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Effect of Temperature on Growth and Survival in Juvenile Opossum Pipefish, *Microphis brachyurus*: First Observations on the Species in Culture Conditions

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Abstract

In Mexico, natural populations of the opossum pipefish, *Microphys brachyurus*, are under pressure by anthropogenic alteration of their habitat and unregulated fishing as this species is traded as an aquarium fish, where its survival is low due to inadequate culture practices. The development of culturing techniques has been a successful strategy to protect many exploited species through providing an alternative source to wild exploitation. The aim of this study was to examine the effect on growth, condition, and survival of pipefish cultured for 6 wk at temperatures of 26, 28, and 30 C. Pipefish were fed enriched *Artemia* nauplii maintaining a ration rate of 14% body weight per day (dry weight *Artemia* : wet weight fish). The pipefish cultured at 26 and 28 C were longer, heavier, grew faster, and presented better condition than those at 30 C. The survival of pipefish cultured at 26 C was greater (83%) than at 30 C (30%) but not different than at 28 C (50%). This study is the first to report the acceptance of *Artemia* nauplii by juveniles of the species and that a temperature of 30 C is not suitable for juvenile *M. brachyurus* culture.

In Mexico, wild populations of native fishes are under pressure due to anthropogenic alteration of their habitat and the introduction of exotic species (Jelks et al. 2008). The opossum pipefish, *Microphys brachyurus*, is a subtropical estuarine fish that adapts to temperature and salinity fluctuations on a daily basis along the Gulf of Mexico. The subspecies, *Microphys brachyurus lineatus*, has been considered as a species of concern since 1991 in the USA by the National Oceanic and Atmospheric Administration. Natural populations of *M. brachyurus* are affected in Mexico by unregulated fishing as this species is traded as an

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aquarium fish without formal records, as occurs with other exploited syngnathid species around the world (Rosa et al. 2011). *M. brachyurus* often encounters suboptimal culture conditions in the average home aquarium, as it is usually maintained in tanks with conventional freshwater tropical fish. Its survival is compromised by suboptimal aquarium water temperatures and conventional fish diets (flakes, pellets, and frozen *Artemia*), whereas the species naturally preys on live items, mainly crustaceans and fish larvae small enough to be ingested by the characteristic reduced mouth of the Syngnathidae family (Miller et al. 2005).

The development of culturing techniques has been a successful strategy to protect many species. In teleosts, food consumption and food conversion efficiency can be improved by optimizing culture factors, such as temperature, which can lead to growth improvement (Jonassen et al. 2000). Temperature experimentation on fish can be focused on aspects such as temperature extremes (Tucker 1998) and optima for species (Katersky and Carter 2005; Abbink et al. 2012), as a certain temperature can benefit growth but not survival (Barron et al. 2012). Temperature not only affects food intake and nutrient assimilation toward better growth and survival but it can also have an impact on the risk of bacterial diseases (Landis et al. 2012) and parasite infestation (Jee et al. 2001).

The initial allocation of fish to water temperatures used in experiments has been conducted using techniques such as direct transfer (McCarthy et al. 1998; Sheng et al. 2006; Silva et al. 2006) or the gradual acclimation (Suneetha et al. 1999) at asymmetric rates (Aune et al. 1997; Jonassen et al. 2000) or using equal transfer rates (Katersky and Carter 2005; Person-Le Ruyet et al. 2006). In some commercial practices, the temperature levels used for syngnathid culture are selected empirically from a range observed *in situ*, as long as relatively low mortality rates are achieved. However, there are studies that have proven improvement of juvenile seahorse culture in specific temperatures, generally higher than those empirically selected (James and Woods 2000; Wong and Benzie 2003; Lin et al 2007, 2008, 2009; Planas et al. 2012). However, those studies provided limited information on the temperature acclimation prior to the trials.

Due to their peculiar morphology, the weight of pipefish is smaller in relation to their length compared to other teleosts. Therefore, to conduct required culture analyses, specific techniques are used to measure the physiological response of animals to the factors tested. This specificity is related to the scarce sample materials to conduct conventional techniques such as proximate analyses of protein and fat which are used to estimate the nutritional response of fish. Instead, techniques such as the determination of the carbon and nitrogen index (C : N) have been found to be an accurate indicator of the condition of fish. Protein has a carbon and nitrogen ratio close to 3; for instance, in a tissue sample from fish in good condition it is expected to find protein and lipids with a carbon and nitrogen ratio greater than 3. In contrast, the lipids in starved or poor-condition fish are metabolized and the carbon and nitrogen ratio decreases (Westernhagen et al. 1998). Similarly, low moisture content has also been associated with good condition in early stage fish as nutritionally stressed fish consumes body protein (which is replaced with water) to maintain homeostasis (Shackley et al. 1993).

The studies on the response of cultured syngnathids to temperature have focused mostly on seahorses (James and Woods 2000; Wong and Benzie 2003; Sheng et al. 2006; Lin et al. 2007, 2008, 2009; Martinez-Cardenas and Purser 2011; Planas et al. 2012). However, the highest temperature levels tested have not always produced the best growth and survival (Wong and Benzie 2003; Sheng et al. 2006; Martinez-Cardenas and Purser 2011; Planas et al. 2012). In the tropical aquarium trade, for most teleosts, a water temperature of 26 C is typically regarded as being within the "optimal" range, while some tropical syngnathids such as *Hippocampus trimaculatus* are well adapted to temperatures as high as 30 C (Murugan et al. 2009). The primary aim of this study was to compare the effect on growth (wet weight, length, and specific growth rate [SGR]), condition (moisture content, C : N ratio, and Fulton's K), and survival of different temperatures (26, 28, and 30 C) on juvenile *M. brachyurus* in culture conditions.

Materials and Methods

System Design and General Methods

In the Jamapa river (19°05′40.42″N, 96°08′23.40″W), 120 juvenile pipefish (0.18 ± 0.0017 g, 8.56 ± 0.025 cm; mean ± 1 SE) were collected, then transported in 26 C (the temperature recorded at collection) and 8 g/L (the salinity recorded at collection) water inside an insulated water tank from the collection site to the wet laboratory located in Tepic, Nayarit, where experiments were conducted. Continuous aeration was provided during transit. Following a 15-min temperature acclimation period, individuals were allocated to a 200-L holding tank at the same conditions of collection at a temperature of 26 C and 8 g/L.

Each 35-L glass tank used in the experiment had a biological platform filter (3 mm gravel as filtration substrate) activated by continuous aeration provided via an aeration pump (Hagen®; Optima, Mansfield, MA, USA) connected to flexible plastic tubing ending with 2.5-cm air stone diffuser. In each tank, a heater was set to maintain the desired water temperatures. A 12:12 (L : D) photoperiod was provided (lights on at 0800 h and lights off at 2000 h) by a timer-controlled cool white light (35 W; General Electric Company, Fairfield, CT, USA) producing an intensity of 4.8 $\mu E/m^2/sec$ at the water surface. Water quality for both the experiments was maintained as follows: average pH 7.95 (range 7.6–8.3), dissolved oxygen >75%, total ammonia nitrogen (TAN) < 0.5 mg/L, nitrite < 0.25 mg/L, and nitrate < 5 mg/L. For the determination of pH, TAN, nitrite, and nitrate, a colorimetric saltwater liquid test kit (Aquarium Pharmaceuticals, Inc., Chalfont, PA, USA) was used. Salinity and temperature were monitored every 24 h while TAN, pH, nitrite, and nitrate were recorded every 48 h during the experiments. Tanks were inspected daily for mortalities and any excess food and feces were siphoned to waste.

Pipefish were fed live *Artemia* nauplii (enriched with Super Selco®, INVE Asia Service Ltd, Nonthaburi, Thailand, for 24 h at 17 C) maintaining a ration rate of 14% body weight (BW) per day (dry weight *Artemia* : wet weight fish) divided into two equal sized meals (1000 and 1600 h). *Artemia* fed at 1600 h were from the same batch as the morning feed but were enriched for a further 6 h. Feeding adjustments were calculated based on the daily mortality (assigned the previously recorded mean weight) per tank in all trials (the rations corresponding to mortalities were not fed to the remainder of fish). Pipefish length (distance between the tip of the mouth and the tip of the tail) was measured by placing the fish on a 1-mm scaled sheet covered by plastic. Pipefish wet weight was measured on an electronic balance and recorded to the nearest 0.01 g. Weekly growth was also recorded from bulk measures of wet weight. Fish were not fed for 24 h prior to each weighing.

Effect of Temperature Over a 6-wk Period Following a Temperature Acclimation of 1 C Every 24 h

The 90 pipefish used in this experiment were randomly located to each 35 L tank (10 fish per tank, three treatments with three replicates). Prior to the start of the experiment, fish were transferred from 26 C to the next temperature at a rate of 1 C per day until fish were allocated to all the temperatures used in this experiment (48 h to 28 C, 96 h to 30 C). The temperatures tested were selected from the temperature range (25–32 C) experienced by the species in the collection site through the year (Landeros-Sánchez et al. 2012). The lowest temperature (26 C) tested was considered a control as it was the temperature recorded during collection. Therefore, the other two levels (28 and 30 C) were selected in a 2 C interval. After 6 wk, the surviving pipefish were counted and their weight and length measured individually. One replicate from the 30 C treatment presented 100% mortality on Day 26 of the trial. Therefore, the final results from that treatment were calculated from the remaining two replicates. Fulton's K was calculated as $K = (W/L^3) \times$

100 where W = wet weight (g) and L = total length (cm). SGR was calculated as (SGR % increase in BW per day) = $[(\ln W_f - \ln W_i)/t] \times 100$, where W_f = final weight (g), W_i = initial wet weight (g), and t = time (d). Coefficient of variation (CV) of final fish BW was calculated (Kestemont et al. 2003) followed by size heterogeneity = CV_{wf} / CV_{wi}; where wf = final weight, wi = initial wet weight, and CV = coefficient of variation (100 SD/mean).

Moisture and Nitrogen/Carbon Content

The weight of the pipefish did not meet the minimum quantity of tissue required to conduct conventional proximate analyses. Instead, analyses were conducted to quantify moisture, nitrogen, and carbon content in the carcass to determine if pipefish cultured at temperatures above 26 C metabolized food more efficiently than those cultured at 26 C. At the end of the experiment, one pipefish per tank (randomly selected) was euthanized with an overdose of benzocaine (400 mg/L), blotted dry, and weight and length were recorded. Each whole pipefish was freeze-dried until constant weight was achieved. In addition, as low moisture content has been associated with a good condition in fish (Shackley et al. 1993), those dried samples obtained were used for moisture content by determining the difference from wet weight. The pipefish were then individually ground with a mortar and pestle for analysis of nitrogen and carbon by oxidation/infrared detection, using a CHNS auto-analyzer.

Statistical Analysis

A one-way analysis of variance (ANOVA) (SPSS 17.0) was used to compare the means among treatments of: survival, initial length, final length (mm), initial weight, final wet weight (g), CV (fish BW g), size heterogeneity (fish BW g), moisture (%), C : N ratio, Fulton's K (K), and SGR (%/d). A significance level of $P < 0.05$ was used. Levene's test and residual plots were used to test homogeneity of variance. Tukey's HSD *post hoc* test was used to identify differences among treatment means (SPSS 17.0). Levene's test and residual plots were used to test homogeneity of variance.

Results

Effect of Temperature Over a 6-wk Period Following a Temperature Acclimation of 1 C Every 24 h

There were no significant differences in either juvenile length ($F_{2,6} = 1.383$, $P = 0.321$) or wet weight ($F_{2,6} = 1.820$, $P = 0.241$) among treatments at the start of the trial (Table 1). After 6 wk, there were significant differences in length ($F_{2,5} = 7.768$, $P = 0.029$), wet weight ($F_{2,5} = 9.965$, $P = 0.018$), Fulton's K ($F_{2,5} = 7.32$, $P = 0.033$), and SGR ($F_{2,5} = 11.34$, $P = 0.014$) (Table 1). The juveniles cultured at 26 and 28 C were longer, heavier, grew faster, and presented better condition than the ones cultured at 30 C. There were also significant differences in survival ($F_{2,5} = 6.27$, $P = 0.043$). The pipefish cultured at 30 C had the lowest survival compared to the pipefish cultured at 26 C. Juveniles cultured at 28 C were not significantly different to those cultured in either 26 or 30 C (Fig. 1). From the first bulk-measuring to the end of the trial, the fish cultured at 26 and 28 C were heavier than those at 30 C (Fig. 2). There were differences in the CV ($F_{2,5} = 20.34$, $P = 0.004$) and size heterogeneity ($F_{2,5} = 19.52$, $P = 0.004$), with the fish cultured at 26 and 28 C having less variation compared to the juveniles cultured at 30 C. There were no significant differences in moisture content of pipefish at different temperatures ($F_{2,5} = 5.48$, $P = 0.055$), but there were significant differences in C : N ratio ($F_{2,5} = 6.04$, $P = 0.046$). The juveniles cultured at 26 C presented better condition than those at 28 and 30 C (Table 1).

Discussion

This study reports a strong negative response in growth of juvenile *M. brachyurus* when cultured at a temperature of 30 C. This temperature also produced 100% mortality in one replicate of that treatment and 70% in the remaining two replicates, which indicates that this is not a suitable culture temperature for this species. Exposure of teleosts to extreme temperatures can alter the function of the cardiovascular system, nerves, proteins, and

TABLE 1. *Survival, initial and final wet weight, initial and final length, coefficient of variation, size heterogeneity, moisture, C : N ratio, Fulton's* K*, and specific growth rate (SGR; mean ± 1 SE of three replicates per treatment) of* Microphys brachyurus *cultured at three different temperatures in a 6-wk growth trial following a temperature acclimation of 1 C per day.*[1]

Temperature (C)	26	28	30
Final observed survival (%)	83.3 ± 3.33^{a}	50 ± 15.27^{ab}	$30 \pm 0.00^{b,2}$
Initial individual weight (g)	0.17 ± 0.002^{a}	0.18 ± 0.002^{a}	0.18 ± 0.003^{a}
Final individual weight (g)	0.38 ± 0.01^{a}	0.40 ± 0.02^{a}	$0.26 \pm 0.03^{b,2}$
Coefficient of variation (final body weight g)	17.88 ± 1.77^{a}	22.5 ± 1.377^{a}	$41.76 \pm 5.44^{b,2}$
Size heterogeneity (body weight g)	0.62 ± 0.1^{a}	0.91 ± 0.05^{a}	$1.59 \pm 0.18^{b,2}$
Initial length (mm)	8.51 ± 0.05^{a}	8.58 ± 0.04^{a}	8.61 ± 0.02^{a}
Final length (mm)	10.15 ± 0.13^{a}	10.29 ± 0.19^{a}	$9.21 \pm 0.28^{b,2}$
Moisture (%)	82.11 ± 0.63^{a}	78.65 ± 0.89^{a}	$84.45 \pm 2.48^{a,2}$
C : N ratio	3.46 ± 0.1^{a}	2.97 ± 0.14^{b}	$2.94 \pm 0.05^{b,2}$
Fulton's *K*	0.037 ± 0.0004^{a}	0.037 ± 0.0001^{a}	$0.034 \pm 0.0013^{b,2}$
SGR (%/day)	1.88 ± 0.07^{a}	1.86 ± 0.15^{a}	$0.93 \pm 0.23^{b,2}$

[1]Means with different superscripts within a row are significantly different (one-way analysis of variance, $P < 0.05$).
[2]Means from the two remaining replicates after 100% mortality was observed in one replicate on Day 26.

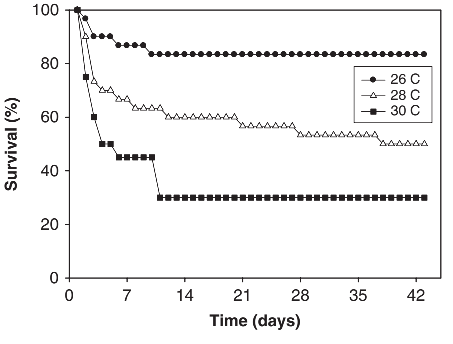

FIGURE 1. *Daily survival (% mean of three replicates per treatment) of* Microphys brachyurus *cultured at three different temperatures in a growth trial following a temperature acclimation of 1 C per day. The mean of 30 C represents the two remaining replicates after 100% mortality was observed in one replicate on Day 26.* M. brachyurus *were fed* Artemia *at a ratio of 14% body weight per day adjusted daily based on growth and mortality. Standard error bars were omitted to aid visualization.*

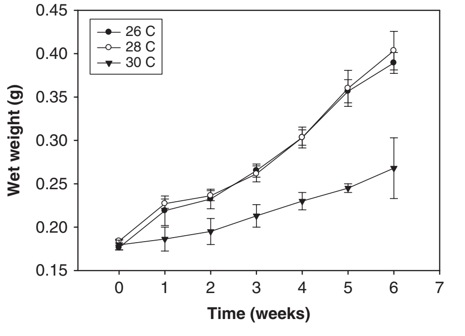

FIGURE 2. *Wet weight of juvenile* Microphys brachyurus *cultured at three different temperatures in a growth trial following a temperature acclimation of 1 C per day. Pipefish were fed* Artemia *at a ratio of 14% body weight per day adjusted daily based on growth and mortality. Values represent the mean of three replicates per treatment ± 1 SE. The mean of 30 C represents the two remaining replicates after 100% mortality was observed in one replicate on Day 26.*

enzymes, especially in juveniles of sensitive species (Tucker 1998). The survival recorded in juveniles cultured at 28 C suggest that the upper temperature limit could be near or below this level, as 50% of the fish died.

Whole-body samples were taken at the end of the trial to achieve a better understanding of the effect of temperature on juvenile *M. brachyurus* metabolism. The greater growth recorded in juveniles cultured at 26 C (considered a reference based on the temperature recorded during collection), compared to the growth at 30 C, was consistent with their significantly greater C : N ratio. A ratio of 3.0 is considered an indicator of good condition in fish (Harris et al. 1986). Juveniles cultured at 26 C not only achieved a 3.0 ratio but also reached 3.46. Although in the rest of the treatments the recorded C : N ratios were significantly lower than the ratio in 26 C, the values of 2.97 and 2.94 (recorded in 28 and 30 C, respectively) were quite close to 3.0, which suggest that the fish were not nutritionally stressed. These results could be related to the provided food ratio of 14%, which despite the poor adaptation of the juveniles to 28 and 30 C was enough to prevent nutritional stress. The 14% feed ratio used in this study aimed to ensure adequate feed intake by *M. brachyurus*, as in a previous syngnathid study (Martinez-Cardenas and Purser 2011) this feed ratio was found to be to excess for experimental seahorse rearing. Moreover, in other temperature studies on teleosts, a 5% feeding ratio has been already considered an excessive amount (Barron et al 2012).

In some syngnathids bred in captivity, the required change of food items due to ontogenetic development affects survival (Sheng et al. 2006). In fish caught in the wild, the transition from the items available to the fish *in situ* compared to the items supplied *ex situ* can have negative effects on growth and survival due to mismatches between the feed supplied and the nutritional requirements/feeding capabilities of the target species. This study is the first to report juvenile *M. brachyurus* acceptance of enriched *Artemia* nauplii from the day of collection, which suggest that the species may be a suitable candidate for commercial aquaculture.

As a response variable the size variation among treatments was recorded, as low variation is considered a desirable characteristic for cultured fish, as it helps to control social problems such as dominance and cannibalism that may result in poor growth, and gives opportunity for fish producers to offer more uniformly sized fish to the market (Streit et al. 2010). In general, syngnathid juveniles are not prone to aggression or cannibalism, perhaps due to their specific feeding requirements such as live items small enough to be suctioned by their snout. During this study, *M. brachyurus* did not display cannibalism or any aggressive behavior. The greater heterogeneity recorded in the juveniles cultured at 30 C could be related to the high mortality in that treatment compared to that at 26 and 28 C. The loss of one replicate during the experiment, and the small number of fish at the end of the trial may explain to some extent the greater variation in the 30 C treatment.

Syngnathid culture studies that have found a benefit of culturing juveniles at specific temperatures, generally higher than the previous levels empirically selected (James and Woods 2000; Lin et al. 2008; Planas et al. 2012), have provided limited information regarding temperature acclimation prior to trials. In the study conducted by James and Woods (2000) on late juvenile *Hippocampus abdominalis*, there is no mention of an acclimation protocol. Two studies (Wong and Benzie 2003; Lin et al. 2009) stated that juvenile seahorses (*Hippocampus whitei* and *Hippocampus erectus*, respectively) were acclimated over 48 h prior to each temperature used in those studies, although the authors did not provide a detailed protocol. Martinez-Cardenas and Purser (2011) used an acclimation protocol of 15-min acclimation to the next 3 C temperature level every 48 h in *H. abdominalis*, which was found to be probably too abrupt. In this study, an acclimation rate of 1 C every 24 h was used. However, 20% mortality was present in the level experienced by the juveniles at collection (26 C) which suggests that it was probably not the temperature changes that caused the mortality. Post-handling stress and poor adaptation skills may explain to some extent the decline in survival during the first weeks of the experiment.

While a proportion of the mortalities may be due to handling and transfer stress, as some mortality was seen in the reference temperature (26 C), the remainder may be due to the initial change in temperature as survival leveled off after the initial 10 d mortality. In addition, the highest variability on survival across treatments was recorded during the first 2 wk of the experiment, perhaps because of a negative response to a new environment resulting in a source of stress. Juvenile fish under stress are more likely to die after they reallocate metabolic energy from investment activities, such as growth, toward activities that require intensification to restore homeostasis (Bolasina et al. 2006).

In this study, juveniles cultured at a temperature of 26 C showed improved growth and survival. These findings are opposite to those of James and Woods (2000), who found improved growth in late juveniles of the temperate seahorse, *H. abdominalis* with increasing temperature, although the highest temperature used in that study was 21 C, and a maximum tolerance temperature was not determined, although this temperate species has been reported in the wild to inhabit water temperatures up to 24 C (Woods 2003). The results of (Lin et al. 2008) on *H. erectus* also differ from the findings of this study as the authors found improvement in growth and survival with increasing temperatures. They observed that neither the lowest (24 C) nor the highest (31 C) temperatures tested but an intermediate level (28–29 C) was the most adequate for the species. This finding is interesting as it indicates that even an eurythermal species that can be found from Nova Scotia to the Venezuelan Caribbean presents a temperature preference under culture conditions. Comparisons to other Syngnathid studies are difficult to establish as the response of each species to environmental factors, such as temperature or salinity, can be age and species specific (Hilomen-Garcia et al. 2003).

This study was based on constant temperatures as commonly found in intensive recirculation systems in aquaculture. In the wild, growth and development of juvenile fish are influenced by variable water temperatures; in syngnathid culture, the ability to provide a constant temperature may therefore significantly benefit the juveniles in terms of producing uniform and optimal growth (Lin et al. 2008). However, there is a need in future studies to test the effect of temperature fluctuations relevant to flow-through systems or cage systems on *M. brachyurus* rearing. In general, it has been found that the optimum temperature for growth is a few degrees lower than the temperature at which food intake is maximal (Burel et al. 1996). However, in this study, food intake was not recorded and was tested a temperature range above the level recorded during collection (26 C) in which was recorded the greatest growth and the highest survival. Future studies can focus on the effect of a lower range of temperatures to assess any adverse response on growth as found in *H. erectus* (Correa et al. 1989) and the effect on food intake. It can be concluded that the use of a temperature of 26 C improved growth and survival, while a temperature of 30 C led to high mortality. Further research would be useful to understand the effect of the temperatures tested at a physiological level of the temperatures tested in each stage of the life cycle.

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