Determining the effects of stocking density and temperature on growth and food consumption in the pharaoh cuttlefish, *Sepia pharaonis*, Ehrenberg 1890

G. J. Barord · K. N. Keister · P. G. Lee

Abstract The purpose of this experiment was to observe the impact of stocking density on growth and food consumption of juvenile *Sepia pharaonis* reared at 23 and 28°C. Two groups of 32 cuttlefish each were reared in closed recirculating seawater systems with water temperatures of 23°C (group A) and 28°C (group B). Each group was divided into three treatments with two replicates per treatment: low-density (equivalent to 20 cuttlefish m^{-2}), medium-density (equivalent to 100 cuttlefish m^{-2}), and high-density (equivalent to 200 cuttlefish m^{-2}). Measured amounts of live food were added three times a day and the wet body weight of each cuttlefish was measured once a week during the 42-day study. Cuttlefish in group B had higher growth rates and food consumption than cuttlefish in group A. The different stocking densities in group B affected the size of the cuttlefish whereas the stocking densities of the cuttlefish in group A treatments did not lead to different sizes between densities. Overall, the gross growth efficiency of the high-density treatments was lower than that of the low-density treatments, as was the weight of the cuttlefish in the high-density treatment. Although the wet weights of group A treatments were not significantly different ($P > 0.05$), the wet weights of the cuttlefish in the high-density, group B, treatment were lower than those in the low and medium density treatments. This decrease in individual size suggests that stocking densities of 100 to 200 cuttlefish m^{-2} may interfere with growth.

Keywords Aquaculture · Cuttlefish · Density · Diet · Growth

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Introduction

Scientists in Europe, Asia, and United States have successfully cultured *Sepia* spp. through multiple generations (Boletzky and Hanlon 1983; Forsythe et al. 1994; Nabhitabhata 1995; Lee et al. 1998; Minton et al. 2001) and growth rates and stocking densities have also been examined in *S. officinalis* (Dominguez et al. 2001; Correja et al. 2005). Forsythe et al. (2002) stated that fast growth, relatively large size, and benthic lifestyle have made *S. officinalis* an accepted species for culture in the laboratory. There has been no attempt at commercial-scale culture of cephalopods for human consumption but the prospect is anticipated because of the continual decline in the world's fisheries (Hanlon and Forsythe 1990; Lee et al. 1998).

Sepia pharaonis has a tropical distribution from 35°N to 30°S and from 30° to 140°E in the Indo-Pacific (Nabhitabhata and Nilaphat 1999) and is generally found in coastal waters to 100 m depth (Norman and Reid 2000b). *Sepia pharaonis* is an attractive species for commercial production because it is one of the primary fisheries in the Suez Canal and a commercially valuable cephalopod in the northern Indian Ocean (Gabr et al. 1998).

There is little information on the biology of *S. pharaonis* but fishery studies have been conducted in Australia (Dunning et al. 1994), Yemen (Aoyama and Nguyen 1989), Taiwan (Lin and Su 1994), Thailand (Chotiyaputta 1993), and Egypt (Gabr et al. 1998). Large-scale culture of *S. pharaonis* began with Nabhitabhata's work in 1978 in Thailand (Nabhitabhata 1995). *Sepia pharaonis* has also been cultured and used for biomedicine at the National Resource Center for Cephalopods since 1998 (Minton et al. 2001) and reared to adulthood in other instances (Anil et al. 2005).

The objective of this study was to determine the optimal stocking densities of juvenile *S. pharaonis* in aquaculture considering growth rate, size, and the space required. The behavior and feeding habits of this species were also observed to evaluate feeding response, injury, and stress. In addition, a temperature study was conducted to determine the effects of two different temperature regimes on growth rate and food consumption.

Materials and methods

Tank systems

The two systems used to rear *S. pharaonis* in this study were closed, recirculating seawater systems described in detail in previous publications (Lee et al. 1998; Minton et al. 2001; Forsythe et al. 2002). The warm-water tank (28°C) used in this study comprised two circular tanks; the filter bed and the rearing tank. Each tank had a diameter of 1.8 m and a total system volume of 3,600 l. The cold-water tank (23°C) was rectangular in design (185 cm × 417 cm × 61 cm), with a smaller biological filter bed (91 cm × 198 cm × 46 cm). Each of the systems facilitated biological filtration in the same way. A high-rate airlift pumped seawater through both of the systems. In either case, the sea water first passed through a polyester batting particle filter (35 μm pore size), through an activated carbon filter, and finally through an under-gravel biofilter made of crushed oyster shell. The 28°C rearing tank was equipped with an ultraviolet sterilizer as a final filter before the water was passed back into the tank.

Six cylindrical holding chambers, height of 28 cm and radius 15 cm, were placed into each of the culture systems, for a total of 12 chambers. The chambers were fitted with styrofoam floats glued near the top so the chambers would float. Additionally, two slots

were cut opposite each other in each chamber and covered with a 0.5 mm plastic mesh. The slots and the ability to float freely allowed water to flow through the chambers, supplying sufficient dissolved oxygen to the cuttlefish. The chambers were tied in place to prevent tipping. Air stones were placed next to the chambers to further facilitate aeration; after day 25, the air stones were placed directly into the chambers to ensure an ample oxygen supply.

Water quality characteristics remained within acceptable safe ranges (Forsythe et al. 1994). Both ammonia and nitrite levels remained below 0.05 mg/l and were normally around 0.001 mg/l. Nitrate levels remained below 20 mg/l and averaged 10 mg/l. Temperatures remained within a ±2°C of the set temperatures. Salinity ranged from 34 to 39 ppt.

Stocking densities

The *S. pharaonis* used in the study were of the Type II species (Norman and Reid 2000a) and the first captive-born (F_1) generation from eggs collected in Thailand by the Georgia Aquarium and shipped to Galveston. A total of 64 newly hatched (0-day-old) cuttlefish were divided throughout the twelve (12) chambers. Four (4) of the chambers (two at 28°C and two at 23°C) were stocked at a low-density treatment of one cuttlefish (equivalent to 20 individuals m^{-2}). Four of the chambers (two at 28°C and two at 23°C) were stocked at a medium-density treatment of five cuttlefish (equivalent to 100 individuals m^{-2}). The final four chambers (two at 28°C and two at 23°C) were stocked with a high-density treatment of ten cuttlefish (equivalent to 200 individuals m^{-2}). The length of the study was 42 days. The initial stocking densities were based upon the assumption that juvenile cuttlefish would remain at the bottom of the baskets, thus being measured in m^2, whereas older cuttlefish tended to inhabit all three dimensions of the baskets with their stocking densities being measured in m^3.

Sepia pharaonis weight

Wet weights (0.01 g) and mantle lengths (0.01 mm) were recorded for every cuttlefish on a weekly basis beginning on day 1 and ending on day 42. The mantle lengths were measured using Vernier calipers. No anesthetic was required for these procedures. The total survivorship was calculated at the end of the study.

Food consumption and growth

The main food item given to *S. pharaonis* was the grass shrimp, *Palaeomonetes pugio*, but other food items included *Penaeus* sp. and *Fundulus* sp. when there were no grass shrimp available. All live food items were collected from Galveston Bay, TX, USA, using seine nets; these were transported and held in the laboratory in recirculating seawater filtration systems. All food items were used within the first 48 h of holding and received no food during holding. Each treatment received the same amount of food throughout the study. The replicates were fed three times a day and the food was measured out for each of the three feedings on a daily basis. Waste was siphoned out of the chambers into Erlenmeyer flasks and placed into a refrigerator. The contents of the flasks were weighed weekly.

The total weight of food consumed (TFC) was calculated on a weekly basis for each group by subtracting the amount of waste removed from the amount of food offered (TFC = (Amount offered (g) − amount removed (g)) $week^{-1}$). Individual food

consumption (IFC) was calculated by dividing the TFC by the respective number of cuttlefish in that particular chamber. Furthermore, the percentage consumed (PC) was the percentage of body weight consumed and was calculated by dividing the TFC by the number of cuttlefish in a replicate, dividing that by the average weight of the cuttlefish in that replicate and then multiplying by 100 (PC = [(TFC (number of cuttlefish)$^{-1}$) (mean weight (g))$^{-1}$] × 100).

Gross growth efficiency (GGE) was calculated each week for each of the replicate groups. The GGE measures how efficient the animal is at converting food intake into biomass. It was calculated by dividing the weekly increase in average weight (g) of a cuttlefish group by the average weight (g) of food consumed by the group in the same week, multiplied by 100.

The instantaneous relative growth rate (IRGR) was calculated weekly for each of the replicates. The IRGR measures the percentage increase in body weight per day and is calculated using the equation: IRGR = (ln W2 − ln W1)(100t)$^{-1}$ where ln W2 and ln W1 are the natural logarithms of weight measurements 1 and 2 and t is the time between weight measurements in days.

All data were analyzed and graphed using Microsoft Excel for Windows. A MANOVA-style analysis was run using SPSS for Windows (Zar 1998). Temperature and stocking density were independent variables, and weight, length, IFC, PC, GGE, and IRGR were all dependent variables.

Results

Behavioral observations

The cuttlefish kept at 28°C were most active when observed at feeding, cleaning, and weighing times. During the most active times, the cuttlefish jetted around the chamber into the walls and were frequently inking. During periods of observation only with no interference, the cuttlefish were still seen swimming around the chamber although no inking or erratic jetting occurred. A large mortality occurred from day 22 to day 25. The water in each of the chambers that had mortalities was thick and cloudy from ink. Low dissolved oxygen in the higher density chambers was thought to be the cause; air stones were placed directly in all of the chambers after the large mortality to ensure a sufficient amount of aeration and water movement. Table 1 presents survivability for each stocking density at each temperature.

The cuttlefish reared at 23°C were observed to be most active during feeding, cleaning, and weighing times also, but were observed to be sedentary during all other times. During times of low activity the cuttlefish would lie at the bottom of the chamber equidistant from one another, whereas in times of high activity the cuttlefish would be in the water column jetting around the tank into the walls and inking. On several occasions, a cuttlefish from one of the chambers jumped out and was found in the main tank and was subsequently placed back into the chamber without any change in behavior. None of the cuttlefish in either of the temperature conditions was observed to have any caudal damage as a result from bumping into the walls.

Stocking densities

Stocking densities declined during the 42 day experiment except in the low-density group. The dead cuttlefish were not replaced with new ones because no similar sized cuttlefish

Table 1 *Sepia pharaonis* survival (%) for the experiment by replicate groups and treatments

	Survival per replicate group (%)	Mean group survival (%)
23°C low density	100	100
	100	
23°C medium density	80	80
	80	
23°C high density	100	95
	90	
28°C low density	100	100
	100	
28°C medium density	60	50
	40	
28°C high density	10	0.05
	0	

were available. The surface area covered by the cuttlefish increased, because of the growth of the cuttlefish, particularly in the group B. The average mantle length of group B surpassed the final mantle length of group A by the third week, resulting in greater crowding for the duration of the study.

The dorsal surface area of the cuttlefish was approximated by calculating the area of an oval with a length equal to the average mantle length of the cuttlefish at each density and the width equal to the width of *S. officinalis* of the same age and size. *Sepia officinialis* was used for this calculation, because these individuals most closely matched the size and age of the test group. This calculation showed that the initial surface area of a single *S. pharaonis* was 3.0% and the final surface area was 9.0% of the available space in the 28°C treatment, throughout each of the different densities. For the 23°C treatment, the initial and final surface areas occupied by a single *S. pharaonis* were 2.0 and 5.0%, respectively. The final, total dorsal surface area for the low-density, 28°C, replicates was 11% of the bottom area of the rearing chamber. In contrast, the final, total dorsal surface area of the low-density, 23°C, replicates was 0.05%. The survivorship of each group is shown in Table 1.

Food consumption

The feeding conditions for group B were kept constant at a 2:1 ratio of *P. pugio* to cuttlefish. All of the food was eaten until the high mortality that occurred in group B because of low oxygen. Thereafter, the cuttlefish ate less for one week, at which time the feeding ratio was reduced to a 1:1 ratio. The cuttlefish then returned to eating their normal 2:1 ratio for the duration of the study. The cuttlefish reared in group A were fed a 2:1 ratio at the beginning of the experiment with much of the food still remaining in the chambers at the end of the day. As a result, the feeding proportions were changed to a 1:1 ratio, though some of the food was still uneaten. At day 28, the feeding proportions were increased back to a 2:1 ratio because of increased food consumption. It should be noted that during the first 22 days, group B cuttlefish were observed to eat at each of the three different feedings whereas group A were observed to initially disregard the food when it was added to the chamber. Additionally, there was a span of three days (day 22 to day 25) when there was no *P. pugio* so the cuttlefish were fed various estuarine fishes.

The total food consumed was calculated every week for each of the groups (Fig. 1a, b). Group B showed distinct food consumption values for each of the three different stocking densities. The high-density group had the highest TFC values until day 21, when only one cuttlefish remained because of high mortality, and showed a range of 9.55–73.39% (Fig. 1a). The medium-density treatment also showed a decrease at day 21 that corresponded to mortality in the medium-density replicates (Fig. 1a). The range of TFC in the medium-density treatment was 23.56–44.06%. The lowest TFC values were seen in the low-density treatment and ranged from 8.14 to 14.22%. The TFC values in group A were consistent throughout the 42-day study. The low-density groups had a range of 2.27–5.08%. The medium-density and high-density groups had ranges of 12.52–21.34% and 32.02–52.74%, respectively. In both the medium-density group and the high-density group there was a decrease in the TFC values at day 14 that may have been a result of a temporary reduction in food available to the cuttlefish (Fig. 1b).

The IFC values were significantly different because of stocking density and temperature ($P = 0.000$). Overall, the IFC values for group B were higher than for the low-density group, as shown in Table 2. Both the medium-density group and the high-density group had comparable trends in their IFC values during the first 21 days. The increase at day 28
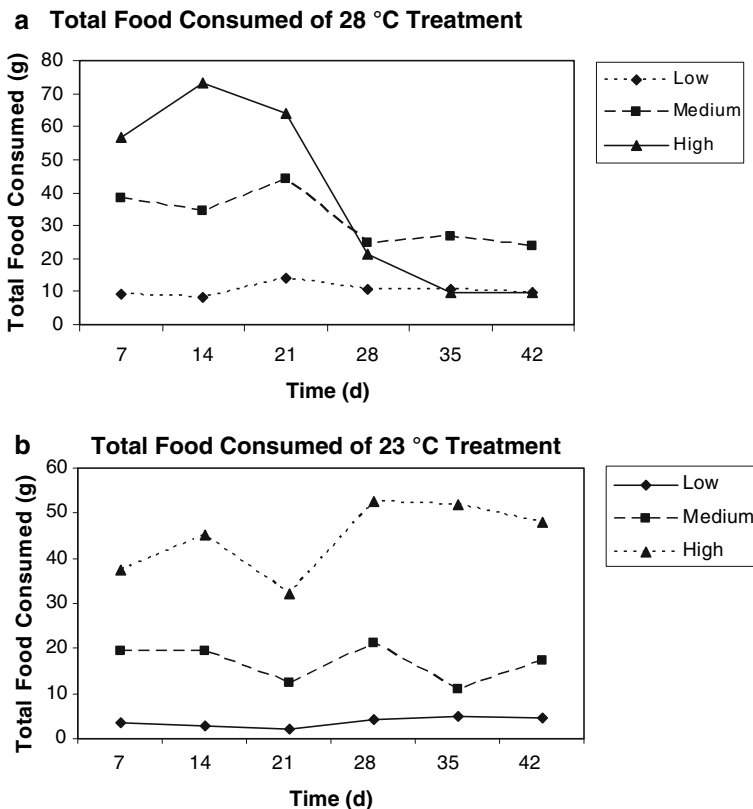

Fig. 1 **a** Average total food consumed (g) over each seven-day period during a 35-day period per density group in the 28°C treatment. **b** Average total food consumed (g) over each seven-day period during a 35-day period per density group in the 23°C treatment

Table 2 Average individual food consumed for the 28°C and 23°C groups during the 42-day study

Time (days)	28°C			23°C		
	Low density	Medium density	High density	Low density	Medium density	High density
7	8.99	7.68	6.59	3.45	3.91	3.72
14	8.14	6.90	7.34	2.90	3.92	4.52
21	14.22	8.81	6.39	2.27	2.50	3.20
28	10.81	4.93	21.10	4.25	4.27	5.27
35	10.88	5.32	9.55	5.08	4.17	5.20
42	9.47	4.71	9.64	4.52	3.42	4.78

in the high-density group corresponded to mortalities suffered during that week. The two remaining values at day 35 and day 42 are calculated from only one cuttlefish, thus these two lower values are analogous with the low-density group at the same week. The cold-water group had smaller IFC values at all stocking densities when compared with the warm-water cuttlefish.

The percent consumption (PC) of both the 28°C and 23°C treatments were consistent throughout the 42-day study based on stocking density and showed no significant differences ($P = 0.402$). As expected, the 28°C groups all had considerable decreases in PC by the end of the 42-day study with the only fluctuation being at day 21 during the time of high mortality in the high-density group (Fig. 2a). Furthermore, the 23°C groups had an overall decrease at the end of the study (Fig. 2b). The decrease at day 14 was a result of restricted food availability. While the larger group B cuttlefish were able to adapt to the substituted food as a result of their large size, the smaller group A cuttlefish were not able to consume the substituted food types (large shrimp and fish).

Sepia pharaonis average weights and mantle lengths

Growth trends were observed during this study, increasing at day 21 and continuing until the end of the study. The 28°C, low-density group of only one cuttlefish weighed the most at the end of the study with an increase of 26.42 g in 42 days (Fig. 3). After day 28, the data for the high-density group is from one cuttlefish because of mass mortalities that occurred from day 22 to day 25. The three groups were growing at the same rate until day 21 (Fig. 3). The medium-density group showed an overall increase of 20.76 g while the high-density group showed the lowest growth, 14.9 g.

The three groups in the 23°C treatment did not show any noted difference in growth until the final weights taken on day 42 (Fig. 3). The three groups, low, medium, and high, had steady weekly weight increases and had final weight gains of 12.63, 11.63, and 10.67 g, respectively. There was a significant difference in weight gain between the interaction of the two temperature treatments and the three stocking densities ($P = 0.009$).

Mantle lengths of group B showed a trend among the three different stocking densities (Fig. 4). The low-density replicate had the longest mantle length at day 42 of 59.20 mm. The medium stocking density replicates had an average mantle length of 54.18 mm at day 42 while the high-density group had an end mantle length of 42.60 mm. Group A had consistent increases in mantle lengths during the entire study, irrespective of density (Fig. 4). The low-density group had an increase of 16.98 mm at day 42 while the medium and high-density groups showed increases of 14.25 and 16.81 mm, respectively.
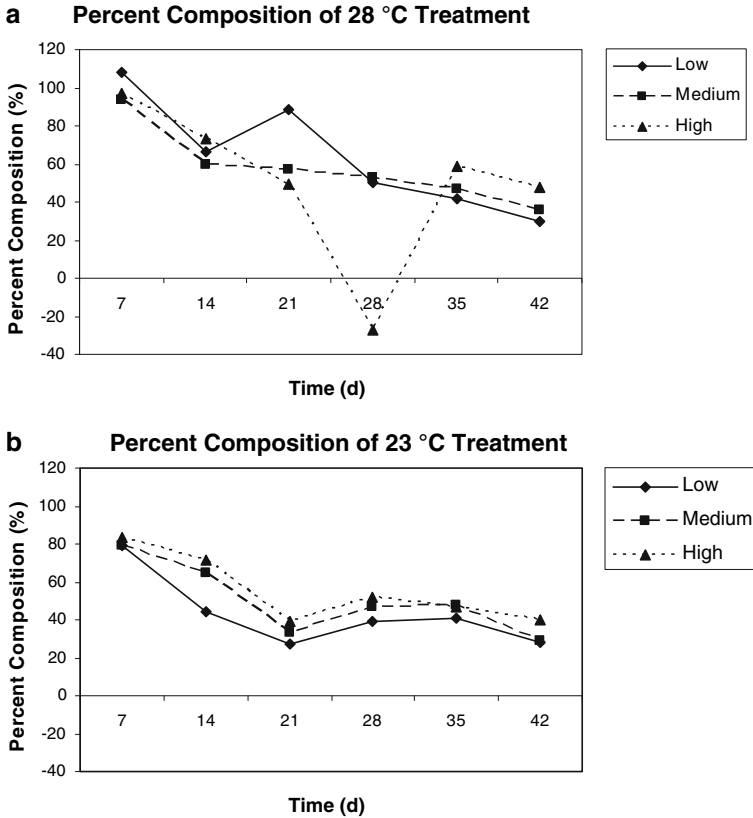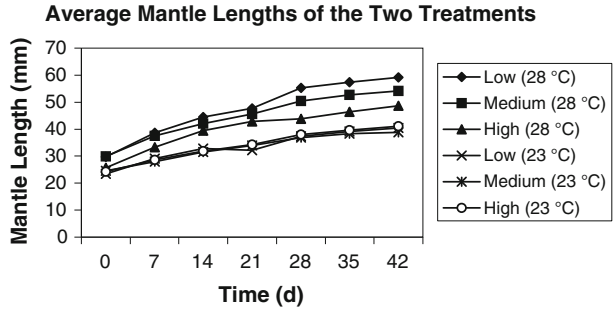
a   Percent Composition of 28 °C Treatment

b   Percent Composition of 23 °C Treatment

Fig. 2 **a** Percent consumption over each 7-day period during the 42-day period per density group in the 28°C treatment. **b** Percent consumption over each 7-day period during the 42-day period per density group in the 23°C treatment

Fig. 3 Average weight (g) of the two treatments (28 and 23°C) over each 7-day period during a 42-day period per density groups (low, medium, and high)

Average Weight of the Two Treatments
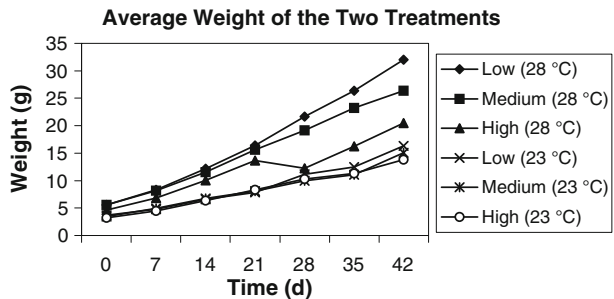
The 28°C treatment showed increases in mantle lengths that corresponded directly to the increases in mantle weight in each of the treatments among the three different stocking densities. The low-density group had both the highest weight and the greatest mantle length at day 42 whereas the medium-density group had the next highest weights and mantle lengths and the high-density group had the lowest weights and mantle lengths at day 42. Mantle lengths showed significant differences ($P = 0.000$) between the two temperature groups and between the three stocking densities.

Fig. 4 Average mantle length increase (mm) of the two treatments (28 and 23°C) over each 7-day period during a 42-day period per density groups (low, medium, and high)

Average Mantle Lengths of the Two Treatments

Legend:
- Low (28 °C)
- Medium (28 °C)
- High (28 °C)
- Low (23 °C)
- Medium (23 °C)
- High (23 °C)

Gross growth efficiency

Each week, the gross growth efficiency (GGE) was calculated and the average of the replicate groups in each treatment was taken for the 42-day study. The GGE values were significantly different between the temperature and the stocking densities ($P = 0.000$).

The GGE of group B remained consistent in the high-density and medium-density groups ranging from 4.4 to 15.65% (Fig. 5a). The low-density group, though, was
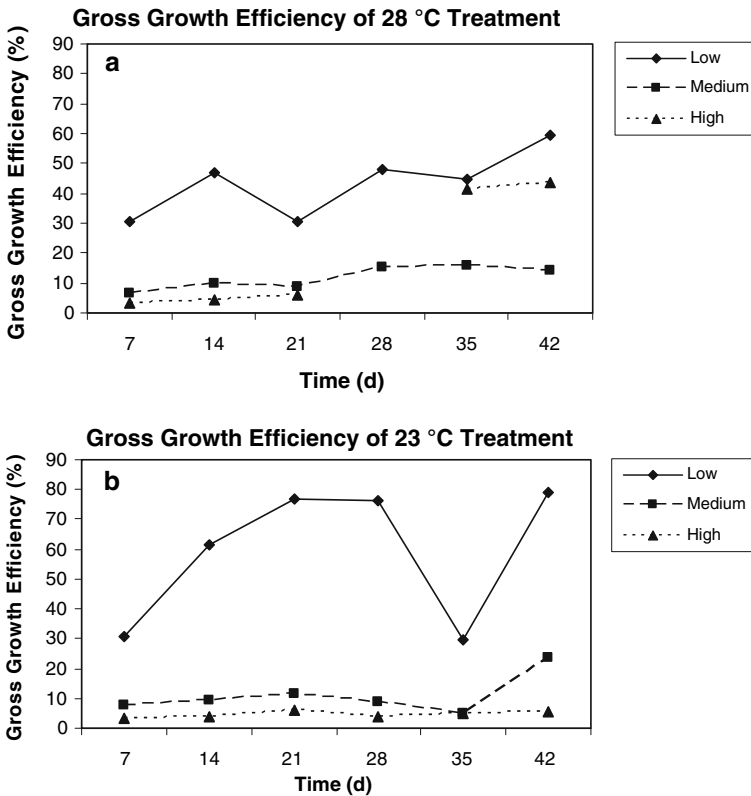

Gross Growth Efficiency of 28 °C Treatment

a

Legend: Low, Medium, High

Gross Growth Efficiency of 23 °C Treatment

b

Legend: Low, Medium, High

Fig. 5 a Average gross growth efficiency % over each 7-day period during a 42-day period per density group in the 28°C treatment. Note that mass mortalities occurred from day 22 to day 25 in the high-density group and all data after day 28 are from one cuttlefish. **b** Average gross growth efficiency % over each 7-day period during a 42-day period per density group in the 23°C treatment

inconsistent ranging from 30.45 to 59.2% (Fig. 5a). Group A showed comparable values in the medium and high-density groups ranging from 3.7 to 23.5% (Fig. 5b). The low-density group was again, inconsistent with a large decrease at day 28 and a range of 26.95–79.2% (Fig. 5b).

It should be noted that in both treatments the low-density group had very inconsistent GGE values while the high-density group was much more consistent through the entire study. The 28°C high-density group increased from a GGE of 5.75% at day 14 to a GGE of 41.68% at day 28 (Fig. 5a) but this group had experienced high mortalities from day 22 to day 25 and had one cuttlefish (same as the low density group) in it at the day 28 GGE value.

Instantaneous relative growth rates (IRGR) were calculated weekly for each of the treatments. The IRGR showed no significant differences between temperature and stocking density ($P = 0.423$). Group B showed IRGR values similar for all three stocking densities ($R^2 = 0.969$). IRGR values consistently decreased each week with the increase in body weight and mantle length. The IRGR values for group A were very erratic from week to week ($R^2 = 0.223$). The R^2 values correspond to the temperature that they were in. Group B, where the cuttlefish fed consistently, had a much higher R^2 value than group A, where the cuttlefish fed irregularly.

Discussion

Temperature has been shown to significantly affect growth rates and food consumption in S. officinalis (Forsythe et al. 2002; Dominguez et al. 2001) and at 21°C there has been little success in culturing S. pharaonis through consecutive generations (Minton et al. 2001). During this study, temperature significantly affected growth rates ($P = 0.000$) and food consumption while GGE values ($P = 0.667$) and IRGR values ($P = 0.136$) were not significantly affected by temperature. At day 42, the 28°C treatment produced sizeable differences in cuttlefish weights, though mantle lengths remained similar. The low-density group in this treatment had grown to a larger size than at both of the higher stocking densities. The lone cuttlefish in this group was fed the same amount of food, but unlike the other larger density groups, did not have to compete for food or share the chamber with any other cuttlefish. Thus, the lone cuttlefish did not have to expend a large amount of energy in competition for food enabling it to use more of its food intake for somatic growth.

Decreased growth in high-density groups was also shown in S. officinalis. Forsythe et al. (1994) stated that because food consumption was equal in low and high-density groups, high-density groups were expending a larger amount of energy in social interactions and less on growth. The GGE values for the lone cuttlefish in the low-density group were 300% higher than for the medium and high-density groups in both of the temperature controls ($P = 0.000$). The cuttlefish at the higher densities were forced to compete for their food, thus expending more energy to obtain food so less of their food intake was available to use for growth. Although a sufficient amount of food was offered to each group, allowing all of the cuttlefish to feed, the predatory behavior of locating prey and capturing it played a greater role in the high-density groups. While the lone cuttlefish was able to capture its food on the first try, the cuttlefish in the medium and high-density groups were repeatedly striking at the food items. The cuttlefish that did not capture a shrimp were then observed to actively search the chamber and strike several more times before finally capturing their food. These direct observations explain why each of the higher stocking density groups comprised one to two cuttlefish that were much larger than their conspecifics. The larger

cuttlefish were able to capture their food on the first try while the smaller cuttlefish had to consistently search for and strike at prey repeatedly. It should be noted, once again, that all cuttlefish were fed a fixed number of equally sized food items and all cuttlefish were able to find food at nearly every feeding so the probability that one to two random cuttlefish could eat all of the food was not likely. This competition amongst conspecifics corresponds to stocking density. While individual competition did not change, Minton (2004) stated that animals in solitary chambers feed irregularly whereas animals in low-density groups tended to feed more often, because of foraging competition, but this competition decreases when crowding is further intensified. Each stocking density in the 28°C treatment fed consistently from day-to-day giving no validation to Minton's foraging theory occurring within the groups.

GGE values of individuals have been reported to be between 40 and 60% for octopus and cuttlefish and 25% for squid (Boyle 1991). *Sepia officinalis* has been reported to have GGE values of 45.1 and 44.6% within temperature treatments of 17 and 25°C, respectively (Forsythe et al. 2002). The results fall close to this range for both *S. pharaonis* of the low-density groups in both temperature treatments. However, when an increased number of cuttlefish is placed in close proximity given the same ratio of food per cuttlefish, GGE values drop substantially. Although IRGR values were not significantly different, there were observable trends between the two temperature treatments. The cuttlefish in group A had IRGR values very similar among the stocking densities and the IRGR decreased from week to week, accompanied by cuttlefish growth, as expected. Group A yielded erratic IRGR values from week to week possibly because the cuttlefish in the colder temperature had not adjusted their metabolic activity to the new environment. At day 42, IRGR values for each stocking density in group A increased. Further studies are needed in order to determine how growth phases are different between *S. pharaonis* in warmer and colder temperatures and to determine if this increase in growth rate trend would continue.

Group A yielded comparable weights and mantle lengths throughout the study. The colder water temperature caused the cuttlefish in this treatment to metabolize at a lower rate and consume a smaller portion of food than group B. GGE values remained the same between the two temperature treatments but the cold-water cuttlefish grew to a smaller size by day 42. The colder water produced a greater effect on cuttlefish growth in this treatment than stocking density did. The findings show that the cuttlefish were comparable in weight and size regardless of their density. Koueta and Boucaud-Camou (1999) reported that *S. officinalis* cultured at lower temperatures had decreased feeding rates compared to cultures at higher temperatures. This study confirms that this statement is also true for *S. pharaonis*. While fecundity and reproduction were not reported in this study, Minton et al. (2001) failed to culture *S. pharaonis* at 21°C to a second generation. This temperature may not allow for natural maturation of the cuttlefish reproductive system and subsequent mating. *Sepia pharaonis* may be better suited for temperatures at the warmer range of their limit.

Stocking densities have been reported for *S. pharaonis* and *S. officinalis*. Optimal stocking densities for *S. pharaonis* have been reported to be 500 m^{-3} (<30 mm ML) for hatchlings and 10 m^{-3} for juveniles (<150 mm ML) (Nabhitabhata 1995). Forsythe et al. (1994) estimated stocking densities for *S. officinalis* to be 250–300 m^{-2} for hatchlings (<30 mm ML), 20 m^{-2} for juveniles (<150 mm ML), and 2 m^{-2} for adults (>150 mm ML). Findings in this study support a stocking density of juvenile *S. pharaonis* of higher than 10 m^{-3}, although increased food rations and ample oxygen supply is required. For *S. pharaonis* with mantle lengths less than 60 mm, stocking densities of 100–200 m^{-2} would be appropriate.

Forsythe et al. (2002) reported that behavioral aspects of *S. officinalis* would play a role in determining stocking densities. Boal et al. (1999) reported that *S. officinalis* at higher densities show signs of stress such as avoiding same-gendered conspecifics and hovering above the bottom of the tank. This type of "hovering" behavior was witnessed for *S. pharaonis* in group B but not in group A. It seemed that the colder water temperature, and its subsequent effects on cuttlefish activity levels, allowed each cuttlefish to tolerate higher densities. The hovering behavior witnessed in this study seemed to be merely a result of inadequate space for the number and size of the cuttlefish in each chamber as the study progressed. Furthermore, the increased density levels caused highly competitive feeding behaviors that would lead to fast jetting and bumping into the chamber walls but these behaviors caused occasional caudal tissue damage that healed within seven days. Tissue damage was not seen in any substantial number of cuttlefish. Although there were signs of stress-related behaviors, this additional stress did not seem to reduce the animal's fitness. There were no mortalities due to cannibalism or aggression; rather, the mortalities presumably occurred as a result of insufficient water flow within the chambers that led to reduced dissolved oxygen in the chambers.

Sepia pharaonis proved to be highly tolerant to varying densities and temperature. While stocking densities of such high proportions are not natural, they are required for large-scale aquaculture. High stocking densities can be accomplished with proper feeding and adequate oxygen supply. While stocking density based on individuals remains constant except for mortalities, the actual space used and occupied by the cuttlefish will increase as the cuttlefish grow and this must be taken into consideration. Further study is needed in order to determine a critical value of stocking density that would begin to negatively affect growth rate, food consumption, fecundity, and dissolved oxygen concentration. The target would seem to be between 50 and 100 cuttlefish m^{-2}.

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