Growth performance of the early life stages of broad-nosed pipefish, *Syngnathus typhle* (L.) fed different natural diets

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Abstract

This work, divided in two experiments, aimed to test the effect of using different live and frozen feed on the growth performance of *Syngnathus typhle*. In experiment I, *Artemia* and Atlantic ditch shrimp (*Palaemonetes varians*) larvae were used as live diets, whilst in experiment II, frozen mysids *Mesopodopsis slabberi* and frozen *P. varians* were used. At the end of the first experiment, Juvenile pipefish grew significantly more when fed *P. varians* ($P<0.05$) with an overall Weight Gain (WG) of 914.8 ± 79.3% bw d^{-1}, when comparing to *Artemia* fed fish (WG = 683.2 ± 14.7%). Both Mean Specific Growth Rate (SGR) and survival where similar between dietary treatments and did not vary significantly ($P>0.05$). In the experiment II, juveniles were weaned to frozen diets but no significant differences were found between the two tested diets in all parameters tested. Final WG was 516.5±63.3% and 566±17.6%, and Feed Conversion Ratio (FCR) was 30±1.5% and 28.2±1.2%, for animals fed *P. varians* and *M. slabberi*, respectively. Results indicate that *P. varian*s is an adequate diet to feed initial stages of *S. typhle* life cycle and should be considered as a frozen diet for subsequent life stages of this species, as an alternative to currently known natural diets.

Keywords: *Syngnathus typhle*; natural diets; *Artemia*; shrimp*;* mysids

Introduction

The Syngnathidae are considered to have exceptional reproductive behaviour as developing embryos are reared on the male's ventral surface (Vincent *et al.* 1992). Males are responsible for the fertilization, carrying and incubation of the eggs in a brood pouch. Syngnathid fish, including seahorses, seadragons and pipefish, attract

economic interest for their value on the ornamental fish trade and as ingredients in traditional Chinese medicine (Payne *et al.* 1998).

Traditionally, the trade of Syngnathid fishes as ornamental fish has been mainly dependent of their collection in the wild, which may lead to significant impacts on local communities. Such impact may be also due to habitat degradation, which has resulted in the extinction of at least one Syngnathid species (Whitfield 1995). Therefore, several authors have tried intensive commercial cultivation of some species of Syngnathids (Xu 1985, Prein 1995, Payne et al. 1998). The inclusion of all *Hippocampus* species and 27 pipefish species in the Appendix of CITES (2004), the increased conservation awareness and the significant breakthroughs in Syngnathids cultivation techniques have encouraged the interest to breed these species in captivity. However, most of the Syngnathid breeding research is mostly focused on *Hippocampus* species (Wilson and Vincent 2000, Woods 2000, Palma *et al.* 2008b, Lin *et al.* 2009, Palma *et al.* 2011).

The broad-nosed pipefish *Syngnathus typhle* generally inhabits shallow waters (up to 20 meters depth) in algae/*Zostera* beds. It has been recorded in the coastal waters of Europe, from Norway to Morocco, in the Baltic Sea, Azov Sea, Black Sea and in the Mediterranean Sea (Dawson 1986). This species presents a colour range from light green to dark brown (Dawson 1986, Ahnesjo 1996), and is considered to be a predator of limited mobility, feeding mainly on small crustaceans such as mysids and shrimps (Ryer and Orth 1987, Campolmi *et al.* 1996, Berglund and Rosenqvist 2003). In addition, copepods have been reported to be the main food items in the early life stages of most Sygnathid species (Ryer and Orth 1987, Tipton and Bell 1988, Franzoi *et al.* 1993, Teixeira and Musick 1995), including *S. typhle* (Oliveira *et al.* 2007). Therefore, it is assumed that when rearing this species, an abundant variety of organisms must be provided. Knowledge on the nutritional requirements of juvenile Syngnathids is scarce, and no optimal feed as yet been reported for this species. Culture of live food is generally expensive and their collection from the wild is unreliable, non-sustainable and may have deleterious effects on the natural ecosystems. In alternative, due to its high survival rate and the possibility to be weaned with artificial diets during the larval stage (Palma *et al.* 2008a, Palma *et al.* 2009), *P. varians* can be produced as an alternative live diet for aquaculture. *P. varians* has been successfully used to improve the condition of wild captured *Hippocampus guttulatus* (Palma et al. 2008b) and the different life stages can be used to feed different sized *H. guttulatus* juveniles (Palma et al. 2011) .

To our knowledge, no artificial diet has yet been tested to feed this species. Thus, this study aimed to test the effectiveness of using different live and frozen natural diets on the growth performance of the early stages of the broad-nosed pipefish *S. typhle*.

Material and Methods

Feeding experiments

Two experiments were conducted using captive bred F3 *S. typhle* juveniles obtained from a natural breeding broodstock kept at the Ramalhete Aquaculture Field Station of the University of the Algarve (South Portugal). After hatching and until the beginning of the experiment I, fish were kept in the same rearing conditions. Animals were daily fed with newly hatched *Artemia* nauplii (Sanders®, Ogden, Utah, USA) (2000 nauplii l^{-1}) until the beginning of the experiment. *Artemia* was hatched according to the proceedings described by Lavens & Sorgeloos (1996).

In experiment I, 108 10-day-old *S. typhle* (Mean Weight = 0.048 ± 0.004g) were randomly selected from the captive population referred above and allocated in 6 replicate tanks (18 animals per tank). Experiment I was conducted in a flow-through culture system composed of six plastic rectangular tanks (38 x 28.5cm) with 12cm water depth, 10 l volume (Fig.1) and constant water inflow of 10 l h^{-1} per tank and moderate aeration. After continuous sand and biological filtration, seawater flowed into the tanks through a black polystyrene tube placed approximately 3 cm from the water surface. The water outflow structure was assembled in one of the corners of the tanks and was composed of a PVC tube covered, at the water surface, with a mesh screen of 150µm diameter to prevent feed flush. Tanks were illuminated from above with fluorescent light (2×18W white tubes), with an intensity of 425.5 ± 20.5 lux at the water surface and a photoperiod controlled by a timer at 12L:12D (8am – 8pm). Temperature and salinity were kept at 18.5 ± 0.5 ºC and 36 ± 1 ‰, respectively. Water quality parameters remained stable throughout the experiment. Ammonia values were always below detectable levels, nitrate <0.3 mg L^{-1} and nitrite <1.25 mg L^{-1}.

In 3 replicate tanks, animals were fed live *Artemia* metanauplii until day 20 and juvenile *Artemia* until the end of this experiment. In the remaining 3 replicate tanks, fish were fed *P. varians* larvae.

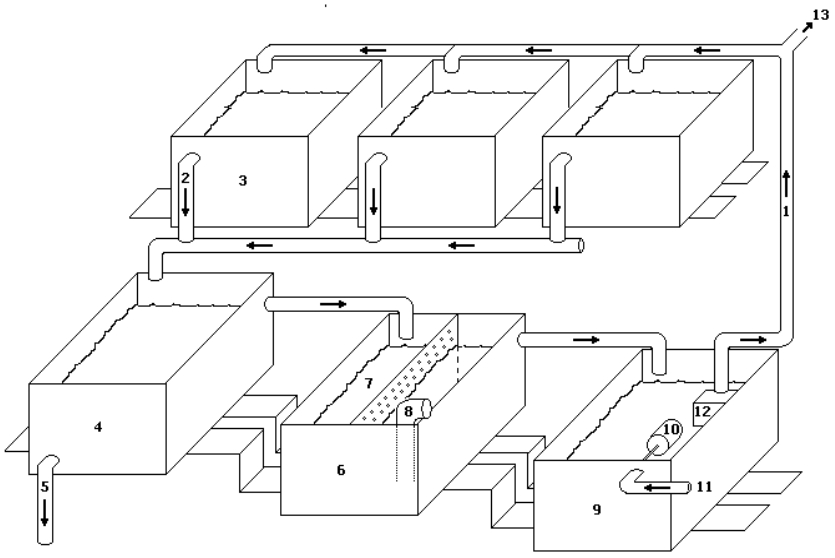

Fig. 1 – Rearing system representing the schematics of the experiment tanks; (1) inflow pipes; (2) outflow pipes; (3) rearing tanks; (4) settling tank; (5) outflow during semi-open system; (6) filtering tank; (7) bio-filter; (8) protein skimmer, (9) reservoir tank; (10) leveller; (11) inflow during semi-open system after passing through a ultra-violet light filter; (12) water pump; (13) inflow to the other rearing tanks.

Artemia metanauplii (24 to 48 hour old) were daily enriched with 150 mg L^{-1} of *Spirulina* (New Era Aquaculture, Lda., Thorne, UK) for 24 h in two 20 L acrylic cylindrical-conical tanks at a maximum concentration of 50.000 *Artemia* L^{-1} held at room temperature (20–22 °C) and under constant moderate aeration. *P. varians* larvae were obtained from ovigerous females kept in two 40 L square fiber glass tanks assembled in a flow through system (temperature and salinity, respectively, 23.3±0.5 °C and 37.1±0.1‰) and fed an artificial dry shrimp diet (CP, 39%, L, 8%). Juvenile *Artemia* (sized 3mm approximately) used from day 20 onwards was grown out using *Spirulina* as described above. Both diets were provided enough quantity to ensure *ad libitum* conditions (aprox. 5000 metanauplii L^{-1} and further on 1000 juvenile *Artemia* L^{-1}; and 20 *P. varians* larvae L^{-1}). This experiment lasted for 60 days. Faeces and other debris were removed from the tanks by siphon method on a daily basis. Any dead animals were removed and mortality registered.

In experiment II, 60 juveniles (Mean Weight = 0.487 ± 0.052g), aged 60 day-old, were randomly selected and allocated into 6 tanks, obtaining 3 replicate per treatment with a final density of 10 animals per tank. These fish were breed from hatch to 60

days-old in a 90 litre tank and fed *Artemia* (as described above) until 10 day-old followed by live *P. varians larvae* until the beginning of experiment II. Experiment II was conducted in a flow-through culture system composed of six fibreglass square tanks (46 x 46cm) with 25cm water depth, 40 L volume and constant water flow of 20 l h^{-1} per tank. All the remaining experimental design was the same as described above for the first experiment.

In both treatments, animals were fed with a daily feed ration of 10% of their wet bodyweight at 09:00 and 15:00 h, with each treatment's feed ration divided equally amongst these two feedings (5% at each feeding). This value was chosen based on preliminary visual observation of consumed food. Following each sampling, the daily wet weight of each ration was adjusted according to the average wet weight increase for each treatment, to maintain the appropriate feed rations (i.e. 10 day ration adjustment). Each feed ration adjustment was done to provide *ad libitum* conditions. After three hours, any food remaining was collected, dried (24h at 65ºC) and subtracted from the offered amount. Shrimps (*P. varians*) and mysids (*M. slabberi*) used in experiment II were all captured in a single fishing event in the surrounding ponds of the Ramalhete Aquaculture Field Station. *P. varians* ranged in length from 10 to 15 mm, whereas *M. slabberi* ranged between 10 and 12 mm in. Diets were immediately frozen and kept at -18ºC until use. The daily ration of each natural diet was thawed in seawater prior to use. After defrosting, the water was drained and the diets were gently dried on paper towel, weighed and then supplied to the animals.

All the remaining feeding protocol was the same as described above for the first experiment. Experiment lasted for 40 days and sampling was done at 10 day intervals.

Proximate analysis

Analyses were performed on 100 g samples of shrimp (*P. varians*), mysids (*M. slabberi*) and *Artemia*. Analyses were performed on triplicate sub-samples from the 100 g sample. Diet samples were analyzed for dry matter and ash contents according to the methods of AOAC (1995), crude protein (N × 6.25) by Kjeldahl method using a Kjeltech auto-analyzer (Model 1030, Tecator, Höganäs, Sweden), and total lipid by petroleum ether extraction using a XT20 ANKOM analyzer (Ankom Technology, Macedon, NY).

Data analysis

All fish were sampled every 10 days and collected data was used for the following calculations: Mean Specific Growth Rate (SGR) (% bw d^{-1}) = ((lnW_2-lnW_1)/t*100), where W_2 and W_1 are the final and initial weights of the pipefish, respectively, *ln* the natural logarithm and *t* the number of days of the time period; Feed Conversion Ratio (FCR) (%) = (B_f-B_i)*100/TFO, where B_f-B_i is the total biomass gained by the pipefish during the entire experiment and TFO the total wet feed offered for that same period of time (this was only calculated in experiment II) ; Weight Gain (WG) (%) = (F_w-I_w)/ I_w*100, where F_w is the final mean wet weight of pipefish in each replicate and I_w the initial mean wet weight; Survival was calculated for each sampling, using the expression Survival (%) = (n_t-M_t)/n_t*100, where n_t is total number of individuals at the beginning of the experiment, and M_t, number of dead animals recorded for that time period. Comparisons (one-way ANOVA; Zar, 1999) were done using all individual weights from each replicate in each treatment of the same diet tested. If during that period no differences were found in the three replicates of each density, all weights of the three replicates were pooled and *t*-test (Zar, 1999) was used to compare weights of all individuals in the two treatments. In all statistical procedures, data was tested for normality and homogeneity. Whenever one of these requisites was absent, alternative non-parametric Mann-Whitney tests (Zar, 1999) were used.

Results

Experiment I

At the end of the experiment, *S. typhle* fed *P. varians* larvae grew significantly more ($P<0.05$) attaining a final wet weight of 0.48 ± 0.06g when comparing to juvenile fed *Artemia* (0.38 ± 0.04g). This corresponded to a WG of 683.2 ± 14.7% and 914.8 ± 79.3% for animals fed *Artemia* and *P. varians*, respectively and was significantly different between groups ($F=24.715$; $P=0.008$). Data on the Standard Length, Daily Length Increase (DLI), Wet Body Weight, WG, SGR and survival are reported in Table 1.

Average growth rates (SGR) were respectively 3.4 ± 1.1% and 3.6± 1.3% bw d^{-1} for juvenile fed *Artemia* and *P. varians* (Fig.2). Final mean length was 110.8 ± 3.1 mm and 120.1 ± 5.2mm, which corresponds to a DLI of 0.98 ± 0.04mm d^{-1} and 1.25 ± 0.07mm d^{-1}, for juvenile fed *Artemia* and *P. varians*, respectively.

Table 1: Standard length (cm), Daily Length Increase (DLI) (mm d^{-1}), Wet Body Weight(g), Weight Gain (WG), Specific Growth Rate (SGR) and Survival (%) of juvenile *S. typhle* during the 60 day study.

	T0	T10	T20	T30	T40	T50	T60
Artemia							
Standard length (cm)	4.217 ± 0.069	5.800 ± 0.069*	7.022 ± 0.048	8.196 ± 0.003	9.221 ± 0.191	9.706 ± 0.259	11.081 ± 0.313*
DLI (mm d^{-1})	---	1.583 ± 0.069*	1.223 ± 0.096	1.174 ± 0.051	1.024 ± 0.190	0.485 ± 0.101	1.375 ± 0.059*
Body weight (g)	0.048 ± 0.004	0.074 ± 0.006	0.101 ± 0.002	0.162 ± 0.010	0.203 ± 0.018	0.308 ± 0.028*	0.377 ± 0.035*
WG (%)	---	53.92 ± 23.72*	38.58 ± 10.65	59.90 ± 11.37	25.20 ± 7.94	52.14 ± 9.28	22.32 ± 1.61
SGR (% bw d^{-1})	---	4.23 ± 1.62*	3.24 ± 0.75	4.68 ± 0.71	2.23 ± 0.63	4.18 ± 0.62	2.01 ± 0.13
Survival (%)	100	100	96.30	87.04	85.19	83.33	83.33
P. varians							
Standard length (cm)	4.475 ± 0.080	6.745 ± 0.212*	7.573 ± 0.144	8.557 ± 0.236	9.426 ± 0.320	10.038 ± 0.306	12.002 ± 0.515*
DLI (mm d^{-1})	---	2.270 ± 0.279*	0.828 ± 0.178	0.984 ± 0.214	0.868 ± 0.493	0.613 ± 0.133	1.963 ± 0.210*
Body weight (g)	0.047 ± 0.003	0.097 ± 0.011	0.123 ± 0.004	0.180 ± 0.013	0.233 ± 0.017	0.331 ± 0.018*	0.475 ± 0.057*
WG (%)	---	108.64 ± 26.77*	27.34 ± 11.82	46.70 ± 14.68	29.67 ± 6.93	42.29 ± 4.27	42.91 ± 9.95
SGR (% bw d^{-1})	---	5.38 ± 1.32*	2.99 ± 0.92	4.74 ± 0.98	2.04 ± 0.53	3.83 ± 0.30	2.68 ± 0.71
Survival (%)	100	96.30	85.10	83.33	77.78	77.78	77.78

* Values were significantly different (P<0.05) between treatments within that period.

Survival was not significant different between the two tested diets (P>0.05). At the end of the experiment, survival recorded for juvenile fed *Artemia* and *P. varians* was 83.33 ± 14.70 and 77.78 ± 11.11%, respectively.

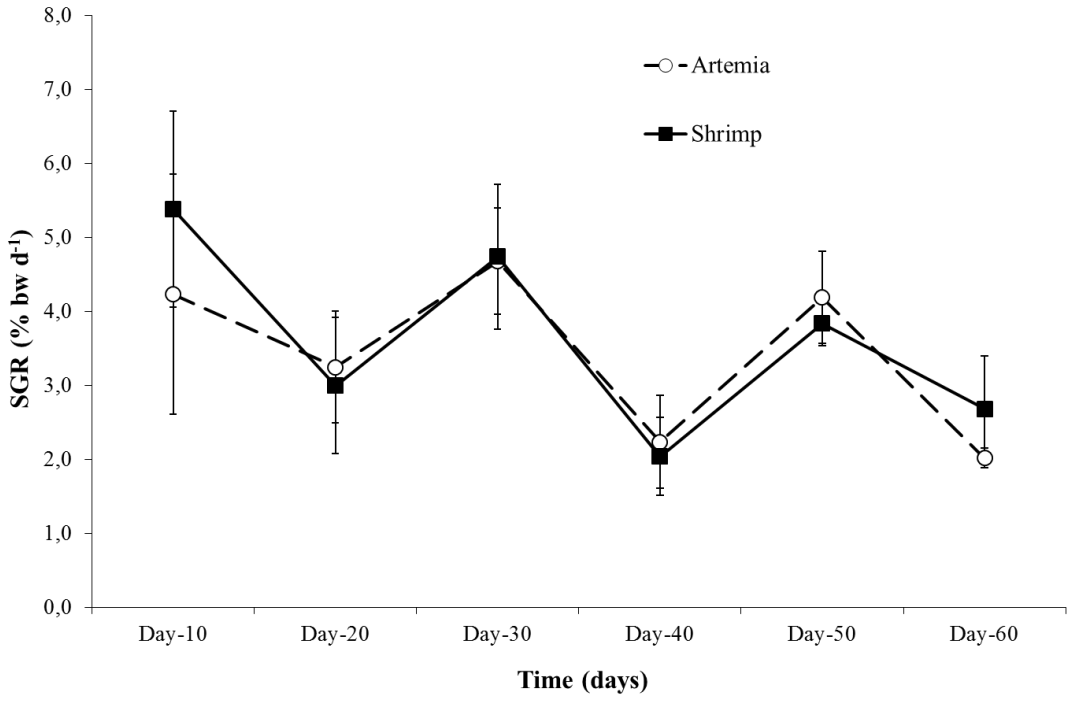

Fig. 2: Specific Growth Rate (SGR) (% bw d^{-1}) for pipefish fed *Artemia* and Shrimp (*P. varians*). Vertical lines represent standard deviations.

Experiment II

Data on the Standard Length, Daily Length Increase (DLI), Wet Body Weight, WG, SGR and survival is reported in Table 2. The average wet weight of *S. typhle* fed *P. varians* and *M. slabberi* at the end of the experiment was 3.1 ± 0.1 g and 3.2 ± 0.1 g, respectively. This growth corresponds to a weight gain (WG) of 516.5 ± 63.3% and 566 ± 17.6%, respectively and no significant differences were found between the two tested groups (F=1.707; P=0.261).

Table 2: Wet Body Weight(g), Weight Gain (WG), Specific Growth Rate (SGR) and Survival (%) of pipefish *S. typhle* during the 40 day study

	T0	T10	T20	T30	T40
P. varians					
Standard length (cm)	12.595 ± 0.990	14.819 ± 0.515	16.068 ± 0.424	16.949 ± 0.338	18.191 ± 0.416
DLI (mm d^{-1})	---	2.224 ± 0.754	1.250 ± 0.911	0.880 ± 0.753	1.242 ± 0.743
Body weight (g)	0.499 ± 0.004	1.153 ± 0.012	1.775 ± 0.071	2.373 ± 0.033	3.059 ± 0.028
WG (%)	---	132.08 ± 16.24	53.90 ± 11.37	33.80 ± 4.35	28.91 ± 4.70
SGR (% bw d^{-1})	---	8.40 ± 0.71	4.31 ± 0.34	2.91 ± 0.33	2.53 ± 0.36
Survival (%)	100	100	100	96.67	96.67
Mysids					
Standard length (cm)	11.925 ± 0.250	14.558 ± 1.053	16.649 ± 0.130	17.333 ± 0.381	18.318 ± 0.168
DLI (mm d^{-1})	---	2.633 ± 0.804	2.091 ± 1.180	0.685 ± 0.458	0.985 ± 0.418
Body weight (g)	0.475 ± 0.007	1.145 ± 0.055	1.821 ± 0.112	2.478 ± 0.145	3.162 ± 0.131
WG (%)	---	141.19 ± 8.39	58.90 ± 2.10	36.14 ± 0.52	27.72 ± 3.31
SGR (% bw d^{-1})	---	8.80 ± 0.35	4.42 ± 0.13	2.95 ± 0.04	2.56 ± 0.26
Survival (%)	100	96.67	96.67	96.67	83.33

* Values were significantly different (P<0.05) between treatments within that period.

Likewise, no significant differences were found on SGR (P>0.05) or FCR (F=2.564; P=0.185). SGR was 4.5 ± 2.7% and 4.7± 2.9% bw d^{-1} for pipefish fed *P. varians* and *M. slabberi*, respectively (Fig.3). FCR was 30 ± 1.5% and 28.2 ± 1.2% for animals fed *P. varians* and *M. slabberi*, respectively. Final mean length was 181.9 ± 4.2 mm and 183.2 ± 1.7mm, which corresponds to a DLI of 0.93 ± 0.21mm d^{-1} and 1.07 ± 0.03mm d^{-1}, for juvenile fed *P. varians* and *M. slabberi*, respectively.

At the end of the experiment, there was no significant differences between the survival of juveniles fed the two dietary treatments (P<0.05), with a mean survival of 96.67 ± 5.77 in juveniles fed *P. varians*, compared with 83.33 ± 5.77% of those fed *M. slabberi*. In one replicate tank (mysid treatment), 3 animals were lost (day 30) when the water inflow line was accidentally knocked out overnight. As this was not related to the experiments, this loss was not included in the survival analysis.

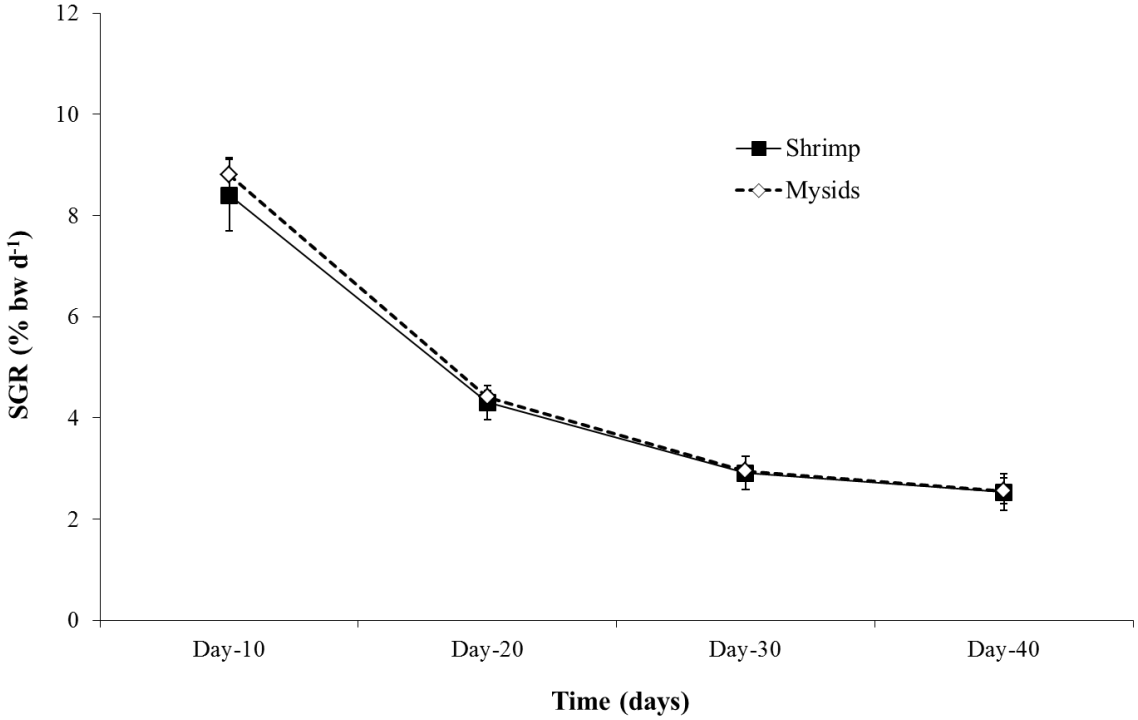

Fig. 3: Specific Growth Rate (SGR) (% bw d^{-1}) for pipefish fed Shrimp (*P. varians*) and Mysids (*M. slabberi*). Vertical lines represent standard deviations.

Proximate analysis

The proximate composition of the three diets used in this study is presented on Table 3. Shrimps had the lowest percentage composition of moisture and the highest in protein, carbohydrates and ash. Mysids had the higher percentage of fat, and Artemia the highest moisture composition.

Table 3: Proximate composition (%) of the 3 different feed used.

	Artemia	**Shrimp**	**Mysids**
Moisture	87.85	74.95	82.93
Fat	0.52	0.5	0.64
Protein	5.85	16.81	12.40
Ash	3.89	4.37	2.92
Carbohidrates	1.90	3.37	1.71

Discussion

Several authors reported copepods as the major food source of *S. typhle* diet in the wild (Franzoi et al. 1993, Teixeira and Musick 1995, Oliveira et al. 2007). In fact, these can represent up to 80% of *S. typhle* natural diet, followed by shrimp (Hippolytidae) 5%, and mysids (Mysidacea) 4% (Oliveira et al. 2007). Although the successful use of copepods as food source for fish larvae has been reported (Fukusho *et al.* 1980, Kuhlmann *et al.* 1981, van der Meeren 1997, Støttrup 1998) its use in aquaculture can be expensive, as they do not reach other live foods higher densities, thus requiring larger volumes of water for its production (Støttrup *et al.* 1986). The use of wild-caught copepods has been suggested has a possibility. Nevertheless, wild copepod populations are subjected to seasonal fluctuations and imply time-consuming efforts in collection and sorting.

In alternative, two of the main natural preys reported by Oliveira *et al.* (2007) were used in this study. Several authors indicated mysids as an important food source for many fish and crustacean species (Mauchline 1980, Lussier *et al.* 1988) including Syngnathids (Woods 2002, Palma et al. 2011) and despite the efforts made in the past few years to obtain a successful culture protocol (Kuhn 1991, Domingues *et al.* 1998, Domingues *et al.* 2000, Domingues *et al.* 2001), mysid culture remains unreliable. As mysids are predators, live *Artemia* has to be used as feed source, when culturing/maintaining this natural diet (Lussier et al. 1988, Kuhn 1991, Domingues et al. 1998). In contrast, *P. varians* has lower feeding demands, compared to mysids (Palma et al. 2008b, Palma et al. 2009) and has been successfully cultured in captivity feeding artificial diets since the early stages of its life cycle (Palma et al. 2009). Furthermore, *P. varians* has been reported as an alternative live diet for several marine species (Sykes *et al.* 2006, Palma et al. 2008b, Palma et al. 2009, Palma et al. 2011, Correia *et al.* 2008a) with encouraging results, when compared to other species. Unlike mysids, *P. varians* can be successfully reared in captivity with moderate costs (Palma et al. 2009). Also, if properly fed, its nutritional profile may be enhanced, which leads to significant improvement in the growth performance of the target species (Correia *et al.* 2008b). Thus, large scale production of *P. varians* is a promising area to be considered in live diet market. This fact configures it as a good quality item for large scale production for the live diet market.

In experiment I, the use of live *Artemia* and *P. varians* larvae showed that fish fed shrimp larvae had better growth performance (WG = 914.8 ± 79.3%) than those fed *Artemia* (WG = 683.2 ± 14.7%). This result emphasizes the usefulness of *P. varians* larvae as an adequate species to feed Syngnathids and agrees with Støttrup (2000) who refers that the poor quality of *Artemia*, even if enriched, when used to feed juvenile fish, may ultimately lead to significant nutritional deficiencies in the on growing species. Thus, the SGR values obtained in the first experiment were much higher than those reported for other Syngnathids (e.g. Woods (2005) for *H. abdominalis*) and similar to those reported by Woods and Valentino (2003) for *H. abdominalis* and Lin et al. (2009) for *H. erectus.* In the first experiment, the daily length increase obtained for animals fed the two diets tested (0.98 ± 0.04mm d^{-1} and 1.25 ± 0.07mm d^{-1}, for pipefish fed *Artemia* and *P. varians*, respectively) was similar to that reported for other Syngnathid species (Takahashi *et al.* 2003, Ripley and Foran 2006).

As in the ornamental fish market, the trade of Syngnathid fish is reliant on length rather than weight, *S. typhle* arise as a promising species, as they may reach market size (10 cm) at 2 month of age. At the end of the 60 days experiments, reared fish already attained the required minimum length for trade.

The nutritional requirements of Syngnathids, in protein and lipids, including highly unsaturated fatty acid (HUFA), are largely unknown. Payne and Rippingale (2000) and Payne et al. (1998) suggested that Syngnathids needs are similar to those of other marine fish. Feed fat content war similar for each diet tested which may indicate that this particular aspect might not be responsible for the growth difference observed. Also, the ≈ 1.7% difference in the carbohydrate content is unlikely to be responsible for the significance or un-significance between the results provided with three tested diets. Furthermore, in the crustacean group, chitin may be the primary source of carbohydrates and despite the apparent simplicity of carbohydrate digestive degradation when compared with protein digestion, animals typically can only digest glycogen and starches with endogenous enzymes and lack the enzymes required to degrade cellulose, chitin, and lignin (Dabrowski and Guderley, 2002). Unlike fat or carbohydrates, the protein percentage in the diets may be a plausible cause for the obtained results. Shrimp and mysids diets contained 16.8 and 12.4% protein in wet weight, respectively. Apparently, the ≈ 4% difference between the two diets did not promote significant differences in the growth parameters, but the ≈ 10% difference between the shrimp and

the Artemia diet is relevant since significant differences were found in all growth parameters between the animals fed these diets. Palma et al (2008b) also obtained similar results when testing these different diets in another Syngnathid fish (*H. guttulatus*).

Woods & Valentino (2003) emphasised the importance to wean Syngnathids onto non-live feeds as far more relevant than the mere demonstration of the usefulness of frozen mysids as promoters of good growth performance and high survival rates. The weaning process is of vital economic importance, since the use of non-live feeds reduces culture costs by decreasing space, material and labour costs involved in the production of live feeds. Also, the possibility to wean cultured Syngnathids onto non-live feeds, before sale in the aquarium trade, should significantly improve their chances of survival. These facts determined the choice of testing two frozen diets in this study, as they can be considered to be a more economical and viable solution than the current use of live diets. Results from the experiment II indicate that *S. typhle* can be successfully weaned from live to frozen diets at 2 months of age with no direct impact on the growth performance or mortality. This fact can greatly contribute to the successful inclusion of *S. typhle* in the ornamental fish trade, since the use of frozen diets can ultimately simplify this species daily care. Moreover adequate feeding will ensure good individual fitness. No significant differences were found in animals fed frozen diets, for any of the parameters tested, which indicates that *P. varians* and *M. slabberi* have the same impact on growth performance of *S. typhle*. However, the above mentioned sustainability of *P. varians* provides to this feed a practical advantage over *M. slabberi* in particular and over mysid shrimp in general.

After the end of these experiments, fish were moved to larger tanks and fed a mix diet of *P. varians* and *M. slabberi*, starting to breed at 5 month old, which led to the successful completion of 4 consecutive live cycles with no apparent negative consequences. This study was a consolidation of the rearing protocol of *S. typhle*. However, further investigation is necessary and should focus on the optimization of daily feed ration, broodstock management, juvenile enhancement and feed optimization.

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