available prey from the wild that are composed essentially of crustaceans and a lesser percentage of fish. This is also similar to the observation of Darmaillacq *et al.* (2004) wherein cuttlefish without previous feeding experience preferred shrimp compared to crabs and fish fry at 3 DAH. In addition, *Artemia* can support the 100% survival of cuttlefish for either 10-20 DAH as reported by Domingues *et al.* (2001). The present data also suggest that size is an important factor rather than nutrient composition in the prey preference of *S. pharaonis*. Next to *Artemia*, mysids is the second preferred prey and tilapia fry was the least preferred prey item of *S. pharaonis* paralarvae. This data suggest the inverse relation of the prey size and the prey consumption of this cephalopod larvae. It was observed in the present study that some tilapia fry are incompletely consumed by the cephalopod larvae indicating the preference of the larvae for prey items that could fit their mouth. The fast swimming characteristics of mysids and tilapia larvae could be also a factor that these prey items could hardly be captured and consumed by the larvae. In a study conducted by Domingues *et al.* (2004), poorest growth was observed in the cuttlefish larvae maintained with fish fry as compared to those fed with crustaceans. This poor larval performance was attributed to lower feed conversion and indicates that fish are not an adequate prey for the culture of the early stages of cuttlefish. The size of the prey might have influence its feeding responses and not the quality of food. These earlier reports on other species of cephalopods support the findings of the current study.

Most cephalopod larvae are highly opportunistic predator and are hatched well-equipped with prey capturing appendages for the efficient utilization of available prey species (Franco-Santos *et al*., 2014; Wakabayashi *et al*., 2005; Shigeno *et al*., 2001). This opportunistic behavior may already manifest after hatching that may explain the significantly higher feed consumption of *S. pharaonis* at higher prey densities as observed in the present study. Although, Rosas *et al*. (2014) stated that prey density (*Palaemonetes* sp.) might not have significant effect on the growth of octopus, however, Jackson and Moltschaniwskyj (2001) reported that high-ration squid reached a significant larger size than the low-ration individuals upon feeding of fish and *Acetes*. Furthermore, prey density had influence the squid growth rate, statolith size and daily statolith increment width. This is also similar to the findings of Correia *et al.* (2008) wherein they stated that prey availability might influence the weight gain, irrespective of the prey used, during the first 2 months of cuttlefish life cycle. Further, the positive influence of prey density on prey ingestion is also well documented in larval crustaceans (Jinbo *et al*., 2005). The high incidence of contact between the prey at high density and the larvae has been suggested as the major reason in the high prey ingestion rate of the larvae. Utilization of less energy to capture and ingest prey when available at high density has been also suggested as a factor for the high incidence of prey ingestion in most marine animal larvae (Ikhwanuddin *et al*., 2005). Overall, these reports support the findings of the present study on the prey consumption of larval *S. pharaonis*.

CONCLUSION

Collectively, the present findings suggest that *Artemia* at higher stocking density could enhance feeding response in *S. pharaonis* paralarvae. The present information adds to the growing knowledge of feeding response, feed preference and general biology of cephalopods specifically the species *S. pharaonis*.

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