Implications of physical key factors in the early rearing of the long-snouted seahorse *Hippocampus guttulatus*

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ABSTRACT:

Although breeding conditions are rather well established for some seahorse species (*Hippocampus* spp.), zootechnics and the effect of non-biological factors are still unknown for many species. The present study is focussed on the effects of aquarium type/design, photoperiod regime and aeration level on the early performance (growth and survival) of *Hippocampus guttulatus* juveniles. Three aquarium types were evaluated: pseudoKreisel, spherical and rectangular. Juveniles grown in pseudoKreisel aquaria showed lower growth rates but significantly higher survivals (69 ± 15%) at day 30 after male's pouch release (DAR) when compared to either spherical (30 ± 22%) or rectangular (16 ± 12%) aquaria. Survival enhancement in pseudoKreisel aquaria was mainly related to the very lower proportion of juveniles showing swim bladder hyperinflation during the first days of life.Conversely, the other aquarium types did not avoid juveniles to remain near water surface and to gulp air in excess for swim bladder inflation. The effects of light regime and aeration level were assayed in *H. guttulatus* juveniles reared in all three aquarium types or in pseudoKreisel aquaria only, respectively. In general, the application of extended (continuous light) or natural photoperiods (day-night cycle; 16h light: 8h dark) did not affect significantly survival nor growth. On the other hand, aeration levels in pseudoKreisel aquaria significantly affected juvenile survival. Survivals in 30 days old seahorses reared under a strong aeration were significantly higher (41 ± 12 %) than when reared under weak aeration (13 ± 0 %). Strong aeration levels enhanced the distribution of juveniles in the aquaria and diminished both their over-exposition to water surface and the resulting appearance of hyperinflation problems. The overall results suggest that the best rearing conditions were met when *H. guttulatus* juveniles grew in pseudoKreisel aquaria under both a strong aeration level and, to a lesser extent, a natural photoperiod regime, due to a slight enhancement in seahorse juvenile performance.

KEY WORDS: Aquaria design; photoperiod; aeration; seahorse; *Hippocampus guttulatus*.

1. Introduction

The rearing of seahorses in captivity has become a palliative activity to reduce the pressure on wild populations (Olivier, 2008) and to meet rising market demand (Koldewey and Martin-Smith, 2010; Olivotto et al., 2011). Concern on the overexploitation of wild seahorses increased in the late 1990's (Vincent 1996) and significant enhancements were achieved in the production of *Hippocampus barbouri*, *H. fuscus*, *H. kuda* (Wilson and Vincent, 2000), *H. abdominalis* (Woods, 2000) and *H. subelongatus* (Payne and Rippingale, 2000). However, the optimal rearing conditions have not been met in most species.

Compared to other marine fish, seahorses largely differ in their biology and, consequently, in their optimal rearing conditions (Olivotto et al., 2011), which are species-specific. Improvements have been made recently in the cultivation of the European long-snouted seahorse *Hippocampus guttulatus*, especially on breeding (Planas et al., 2008, 2009, 2010, 2013) and some important aspects of the early rearing of juveniles such as feeding/nutritional requirements (Olivotto et al., 2011), fatty acid requirements (Faleiro and Narciso, 2010), optimal temperature level (Planas et al., 2012), holdfast preferences (Correia et al., 2013) and genetic inbreeding (López, 2011). However, other aspects of rearing conditions are almost unknown, namely those concerning physical factors.

The early rearing of seahorse juveniles is generally carried out in special aquaria rather than in conventional rearing units. Rectangular aquaria have been traditionally used in the rearing of juveniles of different seahorse species (Payne and Rippingale, 2000; Job et al., 2002; Gardner, 2008; Olivotto et al., 2008; Hora and Joyeux, 2009; Lin et al., 2010; Otero-Ferrer et al., 2010; Palma et al., 2011). Additionally, circular shaped aquaria (from cylindrico-conical to round and circular bowls) have also been used in the culture seahorse juveniles (Woods, 2000; 2003a; 2003b; Wilson and Vincent, 2000; Payne, 2003; Sheng et al., 2007). The use of aquaria with uncommon shapes and types has been also reported, e.g. bamboo cages (Garcia and Hilomen-Garcia, 2009; Garcia et al., 2010) or plastic buckets (Sheng et al., 2006). To fulfil one of the main challenges for sustainable aquaculture, the aquaria design should consider that the fish must be kept under the best conditions for growth and welfare with minimal resource consumption (Duarte et al., 2011). In addition, the engineering and design of the rearing systems should solve biological constraints of fish larvae (Nash et al., 1977). Thus, the use of aquarium designs that provide evenness of the rearing conditions and homogeneous distribution of fish becomes essential to ensure a successful rearing (Ross et al., 1995).

Aquaria design and water movement/turbulence affect water flow pattern in the aquaria, which may result in the presence of dead volumes and stagnant water zones that may compromise fish wellness (Duarte et al., 2011). Therefore, water aeration is essential for an adequate distribution of young juveniles in the water column (Sakakura et al., 2006). Additionally, aeration can be easily manipulated, preventing fish accessing to water surface and potentially providing immediate solution to fish mass mortality (Fui et al., 2012). On the other hand, it has been reported that high turbulence has negative impact on marine fish larvae due to physical injuries by air bubbles and to feeding difficulties by increasing unnecessary energy consumption for hunting (Oshima et al., 2009; Sakakura et al., 2007; Utne-Palm and Stiansen, 2002).

Light regime also affect growth and survival of fish larvae (Partridge et al., 2011; Stuart and Drawbridge, 2012; Vallés and Estévez, 2013). Photoperiod is related to a wide variety of behaviours and biological rhythms in fish larvae, depending on ecological requirements and species-specific characteristics (Eshagh-Zadeh et al., 2013; Fielder et al., 2002). The rearing of seahorses has been generally carried out under day-night cycles of 14L:10D cycle (Wilson and Vincent, 2000; Payne, 2003; Job et al., 2006; Sheng et al., 2007; Faleiro and Narciso, 2010; Zhang et al., 2011; Pham and Lin, 2013) or 12 Light:12 Darkness (Payne and Rippingale, 2000; Woods, 2000; 2003b; Wong and Benzie, 2003; Hora and Joyeux, 2009; Yin et al., 2012; Willadino et al., 2012; Souza-Santos et al., 2013). However, while some species have been successfully reared under a 16L:8D photoperiod (Lin et al., 2010; Lockyear et al., 1997) others have been successfully reared under continuous light (Olivotto et al., 2008; Pawar et al., 2011). The impact of photoperiod regimes on seahorses have been scarcely studied (Martinez-Cardenas and Purser, 2012; Olivotto et al., 2008; Sheng et al., 2006) and, due to its species-specific effects, it becomes of main importance to determine the optimum photoperiod regime for each species.

Studies on the early rearing of *H. guttulatus* pointed out a high percentage of juveniles showing swim bladder hyperinflation (Olivotto et al., 2011), which resulted in extremely low survivals during the first two weeks of development. According to zootechnical, environmental and physical conditions related to hyperinflation problems; aquaria design, photoperiod regime and aeration level need to be addressed in order to reduce the effects of those factors on the problematics. Therefore, the main objective of the present study was to improve the early rearing of *Hippocampus guttulatus* juveniles by assessing photoperiod regimes (Long *vs* short cycle), aeration levels (Strong *vs* weak) and, for the first time in seahorse studies, aquaria designs (rectangular, spherical and pseudoKreisel).

2. Materials and methods

2.1. Broodstock

Adults of the seahorse *Hippocampus guttulatus* were collected by scuba diving from winter to summer in 2010 at several locations of the Galician coast (NW Spain) and properly transported to the facilities of the Institute of Marine Research in Vigo (Spain). Prior to be transferred to 630 L aquaria units, seahorses were gradually acclimatised to room temperature for 3-5 hours, and then weighed and tagged using nylon collars with a unique code for individual identification (Planas et al., 2008).

The broodstock was submitted to standardized photoperiod and temperature regimes (Planas et al., 2010, 2013). The temperature level was gradually adjusted according to a natural-like temperature cycle, ranging from 15 ºC in winter to 19 ºC in summer (± 0.5 ºC), whereas the photoperiod regime was increased from 10L:14D (winter conditions) to 16L :8 D (summer conditions). The breeding aquaria were supplied with 5µm-filtered and UV-treated seawater with a daily renewal of 10-15 % total volume. Water quality was periodically checked for $N0_2^-$, $N0_3^-$ and NH_4^+/NH_3 content (0 mg l^{-1}) by using Sera Test Kits. Salinity and pH levels were 38 ± 1 ppt and 8.1 ± 0.1, respectively.

Adult seahorses were fed twice per day on adult enriched *Artemia* (EG; Iberfrost, Spain) and supplemented with wild-captured Mysidacea (*Leptomysis* sp. and *Siriella* sp.). *Artemia* enrichment consisted on a mixture of microalgae (*Phaeodactylum tricornutum* and *Isochrysis* *galbana*) and a daily dose (0.1 g L^{-1}) of Red Pepper (Bernaqua, Belgium) for at least 5 days. Faeces and uneaten food were siphoned every day before feeding.

Pregnant seahorses were transferred individually from the broodstock aquaria to 30L pseudoKreisel aquaria (18 ºC and 16L:8D light regime) and maintained isolated for a few days until newborn release.

2.2. Rearing system

A total of 15 wild adult seahorses released 7 different newborn batches (October 2011 - January 2013). A total of 4 batches were used in Experiment 1 (photoperiod and aquaria design) and 3 in Experiment 2 (aeration level). All batches used in the present study were transferred to the experimental aquaria at an initial density of 4 seahorses L^{-1}. The rearing aquaria were operated in a semi-opened recirculation system including a degasifying column and two 50 L biofilters with mechanical (up to 20 µm) and biological filters, aerators and skimmers. The seawater was pumped from the biofilters to 36w UV units (AquaMedic, Germany) and from there to 50 l reservoir aquariums, being finally routed by gravity towards the rearing aquaria.

2.3. Experiment 1

2.3.1. Aquaria design

Three types of aquaria were assayed (Figure 1):

- Rectangular (R). The aquaria were 335 x 195 x 220 mm in size (10L useful volume). In addition to black walls (12 cm height) in the upper half of all aquaria sides, black upper covers were used (Figure 1.1). The water inflow was adjusted to 400 ml min^{-1} and placed inside a tube located in the opposite side of the water outlet, which was situated 35mm below the upper part of the aquaria and built on a two mesh-sized T-system (3 cm diameter). Two mesh sizes were used: 500µm during daytime (150mm radius) to allow the exit of remaining prey between feeding times, and 250 µm at evening/night to avoid prey from leaving the aquaria. Aeration of the aquaria was provided by stand-pipes (5mm in diameter) placed at the bottom of the aquaria.

- Spherical (SP). This aquarium (Figure 1.2) was a modification of 9L transparent plastic flat-bottomed circular bowls (7.5L useful volume) commonly used for fish keepers.

The bowls had a 120mm diameter opened top and the biggest diameter of the aquaria, measured at the middle part of the aquaria, was 240mm. The water inflow (300ml min^{-1}) was placed in the upper part of the aquarium, flowing through the wall to avoid a cascade effect while providing top-down water currents. The water outlet was located in the centre of the bottom of the aquaria and consisted of a vertical double tube system. The inner tube (10mm radius and 175mm in length) was placed at the level of water surface and served as water outflow. The external tube (16mm radius and 210mm in length with a 500µm mesh) was used as a protection tube avoiding seahorse juveniles to be drained out while maintaining prey density similar to the other two aquaria designs. Moderated aeration was provided by a stand-pipe attached to the external central tube, in opposite position to the water inflow.

- PseudoKreisel (PK). This polyurethane aquarium (27 L useful volume) was designed as a modification of a common Kreisel aquarium including a combined circular (Upper part) and rectangular (Lower part) shape (Figure 1.3). The water inlet was located in one of the surface corners (flow rate: 700mm min^{-1}) and opposite to the outflow box. The water outlet consisted of an outflow box with two smaller compartments. The first one determined the upper level of the water column. From there, the seawater was transferred to a second compartment from where the water was discarded. The first compartment was screened (9 x 12mm) towards the main compartment of the pseudoKreisel vessel. A 500µm mesh was placed on it during the day-time period of the day and substituted by a 250 µm mesh during the night. Aeration was provided by a stand-pipe placed below the outflow box.

2.3.2. Photoperiod regimes

Two photoperiod regimes, 16L:8D (natural-like) and 24L:0D (extended), were applied to the three experimental aquaria types described above. Lighting was supplied by 20w fluorescent lamps (Power Glo) during the day-time (from 08.00 to 18.00 in the 16L:8D treatment or 24h in the 24L:0D treatment). In rectangular and circular aquaria, the lamps were placed approximately 30 cm above the seawater surface whereas the lightning was lateral in pseudoKreisel aquaria. To avoid light entering from the surface of the water column in rectangular and pseudoKreisel aquaria, the half upper sides of the walls were covered by black plastic films. The top of the rectangular aquaria were also covered by plastic lids. In spherical aquaria, the lamps were covered by a black translucent mesh to avoid brightness on the water surface.

2.3.3. *Feeding conditions.*

For the aquaria design and photoperiod experiments, seahorse newborn were fed on a single daily dose (9 am) of *Acartia tonsa* adult copepods and copepodites (0.11 – 0.90 mm SL; 0.6 copepods ml^{-1}) and *Artemia* nauplii (0.45 – 0.70 mm SL; 1 *Artemia* ml^{-1}) from 0–10 DAR (days after male's pouch release). Three daily doses (1:1, 1 *Artemia* ml^{-1} $dose^{-1}$) of *Artemia* nauplii and 24 h enriched metanauplii (0.82 – 1.23 mm SL) were offered from day 11 until the end of the experiment at day 30. Copepods were cultivated on a mixture of the microalgae *Isochrysis galbana and Rhodomonas lens*. *Artemia* enrichment was performed in 5 L buckets at 26 ºC at initial density of 100 *Artemia* ml^{-1}. The enrichment diet consisted of a mixture of the microalgae *Isochysis galbana* (10^7 cells ml^{-1}) and *Phaeodactylum tricornutum* (1.6 10^7 cells ml^{-1}).

2.4. Experiment 2: Aeration intensity

Strong and weak aeration conditions were assayed in juveniles reared in pseudoKreisel aquaria. Strong aeration was supplied by a stand-pipe producing big air bubbles (24 ± 3 mm in diameter) from the upper half side of the pseudoKreisel aquaria, near the outflow screened window. Approximately 11 ± 1 air bubbles sec^{-1} were supplied at a mean velocity of 42 ± 3 cm sec^{-1}. Weak aeration was provided from the lower half side of the aquaria, below the outflow box (Figure 1.3), by small air bubbles (11 ± 2 mm in diameter) moving at a speed of 37 ± 3 cm sec^{-1} and at a rate of 21 ± 3 bubbles sec^{-1}.

The feeding schedule applied consisted of three daily doses of *Artemia* nauplii (1 *Artemia* ml^{-1}) from 0 to 10 DAR and three daily doses of *Artemia* nauplii and 24 h enriched metanauplii (1:1, 1 *Artemia* ml^{-1} $dose^{-1}$) from 11 DAR until the end of the experiment at day 30. *Artemia* enrichment was carried out as previously described in Experiment 1.

2.5. Seahorse sampling.

Fish faeces and uneaten food were siphoned out twice daily and dead seahorse juveniles removed and counted. Mortalities were daily registered and, at the end of the experiment (30 DAR), all survivors were counted. All procedures involving animals were conducted in accordance with the Spanish laws on animal experimentation and were approved by the Bioethics Committee of CSIC. Sampled seahorses were sacrificed using anaesthetic overdose with MS222 (0.1 g L^{-1}), washed with tap water, individually transferred to Petri dishes and photographed for standard length (SL) measurements. Then, the excess of water was removed and the seahorses were pooled or individually weighted on a Sartorius microbalance MC210P (± 0.01 mg). Seahorses from samples taken at 30 DAR were awaken and transferred to their original aquaria. Estimated final survivals were adjusted considering sampling mortality. In Experiment 1, seahorse samples (n=10-20) were taken at 0, 5, 10, 15 and 30 DAR for wet weight and standard length (Mean ± SD). In Experiment 2, seahorse juveniles were sampled at 0, 15 and 30 DAR.

SL was measured as head + trunk+ tail length (curved measurement), as reported by Lourie et al. (1999). Measurements were made on digital images using an image processing software (NIS, Nikon). Calculations involving development and growth were performed according to the formulations described in Otterlei et al. (1999) and applied to *H. guttulatus* juveniles by Planas et al. (2012). Daily weight-specific growth rates (G; % day^{-1}) were calculated as:

$$G = 100\,(e^{g} - 1)$$

where the instantaneous growth coefficient g was obtained by the following equation

$$g = (\ln W_2 - \mathrm{Ln}\, W_1) / (t_2 - t_1)$$

where W_2 and W_1 are the average seahorse wet weights (mg) on times t_2 and t_1, respectively, being t the time in days.

Fulton's condition factor (K_F) of juveniles was calculated as:

$$K_F = (WW/SL^{b})*1000$$

where WW was the wet weight and SL the standard length, b was the constant from the power tendency of the WW – SL relationship.

2.6. Data analysis

All statistical analyses were done using the software packages SPSS 21.0 and STATISTICA 8.0. To analyse differences in WW and SL among treatments, MANOVA tests were performed considering a significance level of $P < 0.05$. Levene's test and residual plots were used to test homoscedasticity. Bonferroni *post-hoc* test was used to identify differences between treatment means.

One-way ANOVA was used to test for differences among DAR for each experimental treatment (Experiment 1 and Experiment 2). Additionally, in Experiment 1, three-way ANOVA test was used to assess differences between photoperiod, aquaria design and their interaction at each established developmental stage (5, 10, 15 and 30DAR). In both experiments, differences were tested at a significance of $P < 0.05$. Partial squared eta (η^2) was used as a measure of effect size as it is independent of the ANOVA design used (Chu et al., 2012). Levene's test and Shapiro-Wilks normality test were applied to test for homogeneity and normality, respectively, for all samples. Bonferroni *post-hoc* comparisons were analyzed between the different means when statistical differences were found.

3. Results

3.1. Experiment 1: Photoperiod and Aquaria Design

The effect of photoperiod regime and aquaria type on survival rates of seahorse juveniles at different DAR are summarized in Tables 1 and 2 (Three-way ANOVA) and depicted in Figure 2 (Aquaria type: A-B; Photoperiod: C-E). First order and second order interactions were all statistically significant (except for photoperiod x DAR). Overall, survivals were significantly higher in pseudoKreisel aquaria under a 16:8 photoperiod regime and decreased from 0 to 30 DAR, though they remained constant from 10 to 15 DAR.

The photoperiod regime did not affect survivals in pseudoKreisel aquaria ($p=0.805$). However, a long photoperiod had a significant negative impact on survivals in both rectangular ($p=0.026$) and spherical ($p<0.001$) aquaria.

Considering the aquarium type, pseudoKreisel and spherical units performed similarly ($p=0.572$) under a short photoperiod regime. Survivals were significantly lower in the rectangular aquaria ($p<0.001$). Under a continuous light regime, survivals in spherical and rectangular aquaria were not significantly different ($p=0.961$) but lower than in pseudoKreisel units ($p<0.001$, in both cases).

Changes in survival with age were clearly affected by both photoperiod regime and aquarium type. A significant effect of photoperiod was noticed at 10 and 15 DAR ($p=0.001$ and 0.004 respectively) but not at 5 and 30 DAR ($p=0.410$ and 0.129, respectively). Considering aquarium type, survivals did not differ significantly at 5 DAR. In pseudoKreisel and spherical types, seahorses performed similarly at 10 DAR ($p=0.119$), and survivals in the former were significantly higher than in rectangular aquaria ($p<0.001$). Since 10 DAR until the end of the experiment, survivals in pseudoKreisel aquaria were higher than in spherical and rectangular units ($p=0.005$ and $p<0.001$). At 15 and 30 DAR, spherical and rectangular aquaria performed similarly ($p=0.09$ and 0.228, respectively).

Wet weights and standard lengths in seahorses reared in different aquarium types under two photoperiod regimes are shown in Figure 3A.

Mean comparisons showed significant differences in WW and SL between 0 and 30 DAR in all aquaria types and photoperiod regimes (One-way ANOVA, $p < 0.05$). The MANOVA test applied to both WW and SL in 30 DAR juveniles showed no effects for the interaction aquaria type - photoperiod regime (MANOVA $F_{4,\ 104} = 2.16$, $p = 0.08$, $\eta^2 = 0.077$). Additionally, no significance was achieved for main effects aquaria design (MANOVA $F_{4,104} = 2.28$, $p = 0.08$, $\eta^2 = 0.081$) and photoperiod (MANOVA $F_{2,52} = 0.72$, $p = 0.49$, $\eta^2 = 0.027$). However, WW in 30 DAR juveniles from spherical aquaria under the natural photoperiod (130 ± 19 mg) was higher but not statistically different than in juveniles from rectangular (69 ± 20 mg) or pseudoKreisel (77 ± 8 mg) aquaria.

SGRs from 0 to 30 DAR and Fulton's condition factors (K_F) at 30 DAR were not statistically different among treatments except for K_F for the three aquaria types (ANOVA, $F_{2,10}=10{,}96$, $p=0.003$). K_F in juveniles were significantly lower in pseudoKreisel aquaria ($p=0.09$ and 0.010 for spherical and rectangular aquaria, respectively).

3.2. Experiment 2: Aeration

Figures 2F and 3B show survival rates and WW-SL relationships in juveniles submitted to weak and strong aeration. The interaction aeration – age (DAR) in between-subject effect was statistically significant (MANOVA $F_{2,\ 434} = 10.35$, $p < 0.001$) for both WW and SL. Pair-wise statistical differences for WW and SL were obtained at 15 and 30 DAR. Average (± SD)

WW in 15 DAR juveniles reared under weak aeration was higher than in those submitted to strong aeration (19.7 ± 1.4 and 14.7 ± 1.6 mg, respectively); such differences were also found in 30 DAR juveniles (48.9 ± 1.9 mg and 32.8 ± 1.9 mg, respectively). Similarly, seahorses reared under weak aeration showed higher SL than those reared under strong aeration both at 15 DAR (23.4 ± 0.4 mm and 22.1 ± 0.5 mm, respectively) and 30 DAR (32.3 ± 0.6 mm and 29.0 ± 0.6 mm). However, survival rates clearly showed an opposite pattern (One-way ANOVA $F_{1,4} = 11.09$, $p = 0.03$).

Average survivals (± SD) in 15 DAR juveniles from strong aeration aquaria were significantly ($p < 0.001$) higher (71 ± 15 %) than those raised from weakly aerated aquaria (40 ± 6 %). The same finding was observed at 30 DAR (41 ± 12 and 13 ± 0 % for strong and weak aeration, respectively). Differences in survival rates among treatments occurred from 10 DAR onwards ($p < 0.05$).

4. Discussion

The results achieved in the present study have demonstrated that the best performance in the early rearing of seahorses, especially in terms of survival rates, was achieved in pseudoKreisel aquaria under a strong aeration and, to a lesser extent, a day-night photoperiod regime. Clearly, aquaria shape and aeration were pivotal factors interfering with juvenile viability.

The design of aquaculture rearing aquaria should consider some basic principles such as good water mixing, solids removal, minimal stagnant regions and even distribution of food and fish in the media, among others (Cripps and Poxton, 1992). Despite even fish distribution is related to rearing aquarium design (Duarte et al., 2011), there are no previous studies on the survival and performance efficiency of different aquaria shapes in the rearing of seahorses. Our results showed a clear effect of aquaria design on survival of seahorse juveniles. The three types of aquarium tested in the present study differed in shape and also in other characteristics. Darkening different zones of the aquaria has been widely used to reduce upper lightening and maintain phototactic prey away from the surface, which may help to achieve an even fish distribution (Martinez et al., 2005; Moore et al., 1994; Naas et al., 1996). On this regards, rectangular as well as pseudoKreisel aquaria were wall-blacked in the upper half of the aquaria to help with the food and seahorse juveniles (positive phototactic response) on their migration to deeper regions of the water column. On the contrary, due to the reduced water surface of the circular aquaria, their rounded walls were not darkened.

Another important difference among the experimental aquaria was the placement of the water inflow and outflow which could explain the differences among aquaria designs. Spherical and pseudoKreisel aquaria had the inflow placed outside but near the water surface (attached to an aquarium wall) providing a water-cascade and/or a circling water current. Additionally, the opposite placement of the water inlet and the aeration pipe in those aquaria could be related to an improvement of the water circulation and the distribution of prey items and seahorse juveniles. On the contrary, the water inlet in the rectangular type was placed below the water column, inside a protection tube, and the water was projected through a small hole (see Figure 1.1). The circular water flow in spherical aquaria and the top-down flow in rectangular aquaria did not perform differently considering juveniles survival. However, poor water mixing conditions and non-adequate prey/juveniles distribution was observed in the later by creating irregular and unpredictable flow patterns, which is in agreement with Oca et al. (2004). This resulted in earlier mass mortalities (>50% at 15 DAR). Circular water flow in spherical aquaria with a unique water jet might also difficult a uniform fish distribution (Rosenthal, 1989) which might have increased mortalities thorough the experiment (> 60% at 30 DAR).

Changes in physiology, activity and behaviour of fish have been related to poor water mixing (Duarte et al., 2011). Vertical circulation patterns have been reported as beneficial in seahorse rearing by preventing juveniles from becoming trapped at the water surface (Gomezjurado and Gardner, 2005). The use of PseudoKreisel aquaria have been described to alleviate surface overcrowding in jellyfish (Rackmil et al., 2009; Widmer et al., 2005) or lobsters (Tlusty et al., 2005). Additionally, pseudoKreisel aquaria, similar to those used here, has also been reported in the rearing of other seahorse species such as *H. ingens*, *H. erectus* and *H. reidi* (Gomezjurado, 2005; Gomezjurado and Gardner, 2005; Martínez et al., 2005, Burhans, 2004). The design of the pseudoKreisel aquaria used in the present study hugely improved both water circulation and fish distribution avoiding the accumulation of juveniles in stagnant regions and, therefore, the development of swim bladder hyperinflation and further death of the juveniles affected. As a consequence, a huge improvement in the rearing of seahorses and a high final survival (up to 77%) was achieved when compared to the other two aquaria designs under either natural-like or extended photoperiod. The survivals reached in the present study are notoriously higher than those previously reported (up to 25%) in the species by other authors (Faleiro and Narciso, 2010; Palma et al., 2011).

Most problems related to seahorse rearing are related to feeding issues or fish diseases (Olivotto et al., 2008; Planas et al., 2010; Wilson and Vincent, 2000). Seahorse newborn are completely developed and ready to swim and feed. The appearance of dysfunctions in swim bladder inflation (hyperinflation) and the ingestion of air bubbles have been strongly related to the photoperiod regime applied in phototactic marine fish larvae, being the origin of high mortalities (Fielder et al., 2002; Nash et al., 1977; Stuart and Drawbridge, 2012; Woods, 2000). Day-night cycles are highly related to fundamental biological endogenous and exogenous rhythms caused by the pineal organ hormonal secretion (Campagnolo and Nuñer, 2008). Modifications of the factors that influence on the hormonal rhythm secretion affect to fish larvae development and compromise the survival and performance of young fish (Campagnolo and Nuñer, 2008). Olivotto et al. (2008) pointed out that *Hippocampus reidi* juveniles cultured in rectangular aquaria on a mixed diet including copepods performed and survived better when exposed to extended photoperiods than juveniles reared under natural photoperiod conditions. On the contrary, survivals in *H. abdominalis* reared under a 16L:8D cycle did not differ from those achieved under either extended or shortened photoperiods, though juveniles grew better under a day-night cycle (Martinez-Cardenas and Purser, 2012). In the present study, no differences were found on survival or performance of *H. guttulatus* juveniles when comparing extended and natural photoperiod regimes; however, in spherical aquaria, a 16L:8D photoperiod resulted in better juvenile performance both in survival and mass gain. The natural photoperiod was selected as the best rearing light regime since it is the photoperiod commonly applied for most of the whole rearing period. In addition, potential detrimental effects on large juveniles were not tested and we assumed (also based on some preliminary observations) that the natural like photoperiod would be advantageous.

Under culture conditions, the feeding ability of active visual predator fishes has been also widely related to the photoperiod regime (Eshagh-Zadeh et al., 2013; Olivotto et al., 2008; Vallés and Estévez, 2013). In fact, Sheng et al. (2006) observed active feeding and swimming in *H. kuda* during the day-light period but reported abnormally floating juveniles at night. Similarly, a reduction on the activity of *H. abdominalis* at night has been also reported even in 24h of light extended photoperiods (Martinez-Cardenas and Purser, 2012). However, hunting activity in marine fish larvae increases proportionally with the extension of the light period and the food availability which may increase energy expenditure for foraging and swimming (Fielder et al., 2002; Stuart and Drawbridge, 2012). The results achieved on growth and survival of *H. guttulatus* juveniles under both natural and extended photoperiod regimes might be explained through the imbalance between the ingested and mobilized energy. Under a continuous light regime, there is a compromise between the energy expenditure for actively feeding activities and the energy allocated for growth. Under a day-night cycle, however, the energy saved at night due to a reduced locomotion activity might be used for growth, which would be advantageous, especially during the first critical days when the efficiency of food digestion and assimilation is still scarce (Cunha et al., 2007; Blanco et al., 2011, 2013).

Water turbulence is also closely related to energy expenditure for swimming, hunting, feeding and growth (Nash et al., 1977; Oshima et al., 2009; Utne-Palm and Stiansen, 2002). Seahorse specific daily growth rate is enhanced under lower water turbulence environments, which is very likely due to an increasing difficulty for capturing prey in strong water currents (Oshima et al., 2009; Utne-Palm and Stiansen, 2002).

It has been pointed out that survival rates in *H. hippocampus* were quite low (11%) under strong aeration conditions, yet total early mortalities in juveniles reared under moderated aeration were also recorded (Molina et al., 2007). Similar trends were obtained in the present study, in which significantly higher survival rates were achieved in the early rearing of *H. guttulatus* under strong aeration conditions when compared to those raised under a weak aeration, especially from 15 DAR onwards. Final survivals at 30 DAR in strongly aerated aquaria were three-folds higher than in aquaria with weak aeration (41 ± 12 and 13 ± 0%, respectively). However, growth rates were lower in the former, which is in agreement with the statement of higher energy expenditure in more turbulent environments.

Strong turbulence has been considered a mortality causative factor due to potential physical hitting of fish larvae (Sakakura et al., 2007). However, the bony plated skeleton in seahorses as well as their vertical positioning provides them a hardness shielding against impacts and crushing (Porter et al., 2013). In our study, seahorse juveniles under a strong turbulence did not suffer mechanical damages, showing much higher survivals and lack of swim bladder hyperinflation. It has been reported in young fish that high turbulence conditions promote a reduction in swimming activity and avoids their accumulation near water surface (Sakakura et al., 2006; Utne-Palm and Stiansen, 2002). In agreement with that, in the present study *H. guttulatus* juveniles were observed to swim passively due to the water current generated by big bubbling under strong aeration conditions. More developed juveniles were found to be better distributed in the aquaria and actively swimming against the water currents provided by a strong aeration.

The presence of swim bladder hyperinflation has been recently related to the type of first prey supplied during early rearing of *H. guttulatus* (Palma et al. 2013). Prey quality might have influence on the presence of swim bladder hyperinflation, especially when nutritional requirements are not fully met and the energetics of the juveniles is below an optimal level. However, our result suggest that the type of aeration have very likely much more impact on the appearance of swim bladder hyperinflation in young seahorses. These advanced teleost fish are physoclistus, lacking an air transportation duct connecting the lumen of the gas bladder to the external environment. Consequently, they must gulp air at water surface to inflate the swim bladder (Anderson and Petty, 2013; Fielder et al., 2002). Swim bladder inflation in *H. guttulatus* generally occurs during the first hours of life and under adequate conditions all juveniles have the swim bladder normally developed by 1-2 DAR. When present, abnormal swim bladder hyperinflations commonly appeared from day 5 to 10, with juveniles floating passively at water surface, but most affected juveniles died in the following days mainly due to difficulties in prey capture. Swim bladder hyperinflation is a serious and a common problem that may drastically affect to rearing viability, being present when young juveniles with normally developed swim bladders remain for long time near water surface. Conversely to juveniles reared under strong aeration in pseudoKreisel aquaria, the cultures carried out in the present study under weak aeration conditions clearly enhanced the development of hyperinflated swim bladder in juveniles, resulting in considerably lower final survivals.

5. Conclusions

Considering the whole results achieved in the present study on the rearing of juveniles of the seahorse *Hippocampus guttulatus* under different aquaria types, photoperiod regimes and aeration conditions, we recommend for better rearing performances that the early culture be carried out in pseudoKreisel aquaria with strong aeration and a day-night light cycle (16L:8D). Under those conditions, the appearance of swim bladder hyperinflation resulted drastically reduced and the final survivals were radically increased.

Acknowledgements

The study was financed by Project CGL2009-08386 (Spanish Government) and the Regional Government of Galicia (Xunta de Galicia, Project 09MDS022402PR). A. Blanco was supported by a PhD JAE-Pre Grant (Junta para la Ampliación de Estudios Program) from the Spanish National Research Council (CSIC), co-financed by the European Social Fund. We are grateful to S. Valladares and C. Ofelio for their support in seahorse rearing, C. Peake for his help on statistical analyses, and A. Lasen for the drawing of the aquaria design.

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Table 1. Survival, specific daily growth rate and Fulton's condition index (K_F) of seahorse juveniles *Hippocampus guttulatus* under the different experimental conditions of photoperiod, aquaria design and aeration levels.

	pseudoKreisel		*Spherical*		*Rectangular*		*Aeration*	
	16L:8D	*24L:0D*	*16L:8D*	*24L:0D*	*16L:8D*	*24L:0D*	*Strong*	*Weak*
Survival 30 DAR	60±16	63±14	38±1	21±32	17±18	15±6	41±12	13±0.4
Growth 0-30 DAR	9.4	8.9	11.3	9.5	9.0	8.8	6.2	7.9
K_F 0-30 DAR	2.3	2.0	2.8	3.6	3.0	3.3.	3.1	5.1

Table 2. Analyses of variance (3-way ANOVA) on the effects of the photoperiod, aquaria design and age (DAR) in the survival of *Hippocampus guttulatus* juveniles

Source	*df*	*Squared mean*	*F*	*Significance*
Experiment 1				
Aquaria Type	2	6988	40.50	0.000
Photoperiod Regime	1	3506	20.32	0.000
DAR	3	9264	53.69	0.000
Aquaria x Photoperiod	2	1095	6.35	0.000
Aquaria x DAR	6	1001	5.80	0.004
Photoperiod x DAR	3	285	1.65	0.189
Aquaria x Photoperiod x DAR	6	185	1.08	0.390
Error	48	172		
Experiment 2				
Aquaria Type	2	6988	40.50	0.000
Photoperiod Regime	1	3506	20.32	0.000
DAR (Age)	3	9264	53.69	0.000

Figure Captions

Figure 1. Aquaria used in the rearing of seahorse juveniles. Left: 3D drawing, Right: Detailed drawing. 1) Rectangular aquarium; 2) Spherical aquarium; 3) PseudoKreisel aquarium. A) Water outlet; B) Screened mesh (B1: 500 μm and B2: 250 μm); C) Aquaria wall; D) Air pipe; and, E) Water inlet.

Figure 2. Survival rates in seahorse juveniles grown under different conditions. Experiment 1 (A-E): Effect of aquaria type under two photoperiod regimes (A: natural photoperiod 16L:8D; B: extended photoperiod 24L:0D) and effect of photoperiod regimes on three aquaria types (C: Rectangular; D: Spherical aquaria; E: PseudoKreisel). Experiment 2 (F): Effect of aeration (strong and weak) in pseudoKreisel aquaria.

Figure 3. Wet weight (WW) and standard length (SL) relationships in A) Experiments 1: Photoperiod – Aquaria design, and B) Experiment 2: Aeration level.

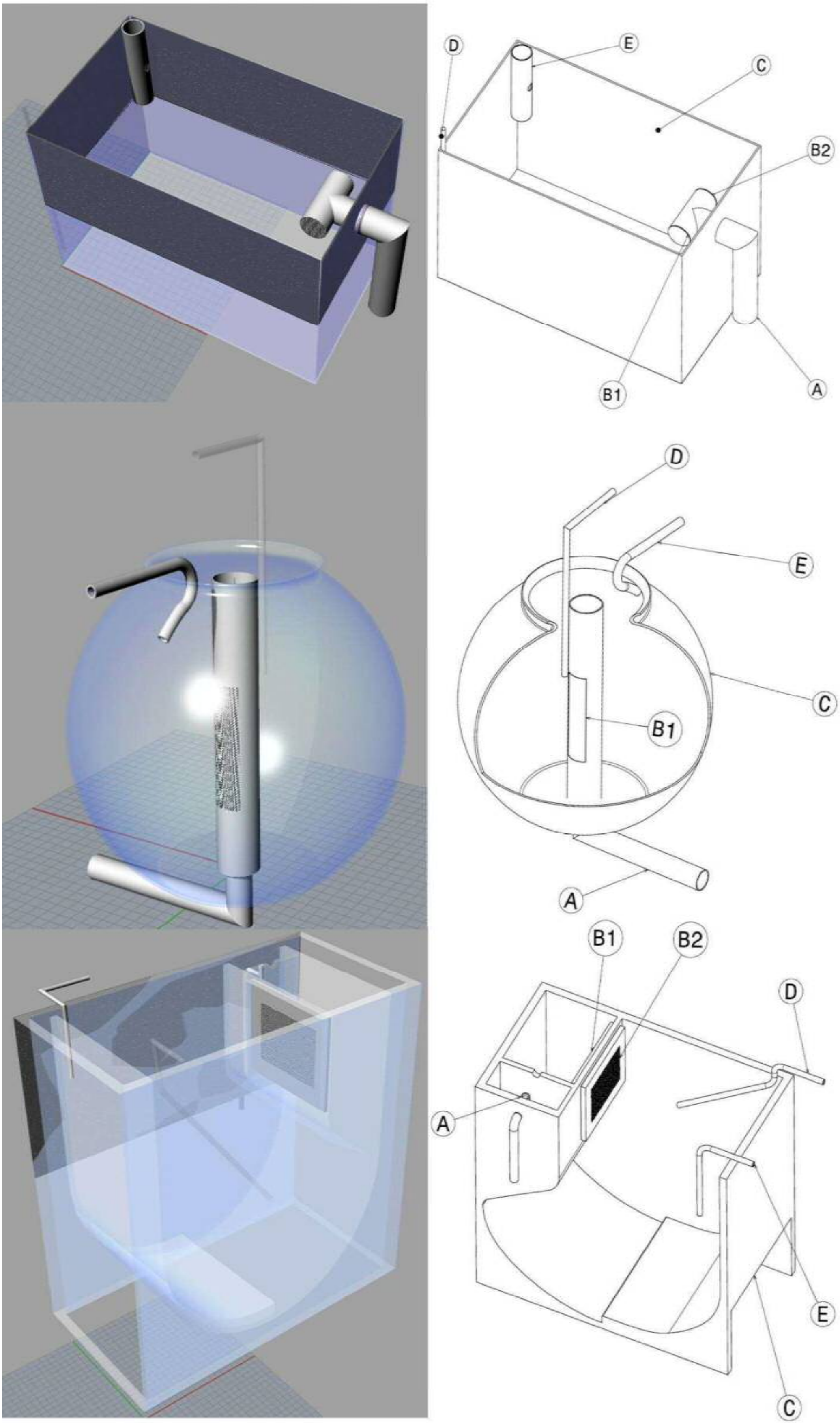

Figure 2

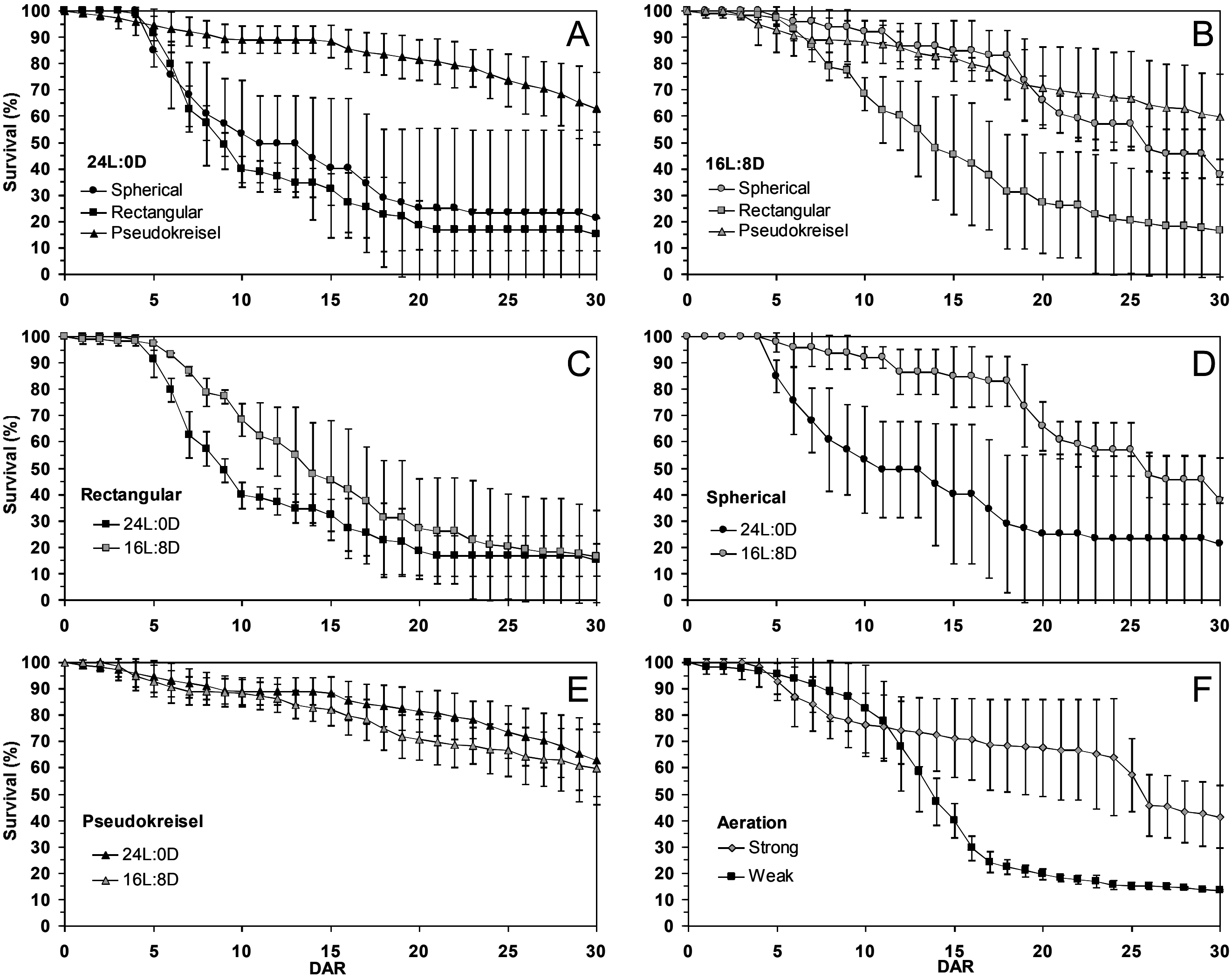
A
24L:0D
Spherical
Rectangular
Pseudokreisel
B
16L:8D
Spherical
Rectangular
Pseudokreisel
C
Rectangular
24L:0D
16L:8D
D
Spherical
24L:0D
16L:8D
E
Pseudokreisel
24L:0D
16L:8D
F
Aeration
Strong
Weak
Survival (%)
DAR
0
10
20
30
40
50
60
70
80
90
100
0
5
10
15
20
25
30

Figure 3

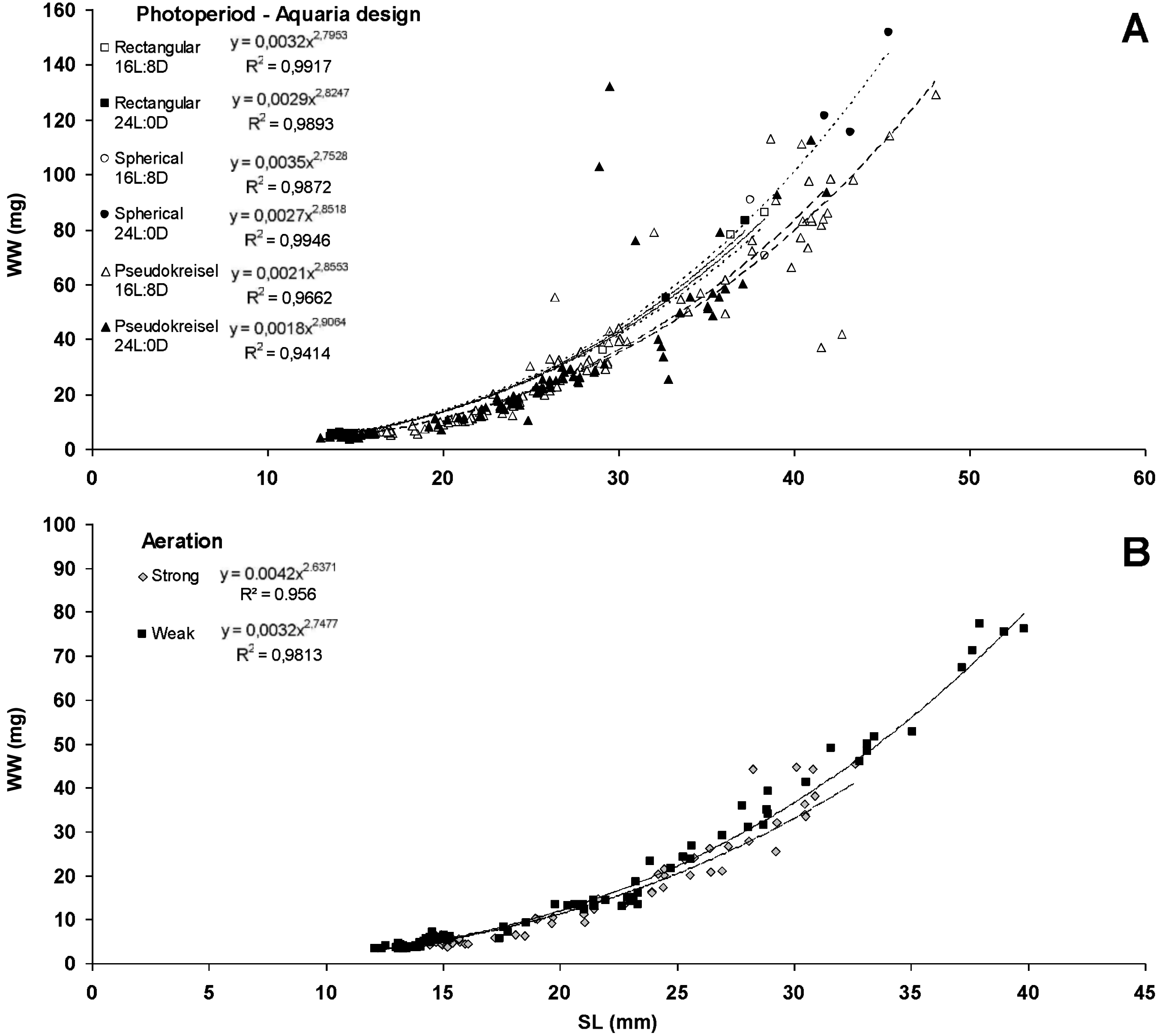
A
Photoperiod - Aquaria design
□ Rectangular 16L:8D
y = 0,0032x^2,7953
R^2 = 0,9917
■ Rectangular 24L:0D
y = 0,0029x^2,8247
R^2 = 0,9893
○ Spherical 16L:8D
y = 0,0035x^2,7528
R^2 = 0,9872
● Spherical 24L:0D
y = 0,0027x^2,8518
R^2 = 0,9946
△ Pseudokreisel 16L:8D
y = 0,0021x^2,8553
R^2 = 0,9662
▲ Pseudokreisel 24L:0D
y = 0,0018x^2,9064
R^2 = 0,9414
WW (mg)
0
20
40
60
80
100
120
140
160
0
10
20
30
40
50
60
B
Aeration
◇ Strong
y = 0.0042x^2.6371
R² = 0.956
■ Weak
y = 0,0032x^2,7477
R^2 = 0,9813
WW (mg)
0
10
20
30
40
50
60
70
80
90
100
0
5
10
15
20
25
30
35
40
45
SL (mm)

Figure 4