Effect of stocking density and photoperiod on growth and survival in cultured early juvenile pot-bellied seahorses *Hippocampus abdominalis* Lesson, 1827

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**Abstract**

The effect of stocking density and photoperiod on *Hippocampus abdominalis* was examined in this study. Stocking densities of 45, 30, 15 and 5 seahorses 3 L\(^{-1}\) were tested on newborns. Growth and survival were independent of stocking density. A second stocking-density experiment aimed to remove the effect of an early mortality experienced in experiment 1 using older juveniles at 25, 15 and 5 seahorses 3 L\(^{-1}\). There were no differences in the parameters measured. Also, the effect of photoperiod was investigated on seahorses cultured under 24:00, 16:08 and 08:16 (L:D) photoperiods. A single *Artemia* meal was delivered at 10:00 hours. Survival and body growth in 16:08 and 08:16 hours were higher than in 24:00 hours. In a second experiment, seahorses were cultured in the photoperiods and conditions described for experiment 1, except they were fed twice the amount in two meals delivered at 10:00 and 16:30 hours. The seahorses in 16:08 hours showed better growth than the other treatments, but there were no differences in survival. These results suggest that early juvenile *H. abdominalis* can be cultured at higher stocking densities than previously reported, without compromising growth and survival, and when feeding was not limiting, grew better in an extended photoperiod (16:08) but not in 24:00 hours.

**Keywords:** early juvenile, pot-bellied seahorse, stocking density, photoperiod, survival, growth

**Introduction**

From the range of factors involved in fish culture, the manipulation of stocking density and photoperiod are considered useful tools for the optimization of culture practices (Tucker 1998). The use of high stocking densities in marine fish culture is required for cost-efficient commercial production of juveniles (Daniels, Berlinsky, Hodson & Sullivan 1996). However, the actual effect stocking density has on fish is species specific as it can be negative, positive or there may be no apparent effect at all (Woods 2003b). Seahorse density in their natural environment tends to be low with *Hippocampus abdominalis* among the species with the lowest mean densities recorded of 0.007 individuals m\(^{-2}\) (Foster & Vincent 2004). However, commercial scale *H. abdominalis* culture is successfully conducted at a maximum stocking density of 100 adults in 1000 L tanks (Seahorse World, pers. comm.). There is considerable information on seahorse culture. However, few studies have focused in early stages due to the elevated possibility of high mortalities. A major constraint in the development of marine fish culture has been ‘early stage’ mortalities, which appear as an interaction of various factors such as chronic starvation (Shackley, Talbot & Cowan 1993) and negative social interaction (Hatzianastasiou, Paspatis, Houbar, Kestemont, Stefanakis & Kentouri 2002). The intensive culture of marine fish larvae also presents problems, such as poor water quality. The deterioration of the culture environment can occur...
at a system level due to overloading of the bio-filtration capacity (Alvarez-Gonzalez, Ortiz-Galindo, Dumas, Martinez-Diaz, Hernandez-Ceballos, Grayeb-Del Alamo, Moreno-Legorreta, Pena-Martinez & Civera-Cercedo 2001). In-tank poor water condition may occur from inadequate maintenance of tanks (in some cases due to design misconceptions) and inadequate water exchange, needed to remove metabolites. This lack of adequate water exchange usually generates anoxic zones that can lead to high mortalities (Tucker 1998). Other causes of mortality at early stages of cultured fish can be their high susceptibility to crowding stress (Van der Salm, Martinez, Flik & Wendelaar Bonga 2004) and the development of negative social hierarchies/ cannibalism at high densities (Hatziantanasiou et al. 2002). In relation to negative social interactions, the culture of early stage seahorses at high stocking density could have an advantage compared with other teleosts, as seahorses do not display cannibalistic tendencies (Bergert & Wainwright 1997).

Literature studies regarding *H. abdominalis* have used a range of stocking densities. Wilson, Carter and Purser (2006) utilized one late-juvenile seahorse L\(^{-1}\) wet mass (M\(_w\)) 1.17 g. Florent (2003) used one newborn seahorses L\(^{-1}\), and Woods (2003b) considered 0.5 and one seahorses L\(^{-1}\) as suitable stocking densities for a variety of different sizes and ages (Woods 2000a, 2003a,c; Woods & Valentino 2003). Also, Woods (2003b) conducted an experiment testing stocking densities of one, two and five seahorses L\(^{-1}\), finding a reduction in survival and a greater incidence of physical interference (i.e. tail grasping and wrestling) between 5-month-old juveniles of 72 mm in standard length (L\(_s\)) and 0.5 g in M\(_w\) cultured at a stocking density of five seahorses L\(^{-1}\) compared with those at one and two seahorses L\(^{-1}\). A stocking density of 15 3-day-old *H. abdominalis* cultured in 3 L tanks was utilized during a 6-week trial on the background colour response of the species conducted by the authors of this study. In that trial, the density used resulted in an overall survival of 75% (Martinez-Cardenas & Purser 2007). In other seahorse species such as *Hippocampus erectus*, stocking density has been approached in preliminary observations testing six newborns L\(^{-1}\), lowered to one seahorse L\(^{-1}\) after juveniles reached 35-day-old (Correa, Chung & Manrique 1989). Wong and Benzie (2003) reported a lack of significant differences in growth of 3-month-old *Hippocampus whitei*, when testing a range 0.5–1 seahorse L\(^{-1}\).

Most of those studies have reported low mortality rates as they have been conducted on late juveniles, when survival is relatively stable compared with early stages, and have tested a limited range of stocking densities at the lower end of the scale.

*Hippocampus abdominalis* does not present a conventional larval stage as in many other marine fish species due to their specialized parental care, where the embryos develop inside the male’s pouch. After approximately 28 days at 17°C, juveniles are released at birth as autonomous individuals at which stage they are referred to as ‘newborns’ in this study. Newborn seahorses are well developed and able to feed on the day of release. Newborn *H. abdominalis* seahorses are distinctive due to their rather large size of approximately 17 mm in standard length (L\(_s\)). Seahorses also give birth to relatively smaller cohorts compared with newborns of other marine teleost (Foster & Vincent 2004), such as summer flounder *Paralichthys dentatus* (newborn approx. total length L\(_t\) 3.5 mm) which are experimentally stocked up to 60 larvae L\(^{-1}\) (King, Howell, Huber & Bengtson 2000), and the pelagic larvae of Atlantic cod *Gadus morhua* (newborn approx. L\(_t\) 5 mm), which have been reported to be cultured at stocking densities as high as 300 individuals L\(^{-1}\) (Baskerville-Bridges & Kling 2000). Juvenile production is the most important factor required to establish the culture of a species with good aquaculture potential (Alvarez-Lajonchere, Cerqueira, Silva, Araujo & Dos Reis 2002). *Hippocampus abdominalis* has been a useful seahorse model for aquaculture and biological research, that breeds small cohorts year round (Lourie, Foster, Cooper & Vincent 2004) for which commercial scale facilities are adapted maintaining a density of approximately 400 newborns per 50 L tank (Seahorse World, pers. comm.). However, breeding peaks occur in summer, and during particularly high productive seasons, water temperature is lowered to suppress mating (Seahorse World, pers. comm.) due to the lack of tank resources to accommodate broods.

Exposure of early stages of fish to photoperiods longer than that of natural conditions has led to an increase in growth rates in some commercial marine species, such as snapper *Pagrus auratus* (Fielder, Bardsley, Allan & Pankhurst 2002), gilthead sea bream *Sparus aurata* (Gines, Afonso, Arguello, Zamorano & Lopez 2004), haddock *Melanogrammus aeglefinus* (Trippel & Neil 2003) and red sea bream *Pagrus major* (Biswas, Seoka, Inoue, Taki & Kumai
2005). As visual feeders, it is important to provide to H. abdominalis conditions to optimize prey ingestion to maximize growth and survival. Conditions which may influence this process include orientation of lights, light intensity and wavelength, contrast of prey against the background, tank colour and photoperiod (Boeuf & Le Bail 1999).

Hippocampus abdominalis exhibits a ‘rearing’ period of approximately 4 months during which they prey on live food until a smooth transition from Artemia nauplii to frozen mysids takes place. A feeding rate of 5% body mass (BM) day\(^{-1}\) has been used previously for H. abdominalis experimentation on newborns (Florent 2003), and juveniles (Wardley et al. 2001; Wilson et al. 2006). Woods (2005) conducted a study comparing different feed rations while culturing H. abdominalis under a 12:12 (L:D) photoperiod, finding that 5% is recommended for cultured seahorses of this species. However, that study was conducted on late juveniles and feeding was provided in the form of frozen mysids. A feeding rate of 14% BM day\(^{-1}\) was utilized in a previous research by the authors of the current study. In those trials, the rate used was found to be in excess for early juvenile H. abdominalis (Martinez-Cardenas & Purser 2007). There are issues regarding food limitation during photoperiod experimentation on early stage fish, in which individuals cultured under longer photoperiods expend more energy swimming and searching for food than the energy they assimilated by actually feeding (Fielder et al. 2002; Gines et al. 2004).

Most seahorse species display diurnal activity in situ as reported by Foster and Vincent (2004). Although a majority of the species observed in that study displayed activity during the day, Hippocampus comes and H. abdominalis recorded both diurnal and nocturnal activities. Natural photoperiod regimes have been utilized in preliminary experiments on seahorse culture to replicate natural conditions, such as the study conducted by Wilson and Vincent (1998) on Hippocampus kuda, Hippocampus barbouri and Hippocampus fuscus and the study conducted on H. erectus by Correa et al. (1989). In experimental husbandry of seahorses, a 12:12 (L:D) photoperiod has been used for different species such as H. whitei (Wong & Benzie 2003) and Hippocampus subelongatus (Payne & Rippingale 2000). A 12:12 (L:D) photoperiod has been used in H. abdominalis experimentation in a range of diverse studies (Adams, Powell & Purser 2001; Shapawi & Purser 2003; Wilson et al. 2006). Woods has covered several topics on H. abdominalis husbandry using a 12:12 (L:D) (Woods 2003a,b,c; Woods & Valentino 2003). Also commercial scale facilities in Tasmania use a range of 12–13 h of light during the entire H. abdominalis life cycle (Seahorse World, pers. comm.).

Literature regarding the effect of different photoperiods on cultured seahorses has reported low activity during the dark period. Karina, Felicio, Rosa, Souto and Freitas (2006) conducted a study for late juvenile Hippocampus reidi in which adults were fed at dawn, noon, dusk and midnight, recording zero feeding activity together with cessation of swimming at midnight. Similarly, Sheng, Lin, Chen, Gao, Shen and Lu (2006) conducted a study on early juvenile Hippocampus trimaculatus, in which they demonstrated that seahorses feed actively in the photophase, but not during the scotophase. In addition, the author tested continuous feeding under a 24-h light regime recording lower feeding incidences during the period corresponding to nighttime (darkness) compared with the period corresponding to daytime (light). Olivotto, Avella, Sampolesi, Piccinetti, Navarro Ruiz and Carnevali (2008), reported for H. reidi, growth improvement on early juveniles cultured under continuous light and a copepod-complemented diet compared with 14:10 (L:D) and a ‘conventional rotifer-Artemia diet’. Ouyang (2005) fed late juvenile H. abdominalis continuously in a behavioural study while recording video images, and found significantly lower locomotor/feeding activity during the dark phase compared with the light phase. A study in adult pot-bellied seahorses H. abdominalis showed elevated levels of melatonin during the scotophase, returning to basal levels during the photophase (Martinez-Cardenas, Porter & Purser 2008). However, no study has been undertaken in relation to the effect of photoperiod on the growth of early juveniles of H. abdominalis, despite the importance of early life-stages in marine fish culture (Tucker 1998).

The aim of this study was to examine the effect of a greater range of stocking densities than reported in the literature (or used commercially) and photoperiods extended/reduced from the photophase used in standard culture practices (12:12 L:D) on replicated groups of early juvenile pot-bellied seahorses H. abdominalis over a 6-week period. Performance parameters such as body growth and survival were measured to determine the effect of these factors on the intensive culture of this species.
Materials and methods

General system design

Juvenile seahorses were transported in seawater and oxygen-filled plastic bags inside an insulated container from a commercial seahorse farm (Seahorse World, Beauty Point) to the marine hatchery area in the Aquaculture Centre at the University of Tasmania, Launceston. After a 15-min temperature acclimation, the fish were allocated to 20-L holding tanks until the start of the experiments. The seahorses for all experiments were exposed to a 12:12 (L:D) photoperiod from birth to the time they were collected from the commercial hatchery. Afterwards, seahorses for photoperiod and density experiments were held at the marine hatchery in the University under the same 12:12 (L:D) photoperiod until the start of the experiments. Transparent 3 L working volume tanks (15 cm diameter, 20 cm high) arranged in a recirculation system were utilized for all the experiments. Inflow to each tank was approximately 15 L h⁻¹ of 20 μm filtered seawater. Steady aeration was provided by aeration tubing ending in a 4 L h⁻¹ plastic water-dripper (Neta®) acting as an air stone, to minimize fish disturbance. Attachment substratum for the fish was provided by a weighted bundle of fishing line filaments. Illumination was provided using a timer-controlled 35 W overhead cool white fluorescent light (General Electric Company, Fairfield, CT, USA) producing an intensity of 4.8 μE s⁻¹ m⁻² at the water surface. For the photoperiod experiments, black plastic screening was installed between and above treatment tanks to isolate the three photoperiods. Treatment tanks were positioned in blocks under each light. Water quality was maintained for density experiments as follows (mean and range): water temperature 16.6 (16–17.5°C), pH 8 (7.8–8.2), salinity 34.2 (33–35 g L⁻¹) and dissolved oxygen >75%, TAN (total ammonia nitrogen) <0.5 mg L⁻¹, nitrite <0.25 mg L⁻¹, nitrate <5 mg L⁻¹. During the photoperiod experiments, the water quality was maintained as follows: water temperature 17.3 (range 17.2–17.6°C), pH 8 (range 7.5–8.4), salinity 32 (range 31–33 g L⁻¹) and dissolved oxygen >75%, TAN <0.5 mg L⁻¹, nitrite <0.25 mg L⁻¹, nitrate <5 mg L⁻¹. For the determination of pH, TAN, nitrite and nitrate, a colorimetric saltwater liquid test kit (Aquarium Pharmaceuticals, Chalfont, PA, USA) was used twice a week. During experiments, the fish were fed Artemia (enriched with Super Selco® for 24 h at 17°C) at a rate of 14% initial BM d⁻¹ (dry mass Artemia: M_w fish); in the density experiments, it was divided into two equal-sized meals delivered at 10:00 and 16:00 hours. During all the experiments, screens (150 μm) were placed over the outlet of the tanks to prevent the loss of Artemia during the day. At the end of the feeding time each day during stocking-density experiments, the screens were replaced with 500 μm screens to flush out the remaining Artemia overnight, ensuring that each day the feeding rate was even for all the tanks and that unenriched Artemia were removed. The feeding and flushing times were different for the photoperiod experiments as it is described in the corresponding section. In all the experiments, the feeding rate was maintained throughout the entire experiment, by adjusting the food on the basis of daily mortality and weekly growth recorded from bulk measurements of M_w per tank (M_b). Initial and final length (L_i and L_f respectively) were considered as the distance between the tip of the coronet and the tip of the uncurled tail. Measurements were conducted by placing the fish on a submerged plastic-covered 1-mm scaled sheet. Seahorses were gently dabbed on a paper towel to remove excess water prior to weighing: individual measurements of initial and final wet mass (M_wi and M_wf respectively), and intermediate bulk mass M_b were measured on an analytical balance and recorded to the nearest 0.0001 g. Fish were deprived of food for 24 h before each weighing. All possible care was taken so that the fish in each treatment passed through equal food deprivation periods before weighing. During experiments, the tanks were inspected daily for mortalities and any excess food and faeces were siphoned to waste. No further cleaning was undertaken to minimize fish disturbance. Water exchanges were used to replace siphoned water and to maintain water quality levels. The parameters measured included L_i, L_f, M_wi, M_wf, M_b, survival, specific growth rate (SGR) and size heterogeneity. Mean SGR of seahorses in each tank was calculated by SGR% day⁻¹ = [(lnM_wf−lnM_wi)/t] × 100, where M_wf = final mass, M_wi = initial wet mass, and t = number of days. Coefficient of variation (CV) of final fish BM was calculated (Kestemont, Jourdan, Houbart, Meillard, Paspatis, Fontaine, Cuvier, Kentouri & Baras 2003) followed by size heterogeneity = CVM_wi/CVM_wf, where M_wi = final mass, M_wf = initial wet mass, and CV = coefficient of variation (100 SD/mean).
Stocking density

Experiment 1: Effect of four stocking densities (45, 30, 15 and 5 seahorses \(3 \text{ L}^{-1}\)) on the growth of early juvenile seahorses

In the commercial-scaled culture of pot-bellied seahorse at Seahorse World, Beauty Point, the allocation of several broods in the same 50 L tank is a regular practice to optimize facilities. Three hundred and eighty juveniles from 385 seahorses collected from four different broods \((n = 25, n = 60, n = 200 \text{ and } n = 100)\) 4, 3, 2 and 1-day-old respectively, were used in this experiment. Four initial densities of 45, 30, 15 and 5 (seahorses \(3 \text{ L}^{-1}\)) each with four replicates were arranged randomly for the experiment. After 1 day in the 20-L holding tank at a density of approximately 20 juveniles \(\text{L}^{-1}\), the appropriate number of juveniles for each 3-L tank was randomly selected, then distributed to each of sixteen 3-L transparent tanks, after the \(L_i\) and \(M_{wi}\) of individual fish were recorded (day 0). After 6 weeks, the surviving seahorses were individually counted and measured.

Experiment 2: Effect of three stocking densities (25, 15 and 5 seahorses \(3 \text{ L}^{-1}\)) on the growth of 3-week-old juvenile seahorses

Four different broods and a total of 560 fish \((n = 139, n = 16, n = 246 \text{ and } n = 159), 21-, 20-, 19- \text{ and } 18\text{-day-old respectively, were held for 3 weeks in a 20-L tank at a density of approximately 20 juveniles \(\text{L}^{-1}\). Then, 180 juveniles were randomly selected for this experiment. The three initial density levels of 25, 15 and 5 seahorses \(3 \text{ L}^{-1}\) each with four replicates were arranged randomly for the experiment. The appropriate number of juveniles for each tank was selected, and then distributed to each of twelve 3-L transparent tanks, after the \(L_i\) and \(M_{wi}\) of individual fish were recorded (day 0). Water quality was monitored during the experiment to ensure adequate conditions.

Photoperiod

Experiment 1: Effect of photoperiod 24:00 LD (T24), 16:08 LD (T16) and 08:16 LD (T8) on the growth and survival of early juveniles fed one meal daily

A total of 225 individuals of 300 juvenile seahorses from a single brood, were used in this experiment. One meal was (14% dry matter of fish BM day\(^{-1}\)) simultaneously provided to early juveniles to examine benefits (in terms of body growth and survival) on early juveniles cultured in three different photoperiods. The 12:12 LD photoperiod was not considered in this study as a reference treatment, as the results of using of 12–13 h of light have already proven to be effective in maintaining pot-bellied seahorses culture (commercial and research) and did not fit the tank configuration available. Instead, it was considered to be of greater interest to compare reduced/extended photoperiods at 8-h intervals against a treatment under continuous light. Five replicates of three treatments were used: 24:00 LD (T24), 16:08 LD (T16) and 08:16 hours LD (T8). Fifteen 2-day-old juveniles were randomly selected, and placed into each of the fifteen 3-L transparent tanks. Their individual \(L_i\) and \(M_{wi}\) were recorded on day 0. The fish were fed daily at 10:00 hours with enriched \(Artemia\) nauplii as previously described and it was always present over the 24-h period. Each day, 1 h before feeding, the screens (150 \(\mu\)m) were replaced with 500 \(\mu\)m screens to flush out the remaining \(Artemia\), ensuring each day that the feeding rate was even for all the tanks through the experiment and that uneaten \(Artemia\) were removed. After 6 weeks, the surviving seahorses were individually counted and measured.

Experiment 2: Effect of photoperiod 24:00 LD (T24), 16:08 L:D (T16) and 08:16 L:D (T8) on the growth and survival of early juvenile seahorses fed two meals daily

In the previous experiment, there was concern about the experimental design in relation to food limitation in the seahorses cultured in T24 and T16. At the end of the photophase for T8, it was observed that although there were nauplii in the tank, most of the \(Artemia\) were in inaccessible areas of the tank (i.e. flushing screens). Therefore, after flushing the remaining \(Artemia\) at 16:15 for a 15-min period, a second meal of 14% BM day\(^{-1}\) (dry mass \(Artemia\): \(M_{wi}\) fish) was simultaneously provided to all treatments avoid any possibility of food limitation. From a total of 460 juvenile seahorses, from four broods \((n = 170, n = 160, n = 80, n = 50); 4-, 3-, 2- and 1-day-old respectively) combined at Seahorse World, 225 juveniles were selected randomly for this experiment. The three photoperiod levels, as well as the husbandry protocols from experiment 1 were used in this experiment. The fish were fed twice a day (at...
10:00 and 16:30 hours) at the rate previously described for experiment 1. Artemia fed at 16:30 hours were enriched for a further 6.5 h. After 6 weeks, the surviving seahorses were individually counted and measured.

**Statistical analysis**

One-way ANOVA tests (SPSS 11.5) were used to compare $L_i$, $L_f$, $M_{wi}$, $M_{wf}$, survival, SGR and size heterogeneity between treatments at a significance level of $P < 0.05$. Levene’s test and residual plots were used to test homogeneity of variance. In both density experiments, a square root transformation was conducted on data of size heterogeneity to satisfy homogeneity of variance requirements. Tukey’s HSD post hoc test was used to identify differences between treatment means (SPSS 11.5).

An orthogonal ANOVA (SPSS 11.5) was used to compare weekly (time as orthogonal factor) $M_b$ between treatments throughout all the experiments. A significance level of $P < 0.05$ was used. Levene’s test and residual plots were used to test homogeneity of variance. In all the experiments, natural logarithm and square root transformation were conducted on data of $M_b$ to satisfy homogeneity of variance requirements.

**Results**

**Stocking density**

**Experiment 1:** Effect of four stocking densities (45, 30, 15 and 5 seahorses $3\text{ L}^{-1}$) on the growth of early juvenile seahorses

The results showed that the stocking densities tested did not affect differently growth or survival over a 6-week period. There were no significant differences in $L_i$, $L_f$, $M_{wi}$, and $M_{wf}$ of the juvenile seahorses between the treatments (Table 1). On the final day of the experiment, there were no differences in survival (Fig. 1), SGR or size heterogeneity between the treatments (Table 1). Also, there were no significant differences in any of the

![Figure 1](image-url)
intermediate $M_b$ measurements. However, the growth profile of stocking densities 45, 30 and 15 seahorse $3\ \text{L}^{-1}$ reached a plateau after week 5, while seahorses at five individuals $3\ \text{L}^{-1}$ maintained steady growth until the end of the experiment (Fig. 2).

**Experiment 2: Effect of stocking density (25, 15 and 5 seahorses $3\ \text{L}^{-1}$) on the growth of 3-week-old juvenile seahorses**

The results showed that stocking density did not affect growth or survival differently over the 4-week period. There were no significant differences in $L_i$, $L_f$, $M_{wi}$, and $M_{wf}$ of the juvenile seahorses, between the treatments (Table 2). On the final day of the experiment, there were no differences in percentage survival (Fig. 1), size heterogeneity or SGR between the treatments (Table 2).

Also, there were no significant differences in any of the intermediate $M_b$ measurements (Fig. 2).

**Photoperiod**

**Experiment 1: Effect of photoperiod 24:00 L:D (T24), 16:08 L:D (T16) and 08:16 L:D (T8) on the growth and survival of early juvenile seahorses fed one meal daily**

There were no significant differences in both $L_i$ and $M_{wi}$ among treatments at the start of the experiment. After 6 weeks, survival of seahorses in this experiment showed a significant difference between juveniles cultured in T24 and T8 (Fig. 3) with higher survival recorded in T8. In T16, seahorse survival was intermediate to and similar to the other treatments (Table 3). There were significant differences in $L_f$, $M_{wf}$ between treatments (Table 3). Juveniles cultured in T24 grew less than those in T16 and T8; such growth was also reflected in the significant differences in $M_b$ between treatments throughout the experiment (Fig. 4). There were no significant differences in size heterogeneity among the treatments.

**Experiment 2: Effect of photoperiod 24:00 L:D (T24), 16:08 L:D (T16) and 08:16 L:D (T8) on the growth and survival of early juvenile seahorses fed two meals daily**

There were no significant differences in both $L_i$ and $M_{wi}$ between treatments at the start of the experiment. After 6 weeks, survival of juvenile seahorses in this experiment showed no significant

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Table 2  Stocking density, experiment 2. Survival, initial and final mass and standard length ($L_i$, $L_f$, $M_{wi}$, $M_{wf}$), size heterogeneity and specific growth rate (mean $\pm$ 1 SE) of 3-week-old seahorses *Hippocampus abdominalis* cultured at stocking densities of 25, 15 and 5 seahorses $3\ \text{L}^{-1}$ ($n = 4$) in a 4-week growth trial

<table>
<thead>
<tr>
<th>Seahorses $3\ \text{L}^{-1}$</th>
<th>25</th>
<th>15</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Final observed survival (%)</td>
<td>45 $\pm$ 9.8</td>
<td>41.3 $\pm$ 6.9</td>
<td>65.0 $\pm$ 9.6</td>
</tr>
<tr>
<td>Individual $M_{wi}$ (mg)</td>
<td>23.3 $\pm$ 0.8</td>
<td>24.2 $\pm$ 1.1</td>
<td>26.4 $\pm$ 2.1</td>
</tr>
<tr>
<td>Individual $M_{wf}$ (mg)</td>
<td>56.6 $\pm$ 1.8</td>
<td>55.4 $\pm$ 3.2</td>
<td>61.4 $\pm$ 4.2</td>
</tr>
<tr>
<td>Individual $L_i$ (mm)</td>
<td>23 $\pm$ 0.4</td>
<td>23 $\pm$ 0.4</td>
<td>24 $\pm$ 0.7</td>
</tr>
<tr>
<td>Individual $L_f$ (mm)</td>
<td>31 $\pm$ 0.4</td>
<td>32 $\pm$ 1.1</td>
<td>33.5 $\pm$ 0.3</td>
</tr>
<tr>
<td>Size heterogeneity (g)</td>
<td>1.4 $\pm$ 0.4</td>
<td>0.7 $\pm$ 0.1</td>
<td>0.5 $\pm$ 0.1</td>
</tr>
<tr>
<td>Specific growth rate (SGR%)</td>
<td>2.9 $\pm$ 0.2</td>
<td>2.6 $\pm$ 0.3</td>
<td>2.7 $\pm$ 0.2</td>
</tr>
<tr>
<td>(day$^{-1}$)</td>
<td></td>
<td></td>
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</table>

Superscripts have been omitted, as there were no significant differences among treatments (one-way ANOVA, $P > 0.05$).
differences among treatments (Fig. 3). There were significant differences in $L_f$ and $M_{w}$, where the juveniles cultured in T16 grew better than fish in T24 and T8 (Table 4). Such growth was also reflected in the significant differences in $M_b$ among treatments throughout experiment (Fig. 4). There were no significant differences in size heterogeneity among the treatments.

**Discussion**

This study is the first to demonstrate that under the experimental conditions described, an initial stocking density as high as 45 early seahorses $3 \text{ L}^{-1}$ did not grow differently from those stocked initially at densities as low as five seahorses $3 \text{ L}^{-1}$. Also, this is the first study to demonstrate under the experimental conditions described that a continuous light regime did not improve seahorse growth, but when feeding was not a limiting factor, early juveniles grew better in an extended photoperiod such as 16:08 L:D.

The densities used in this study were considerably low in comparison with the ones reported in the literature for other marine finfish species (Daniels et al. 1996; Baskerville-Bridges & Kling 2000; Hatzianthasiou et al. 2002), but higher than densities tested on $H. \text{ whitei}$ (Wong & Benzie 2003), late juveniles of $H. \text{ abdominalis}$ (Woods 2003b), and the highest stocking density of seahorses $8 \text{ L}^{-1}$ used in commercial scale systems for $H. \text{ abdominalis}$ rearing during the first 2 months of their life (Martinez-Cardenas & Seahorse World, pers. obs.). Increases in stocking density magnify the competi-

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**Figure 3** Effect of photoperiod on seahorses *Hippocampus abdominalis* survival (% mean, $n = 5$). Experiment 1 (above): 2-day-old seahorses fed one meal daily exposed to 24:00 L:D (T24), 16:08 L:D (T16) and 08:16 L:D (T8) in a 6-week growth trial. Experiment 2: 3-day-old seahorses fed two meals daily exposed to 24:00 L:D (T24), 16:08 L:D (T16) and 08:16 L:D (T8) in a 6-week growth trial. Standard error bars were omitted to aid visualization.

**Figure 4** Photoperiod growth profile as $M_w$ (mean ± 1, SE, $n = 5$) seahorses *Hippocampus abdominalis*. Experiment 1 (above): 2-day-old seahorses fed one meal daily exposed to 24:00 L:D (T24), 16:08 L:D (T16) and 08:16 L:D (T8) ($n = 5$) in a 6-week growth trial. Experiment 2: 3-day-old seahorses fed two meals daily exposed to 24:00 L:D (T24), 16:08 L:D (T16) and 08:16 L:D (T8) ($n = 5$) in a 6-week growth trial.
Stocking density, photoperiod in *H. abdominalis* L. Martinez-Cardenas & G J Purser Aquaculture Research, 2012, 43, 1536–1549

Table 3 Photoperiod, experiment 1. Survival, initial and final mass and length (*L*<sub>i</sub>, *L*<sub>f</sub>, *M*<sub>avg</sub>, *M*<sub>eff</sub>), size heterogeneity and specific growth rate (mean ± 1 SE) of 2-day-old seahorses *Hippocampus abdominalis* exposed to 24:00 L:D (T24), 16:08 L:D (T16) and 08:16 L:D (T8) in a 6-week growth trial (*n* = 5)

<table>
<thead>
<tr>
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<th>24:00</th>
<th>16:08</th>
<th>08:16</th>
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<tbody>
<tr>
<td>Final observed survival (%)</td>
<td>38.7 ± 5.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>44.0 ± 4.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>57.3 ± 3.4&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>Individual <em>M</em>&lt;sub&gt;avg&lt;/sub&gt; (mg)</td>
<td>8.0 ± 0.1</td>
<td>8.3 ± 0.1</td>
<td>8.2 ± 0.17</td>
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<tr>
<td>Individual <em>M</em>&lt;sub&gt;eff&lt;/sub&gt; (mg)</td>
<td>53.7 ± 2.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>86.6 ± 7.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>86.1 ± 5.06&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>Individual <em>L</em>&lt;sub&gt;f&lt;/sub&gt; (mm)</td>
<td>16 ± 0.08</td>
<td>17 ± 0.06</td>
<td>17 ± 0.1</td>
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<tr>
<td>Individual <em>L</em>&lt;sub&gt;i&lt;/sub&gt; (mm)</td>
<td>32 ± 0.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>37 ± 0.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>37 ± 0.7&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>Size heterogeneity (g)</td>
<td>2.8 ± 0.3</td>
<td>3.0 ± 0.4</td>
<td>2.3 ± 0.3</td>
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<tr>
<td>Specific growth rate (SGR% day&lt;sup&gt;−1&lt;/sup&gt;)</td>
<td>4.5 ± 0.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.5 ± 0.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.6 ± 0.1&lt;sup&gt;b&lt;/sup&gt;</td>
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Means with different superscripts within a row are significantly different (one-way ANOVA, *P* < 0.05).

Table 4 Photoperiod, experiment 2. Survival, initial and final mass and standard length (*L*<sub>i</sub>, *L*<sub>f</sub>, *M*<sub>avg</sub>, *M*<sub>eff</sub>), size heterogeneity and specific growth rate (mean ± 1 SE) of 3-day-old seahorses *Hippocampus abdominalis* exposed to 24:00 L:D (T24), 16:08 L:D (T16) and 08:16 L:D (T8) in a 6-week growth trial (*n* = 5)

<table>
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<th>24:00</th>
<th>16:08</th>
<th>08:16</th>
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<tbody>
<tr>
<td>Final observed survival (%)</td>
<td>30.7 ± 4.5</td>
<td>42.7 ± 4.5</td>
<td>48.0 ± 6.8</td>
</tr>
<tr>
<td>Individual <em>M</em>&lt;sub&gt;avg&lt;/sub&gt; (mg)</td>
<td>11.0 ± 0.3</td>
<td>10.6 ± 0.3</td>
<td>10.5 ± 0.3</td>
</tr>
<tr>
<td>Individual <em>M</em>&lt;sub&gt;eff&lt;/sub&gt; (mg)</td>
<td>98.2 ± 9.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>162.2 ± 11.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>107.7 ± 11.8&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td><em>L</em>&lt;sub&gt;i&lt;/sub&gt; (mm)</td>
<td>18 ± 0.1</td>
<td>18 ± 0.2</td>
<td>18 ± 0.1</td>
</tr>
<tr>
<td><em>L</em>&lt;sub&gt;f&lt;/sub&gt; (mm)</td>
<td>37 ± 1.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>42 ± 1.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>47 ± 1.1&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Size heterogeneity (g)</td>
<td>0.5 ± 1.0</td>
<td>0.8 ± 0.1</td>
<td>0.9 ± 0.1</td>
</tr>
<tr>
<td>Specific growth rate (SGR% day&lt;sup&gt;−1&lt;/sup&gt;)</td>
<td>5.2 ± 0.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.5 ± 0.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.5 ± 0.3&lt;sup&gt;a&lt;/sup&gt;</td>
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Means with different superscripts within a row are significantly different (one-way ANOVA, *P* < 0.05).

Survival presented a rapid decline until day 13, when survival across treatments appeared to stabilize for the remainder of the trial. However, the treatments tested displayed an overall survival rate of 59 ± 1.4% (mean ± 1 SE), which, under the experimental conditions of this study, represented a positive indicator for further research and commercial culture. A major constraint in the development of marine fish culture has been the successful rearing of larvae beyond critical stages i.e. first feeding (Odile, Jean-Paul & Elisabeth 1996). Early stage mortality has also been reported in seahorse species such as *H. trimaculatus* and *H. kuda*, where elevated mortalities occur at various critical points, such as the first 2–3 days (first feeding) and after 1 week when the change in prey-item/prey-size from copepods to *Artemia* nauplii takes place (Lin, Lu, Gao, Shen, Cai & Luo 2006; Sheng et al. 2006). The inhibition of feeding during experiments with early juvenile *H. abdominalis* has also resulted in high mortalities (Woods 2000a,b; Woods 2003a). It appears that mortality peaks in early seahorses are related to the transition to different prey item/size. High mortalities in *H. abdominalis* occur generally during the first 2 weeks of their lives and not in several stages as with *H. trimaculatus* and *H. kuda*. This can be explained by the rather large size of the newborns, which allows them to feed directly onto *Artemia* nauplii without several transitions of prey size/item, until a smooth transition from *Artemia* nauplii to mysids (either live or frozen) takes place approximately 4 months after birth (Martinez-Cardenas, pers. obs.). The mortalities displayed in this experiment could be the result of the mentioned early juvenile inherent mortality on *H. abdominalis*, or perhaps due to a negative response to a new environment resulting in a source of stress.
The initial stocking densities were altered due to mortality; these were not replaced, as replacement fish could not be identified through tagging or marking. The small size of the experimental fish complicated the use of conventional tagging techniques. During the preparation for the first experiment, a range of tagging techniques were tried: dorsal fin colouration with alcian blue, the fitting of a distinctive plastic collar and categorizing individuals by natural ‘body markers’ such as slightly deformed tails as in Woods (2003b). Unfortunately, none of these attempts resulted in a reliable way to discriminate the replacements from the experimental individuals, as juvenile seahorses were able to lose fin marking in a very short period of time. Seahorses managed to remove collars, and juveniles with natural ‘body markers’ were very scarce or markers were hardly distinctive at that age.

The experimental design on the second experiment aimed to remove the effect of ‘early stage’ mortality from the stocking density results by using 3-week-old juveniles. However, survival declined in a similar way to experiment 1. At day 13, survival rates appeared to stabilize. Consistent with the first experiment, there were no significant differences between the treatments in terms of percentage survival or growth of the juvenile seahorses at the end of the experiment. However, overall survival at the end of the second experiment (50%) was slightly lower than the first experiment (59%). Perhaps seahorses were less tolerant to high-stock densities as they grew, with a reduction in performance and survival, as suggested by Woods (2003b) who found that 5-month-old juvenile *H. abdominalis* survival was reduced and the physical interference between juveniles (i.e. tail grasping and wrestling) increased as a result of increasing stocking density from one to two seahorses L⁻¹ to five seahorses L⁻¹. Wong and Benzie (2003) reported a lack of significant differences in growth of 3-month-old *H. whitei*, when testing a range of 0.5–1 seahorse L⁻¹. However, the authors of that study acknowledged that this could be explained by the limited stocking-density range tested. A stocking density of fifteen 3-day-old *H. abdominalis* cultured in 3-L tanks utilized during a 6-week trial on the background colour response of the species resulted in an overall survival of 75% (Martinez-Cardenas & Purser 2007), which contrast with the findings of this study. Perhaps, the mentioned early juvenile inherent mortality on *H. abdominalis* or its adaptation skills to a new environment varies between broods.

The lowest performance (growth and survival) displayed by the fish cultured in T24 during the first experiment was possibly caused by the adjusted feeding rate of 14% BM day⁻¹ (dry mass *Artemia: M. marina* fish), and the quality of the *Artemia* after a certain period of time was not adequate to compensate the energy expended by the seahorses cultured in continuous light. However, it would appear unlikely that a feeding rate of 14% BM day⁻¹ would be limiting, as a daily feeding rate of 5% of BM has been used previously for *H. abdominalis* experimentation on newborns (Florent 2003), juveniles (Wardley 2001; Wilson et al. 2006) and late juveniles (Woods 2005). The feeding rate of 14% BM day⁻¹ utilized in the present study was based on previous research on the effect of tank colour, and the rate used was found to be in excess for early juvenile *H. abdominalis* (Martinez-Cardenas & Purser 2007).

During this experiment, although *Artemia* was present in the tanks at all times, not all the live food was available as some nauplii tended to congregate in inaccessible areas of the tanks (i.e. over the filtering screens and near to the surface and walls). Also, the nutritional value of the *Artemia* remaining in the tanks over the 24-h feeding cycle could be low due to evacuation of the enrichment media by the nauplius digestive system. Previous work on juvenile seahorses has shown that the use of unenriched *Artemia* is not recommended to support good growth and survival of juvenile *H. abdominalis* (Shapawi & Purser 2003). In addition, other studies have found possible stress effects associated with continuous light (Stefansson, Fitzgerald & Cross 2002; Turkler 2005) and this may be a cause of the poor performance in T24.

It was assumed that as seahorses are well known for their eager feeding behaviour, which has been reported to be displayed primarily during the light period (Woods 2000a; Ouyang 2005; Karina et al. 2006; Sheng et al. 2006), an extended photoperiod would improve their performance. In contrast, at the end of the experiment, the juveniles cultured in an extended photoperiod (T16) did not show any improvement in terms of growth and survival compared with those in T8. The possibility existed that the *Artemia* remaining in tanks under 08:16 L:D in the present experiment may not have been ingested during the scotophase and that T8 was a sub-optimal feeding duration. Trippel and Neill (2003) found that an extended feeding period for juvenile haddock *M. aeglefinus* did
not enhance growth under equivalent rations, suggesting that haddock may be capable of reaching satiation during a feeding period equivalent to natural day length. Seahorses in T24, T16 and T8 were fed simultaneously (at approximately 10:00 hours) and equivalently on the basis of daily mortality and weekly growth recorded in the $M_D$ measurements per tank. However, the possibility remained that the quality of the Artemia available for the fish in T16 was not adequate towards the end of this photoperiod.

To avoid any possibility of food limitation and the effect of declining nutritional value due to evacuation of enrichment, feeding was doubled in the second experiment compared with that used in the first one. It seemed that the quantity and quality of the food (Artemia fed at approximately 16:30 hours was also maintained in enrichment media prior to feeding) were more adequate for growth of juveniles in T16 compared with those in T16 in the first experiment.

The lower growth rate recorded in seahorses cultured in T8 compared with those in T16 (despite the feeding increase) was consistent with the results of the first experiment. As previously mentioned, this suggests again that seahorses displayed low feeding and locomotor activity during the dark period as previously reported (Ouyang et al. 2008; Sheng et al. 2006).

Seahorses exposed to the 24-h light did not improve their performance despite the additional meal and continuous feeding opportunity. There is a possibility that the juveniles in T24 remained active, spending more energy searching for food than the energy they assimilated by actually feeding. Coincidently, Fielder et al. (2002) found for snapper P. auratus larvae that a light phase, longer than the natural photoperiod, provided longer feeding duration and also extended the duration of the foraging (searching) behaviour resulting in lower mass gain of larvae cultured at 24:00 (L:D) compared with 18:06 (L:D). Similarly, Gines et al. (2004) reported a higher daily growth of juvenile gilthead sea bream S. aurata under an extended photoperiod (16:08 L:D) compared with fish cultured in tanks with continuous lighting. The authors suggested a greater expenditure of non-productive energy associated with increased activity metabolism.

Contrarily, Olivotto et al. (2008) reported improved growth in H. reidi cultured in 24:00 (L:D). In that study, the combination of continuous light and a diet complemented with copepods (Tisbe spp) resulted in a better growth compared with a 14:10 hours photoperiod and a ‘conventional’ rotifer-Artemia diet. The inclusion of Tisbe spp copepods was not considered in this study as the large size of H. abdominalis newborns enables them to prey onto Artemia nauplii (which have proven to be an efficient diet for early stages of this species) on the day of release. However, in further research, the feeding frequency used in H. reidi (08:00, 12:00, 16:00 and 20:00 hours) could improve H. abdominalis growth under 24:00 hours (L:D).

It is not often possible to determine if the light effect on growth depends on food consumption or better food utilization (Trippel & Neil 2003). Chronically stressed teleost fish have lower growth performance than unstrained individuals (Tucker 1998). Diverse sources of stress in marine fish culture could be triggered by continuous light, such as a negative social interaction (Almazan-Rueda, Van Helmon, Verreth & Schrama 2005) leading to lower growth and survival caused primarily by cannibalism. Seahorses do not display cannibalistic tendencies; however, excessive tail grasping has been reported as a cause of stress that easily leads to mortality as juveniles ‘wrestle’ against each other instead of feeding (Woods 2000b). However, in this study, such behaviour was not observed during experiments. In relation to social interaction, Stefansson et al. (2002) suggested that the long-term culture under an extended photoperiod, acted as an irritant inducing stress in juvenile turbot Scophthalmus maximus, suppressing growth and reducing feed utilization compared with juveniles cultured under natural photoperiod. The authors of that study attributed those findings to the development of negative size-dependent hierarchies in experimental fish under continuous light.

In the present study, there were no differences in seahorse size heterogeneity at the end of both experiments, which means that the possibility of larger individuals affecting the performance of smaller juveniles in tanks under continuous light was unlikely.

While no studies have directly examined feeding hierarchies in seahorses, hierarchies would appear unlikely based on seahorse’s general behaviour and dispersed prey availability. It is more likely that continuous light caused a low-level stress through the disruption of physiological processes and rhythmicity. Most mortality occurred in the
first 2 weeks after which it levelled out. It would be of interest to identify the effects of the tested photoperiods over a longer period of time, after conditioning to extended photoperiods.

Some studies related to the effect of photoperiods on juvenile fish have been conducted over periods of time ranging from 60 days (Ergun, Yigit & Tucker 2003) to 12 months (Gines et al. 2004) to observe differences among the photoperiods tested. In contrast, some studies on fish larvae have been conducted for shorter periods of time ranging between 20 and 30 days (Barlow, Pearce, Rodgers & Clayton 1995; Fielder et al. 2002) as younger fish have an inherently faster growth than older individuals (Simensen, Jonassen, Imsland & Stefansson 2000). In the present study, after 42 days, usable data were collected from both photoperiod trials.

In the present study, an extended photoperiod of 16:08 (L:D) improved the growth of early juvenile pot-bellied seahorses *H. abdominalis*, in the presence of a high-feeding ratio supplied in more than one meal. However, further research is required to investigate the optimal combination of photoperiods, feeding rate and meal frequency to be used in commercial seahorse production. It will be of interest to examine the metabolic response of the juveniles exposed to different photoperiods and stocking densities to determine nutritional stress in relation to the C:N ratio as its determination has been used as an indicator of nutritional stress especially in early fish stages, when the small size of the juveniles does not meet the minimum quantity of tissue required to conduct conventional proximal analyses (Harris, Nishiyama & Paul 1986).

The melatonin production pattern of *H. abdominalis* most closely resembles type B, the three main melatonin production patterns described by Reiter (1989), where the highest level of melatonin occurs at the middle of the scotophase followed by a gradual decrease until the photoperiod restarts (Martinez-Cardenas et al. 2008). Based on the knowledge of melatonin profiles, aquaculture practices such as the induction of growth and reproduction have been improved for several commercial species as melatonin has been identified in teleost as a signal to control the timing of daily and seasonal events and the rhythmicity of physiological processes (Randall, Bromage, Thorpe, Miles & Muir 1995). In the present study, the existence of melatonin in *H. abdominalis* adults may explain to some extent the possible influence of photoperiod on its production on early juveniles and the associated reduction in activity during the scotophase. Unfortunately, the small size of the individuals at the end of the photoperiod experiments made impossible the collection of the minimum quantities of blood/plasma required to conduct the radioimmunoassay. Further research could focus on the development of a method to measure melatonin from sample as small as early juvenile seahorse tissue.

The results in this study provide new information from experimental scale trials. It would be of interest for future research to analyse the effect of high stocking densities and photoperiod in the tank distribution of *H. abdominalis* using different orientation and size and quantity of attachment substrate at a pilot scale commercial trial.

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**References**


