

## Effect of Temperature on Growth and Survival in Cultured Early Juvenile Pot-bellied Seahorses, *Hippocampus abdominalis*

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### Abstract

In Tasmania, Australia, commercial seahorse culture takes place in tank systems in which approximately 75% of the water is exchanged daily from the Tamar River estuary. As such, some water conditions such as temperature fluctuate on a daily and seasonal basis. The aim of this study was to examine the effect on growth, condition, survival of, and *Artemia* ingestion by, early juvenile seahorses, *Hippocampus abdominalis*, cultured for 6 wk at temperatures within the species' natural range (8–24 C) and above it (26 C). Seahorses cultured at 20 C were longer and heavier than those at 17 C, although not significantly different to 23 C. There were no differences in survival or *Artemia* ingestion of juveniles cultured at 17, 20, and 23 C. At 26 C, 100% mortality was reached on Day 15. This study demonstrates that *H. abdominalis* can be reared in captivity at a range of 17–23 C in early life stages without compromising growth and survival.

The aquarium trade in seahorses is primarily focused on tropical species, such as *Hippocampus kuda*, *Hippocampus ingens*, and *Hippocampus erectus*, which are compatible with other tropical fish, and thus more marketable as the tropical aquarium trade is much larger compared with the temperate aquarium trade (Koldewey and Martin-Smith 2010). *Hippocampus abdominalis* has been an experimental model in syngnathid research for the last 18 yr. This species is a temperate water species that experiences a temperature range of 8–24 C in the wild (Woods 2003c), whereas a temperature range of 10–19 C has been used in captivity (Woods 2000b). Temperature is one of the most important factors influencing fish growth (Tucker 1998). Food consumption and food conversion efficiency can be improved by optimizing culture temperature, which can lead to growth improvement in teleosts (Jonassen et al. 2000), including the family Syngnathidae (Lin et al. 2006; Silva et al. 2006; Lin et al. 2009).

A temperature range of 17–18 C has been used for *H. abdominalis* experiments on general husbandry (Thomson 1999; Adams et al. 2001; Wardley 2001; Florent 2003; Shapawi and Purser 2003; Woods 2003a; Ouyang 2005; Martinez-Cardenas et al. 2008) and most nutritional studies (Woods 2003b; Woods and Valentino 2003; Woods 2005; Wilson et al. 2006). However, most of these studies have been conducted on late juveniles, with only a few experiments conducted under different temperature regimes (Leef 2001; Woods 2005) or on early stage juveniles (Woods 2000a; Florent 2003; Martinez-Cardenas and Purser 2007). Seahorse World Pty. Ltd. (Tasmania, Australia) also uses a culture temperature of 18 C (Seahorse World Pty. Ltd., pers. comm.). Woods (2003c) conducted a study in which the effects of a temperature range (12, 15, 18, and 21 C) on *H. abdominalis* growth and survival were examined. The authors found improved growth of this species at higher temperatures (18 and 21 C) compared with lower temperatures (12 and 15 C), while mortality was not affected by these temperatures.

Early stage fish research requires the use of specific techniques to measure the physiological response of animals to the factors tested. This

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specificity is related to the small size of the fish; it is not always possible to collect enough 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 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, in poor condition or starved fish the lipids 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 consume body protein (which is replaced with water) to maintain homeostasis (Shackley et al. 1993).

The aim of this study was to compare the effect of a selection of seawater temperatures (17, 20, and 23 C) within the natural range (8–24 C) experienced by early juvenile *H. abdominalis*, and one temperature above that range (26 C).

## Materials and Methods

### *System Design and General Methods*

Juvenile *H. abdominalis* used in the experiments were transported in seawater (32 ppt) and oxygen-filled plastic bags inside an insulated container from a commercial seahorse farm (Seahorse World Pty. Ltd., Beauty Point) to the marine hatchery in the Aquaculture Centre at the University of Tasmania, Launceston. Following a 15-min temperature acclimation period, juveniles were released into a 20-L holding tank at the same conditions of birth at a water temperature of 17 C and a salinity of 32 ppt.

Four separate 35-L recirculation systems were used to maintain four temperatures. Each system had four 3-L tanks connected to a biofilter comprised of two stacked 22-L plastic containers. The upper container was filled with 40-mm bioballs and its floor area was

perforated every 5 cm to allow outflow water from the tanks to trickle down to the container below. This lower container was used as a water reservoir in which a 40 W submersible pump (Resun, Shenzhen, China) of a 2800 L/h delivery volume was installed. The pump provided an inflow of approximately 2.5 L/h/tank of 20 µm-filtered seawater. In the reservoirs, a heater was set to maintain desired water temperatures.

A 12:12 h light : dark 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 µE/m<sup>2</sup>/sec at the water surface. Continuous aeration was provided with flexible plastic tubing ending with a 4-L/h plastic water dripper (Neta) acting as an air stone. Aeration was not located under the substrate but adjacent to and removed from the substrate to avoid direct disturbance to the seahorses. Attachment substratum for juvenile seahorses was provided by a weighted bundle of 55 nylon monofilament segments with a length of approximately 140 mm.

Water quality was maintained as follows: average pH 7.78 (range 7.5–8.0), 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, whereas TAN, pH, nitrite, and nitrate were recorded every 48 h during the experiments. During the experiment, tanks were inspected daily for mortalities and any excess food and faeces were siphoned to waste.

Juvenile seahorses were fed live *Artemia* (enriched with Super Selco for 24 h at 17 C) at a ration rate of 14% initial body weight (BW)/d (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. Screens (150 µm) over the outlet of the tanks prevented the loss of *Artemia* during the day. Feeding time occurred from 0800

to 1900 h daily; at the end of this period the screens were replaced with 500- $\mu\text{m}$  screens to flush out overnight into a central screen the remaining unenriched *Artemia*. Feeding adjustments were calculated based on the daily mortality (assigned the previously recorded mean weight) per tank (the rations corresponding to mortalities were not fed to the remainder of fish). Seahorse length (distance between the tip of the coronet to the tip of the uncurled tail) was measured by placing the seahorse on a 1 mm scaled sheet covered by plastic. Seahorse wet weight was measured on an analytical balance and recorded to the nearest 0.0001 g. Weekly growth was also recorded from bulk measures of wet weight. Fish were not fed for 24 h before each weighing.

*Effect of Temperature over a 6-wk Period  
Following a Temperature Acclimation of 3 C  
Every 48 h*

Two hundred and forty juveniles were randomly selected from 330 fish from three broods ( $n = 100, 60, 170$ ; 7, 6, and 4-d-old, respectively) produced at Seahorse World Pty. Ltd. Prior to the start of the experiment, seahorses were transferred from 17 C to the next temperature in 3 C increments (after a 15 min temperature acclimation) every 48 h until juveniles were allocated to all the temperatures used in this experiment (48 h to 20 C, 96 h to 23 C, and 144 h to 26 C). The fish were fed live *Artemia* under the conditions previously described. After 6 wk, the surviving seahorses were counted and their weight and length measured individually. Fulton's  $K$  was calculated as  $K = (W/L^3) \times 100$ , where  $W$  = wet weight (g) and  $L$  = length (cm). Specific growth rate (SGR) was calculated as (SGR percent increase in BW/d) =  $(\ln W_f - \ln W_i)/t \times 100$ , where  $W_f$  = final weight (g),  $W_i$  = initial wet weight (g), and  $t$  = time (d). CV of final fish BW was calculated followed by size heterogeneity =  $CV_{W_f}/CV_{W_i}$ , where  $W_f$  = final weight,  $W_i$  = initial wet weight, and CV (100 SD/mean) (Kestemont et al. 2003).

*Moisture and Nitrogen/Carbon Content*

The small size of the juveniles did not meet the minimum quantity of tissue required to conduct conventional proximate analyses. Instead, post-experiment analyses were conducted to quantify moisture, nitrogen, and carbon content in the carcass to determine if seahorses cultured at temperatures above 17 C metabolized feed more efficiently than those cultured at 17 C. At the end of the experiment, one seahorse per tank (randomly selected) was euthanized with an overdose of benzocaine (400 mg/L), blotted dry, and weight and length recorded. Each whole seahorse was freeze dried until constant weight was achieved. In addition, as low moisture content has been associated with a good condition early stage fish (Shackley et al. 1993), those dried samples obtained were used for moisture content by determining the difference from wet weight. The seahorses were then individually ground with a mortar and pestle for analysis of nitrogen and carbon by oxidation/infrared detection, using a CHNS auto-analyzer.

*Effect of Temperature on Artemia Ingestion*

To determine how temperature affected the food intake of juvenile *H. abdominalis*, *Artemia* ingestion was recorded during Weeks 1, 3, and 5 of the 6-wk experiment. A single fish was randomly selected per tank (replicate) and the number of feeding strikes produced during a 3-min period, 1 min after the food was introduced into the tank (1000 h) was recorded. One fish was recorded/tank/d over 3 d during each sample week.

*Statistical Analysis*

A one-way ANOVA (SPSS 11.5, North Harbour Portsmouth, UK) was used to compare the means among treatments of survival, initial length, final length (mm), initial weight, final wet weight (mg), 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. Final weight data were natural-log

transformed to satisfy homogeneity of variance requirements. Tukey's HSD post hoc test was used to identify differences among treatment means (SPSS 11.5). An orthogonal ANOVA (SPSS 11.5) was used to compare the means of weekly weights among treatments over the 6-wk trial.

An orthogonal ANOVA (SPSS 11.5) was used to compare the means of feeding strikes among treatments over the 3-d observation (as orthogonal factor) on each sample week. A significance level of  $P < 0.05$  was used. Levene's test and residual plots were used to test homogeneity of variance. A square root transformation was applied to data of Week 1 of the experiment to satisfy homogeneity of variance requirements.

## Results

### *Effect of Temperature over a 6-wk Period Following a Temperature Acclimation of 3 C Every 48 h*

There were no significant differences in either juvenile seahorse length ( $F_{3,12} = 1.146$ ,  $P = 0.371$ ) or wet weight ( $F_{3,12} = 1.64$ ,  $P = 0.23$ ) among treatments at the start of the trial (Table 1). After 6 wk, there were significant differences in length ( $F_{2,9} = 17.170$ ,  $P = 0.001$ ), wet weight ( $F_{2,9} = 9.780$ ,  $P = 0.006$ ), and SGR ( $F_{2,9} = 5.390$ ,  $P = 0.029$ ) (Table 1). The juveniles cultured at 23 and 20 C were

longer and heavier than the ones cultured at 17 C. There were no significant differences in survival among the seahorses cultured at 17, 20, or 23 C ( $F_{2,9} = 2.630$ ,  $P = 0.126$ ). The survival of seahorses cultured at 26 C decreased to 0 on Day 15 (Fig. 1). From the second bulk measuring to the end of the trial, 20 and 23 C fish were heavier than 17 C (Fig. 2). There were no differences in the CV ( $F_{2,9} = 0.746$ ,  $P = 0.501$ ) or the size heterogeneity ( $F_{2,9} = 3.053$ ,  $P = 0.097$ ) among treatments at the end of the trial. There were significant differences in moisture content of seahorses in different temperatures ( $F_{2,9} = 7.90$ ,  $P = 0.01$ ). The juveniles cultured at 23 C contained less moisture than the ones cultured at 17 C, and seahorses cultured at 20 C contained a similar amount of moisture to those cultured at 23 and 17 C (Table 1). There were no significant differences in C : N ( $F_{2,9} = 1.56$ ,  $P = 0.26$ ) or Fulton's K ( $F_{2,9} = 0.94$ ,  $P = 0.42$ ) (Table 1).

### *Effect of Temperature on Artemia Ingestion*

There were no significant differences in *Artemia* ingestion among treatments at Weeks 1 ( $F_{6,36} = 2.02$ ,  $P = 0.08$ ), 3 ( $F_{4,27} = 1.029$ ,  $P = 0.410$ ), or 5 ( $F_{4,27} = 0.547$ ,  $P = 0.703$ ). It was not possible to record *Artemia* ingestion in juveniles cultured at 26 C as all the seahorses died before the first *Artemia* ingestion sample was taken (Table 2).

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 (mean  $\pm$  1 SE of four replicates per treatment) of early juvenile *Hippocampus abdominalis* cultured at four different temperatures in a 6-wk growth trial following a temperature acclimation of 3 C every 48 h.<sup>1</sup>

Temperature	17 C	20 C	23 C	26 C
Final observed survival (%)	68.3 $\pm$ 7.4 <sup>a</sup>	68.3 $\pm$ 8.3 <sup>a</sup>	50.0 $\pm$ 2 <sup>a</sup>	0
Initial individual weight (mg)	15.1 $\pm$ 0.8 <sup>a</sup>	15.9 $\pm$ 2.8 <sup>a</sup>	13.9 $\pm$ 0.4 <sup>a</sup>	14.0 $\pm$ 0.6 <sup>a</sup>
Final individual weight (mg)	131.8 $\pm$ 7.5 <sup>a</sup>	199.9 $\pm$ 6.1 <sup>b</sup>	216.8 $\pm$ 23.0 <sup>b</sup>	2
Coefficient of variation (final body weight [g])	40.3 $\pm$ 4.1 <sup>a</sup>	31.9 $\pm$ 5.4 <sup>a</sup>	31.2 $\pm$ 7.4 <sup>a</sup>	2
Size heterogeneity (body weight [g])	1.60 $\pm$ 0.17 <sup>a</sup>	1.04 $\pm$ 0.17 <sup>a</sup>	1.00 $\pm$ 0.25 <sup>a</sup>	2
Initial length (mm)	19.9 $\pm$ 0.5 <sup>a</sup>	19.5 $\pm$ 0.1 <sup>a</sup>	19.3 $\pm$ 0.2 <sup>a</sup>	19.9 $\pm$ 0.2 <sup>a</sup>
Final length (mm)	40.5 $\pm$ 0.8 <sup>a</sup>	47.4 $\pm$ 1.0 <sup>b</sup>	48.3 $\pm$ 1.3 <sup>b</sup>	2
Moisture (%)	82.8 $\pm$ 0.5 <sup>a</sup>	81.9 $\pm$ 0.2 <sup>ab</sup>	80.4 $\pm$ 0.4 <sup>b</sup>	2
C : N ratio	3.70 $\pm$ 0.1 <sup>a</sup>	3.54 $\pm$ 0.03 <sup>a</sup>	3.78 $\pm$ 0.14 <sup>a</sup>	2
Fulton's K	0.170 $\pm$ 0.005 <sup>a</sup>	0.160 $\pm$ 0.008 <sup>a</sup>	0.160 $\pm$ 0.006 <sup>a</sup>	2
Specific growth rate (% d)	5.1 $\pm$ 0.2 <sup>a</sup>	6.4 $\pm$ 0.1 <sup>ab</sup>	6.5 $\pm$ 0.3 <sup>b</sup>	2

<sup>1</sup>Means with different superscripts within a row are significantly different (one-way ANOVA,  $P < 0.05$ ).

<sup>2</sup>No data because of 100% mortality.

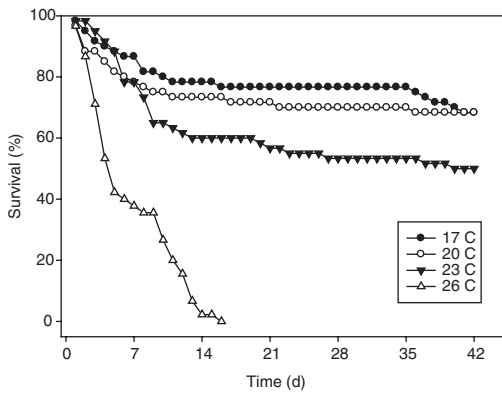


FIGURE 1. Daily survival (percent mean of four replicates per treatment) of early *Hippocampus abdominalis* cultured at four different temperatures in a growth trial following a temperature acclimation of 3 C every 48 h. Seahorses were fed *Artemia* at a ratio of 14% body weight d adjusted daily based on growth and mortality. SE bars were omitted to aid visualization.

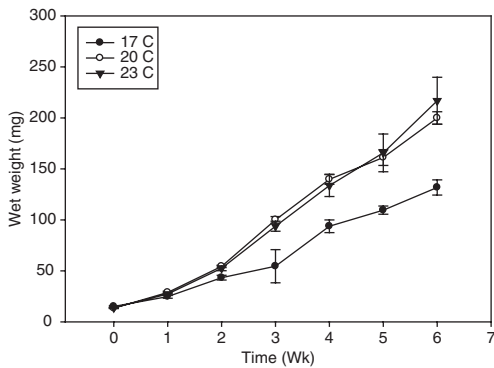


FIGURE 2. Wet weight of juvenile *Hippocampus abdominalis* cultured at four different temperatures in a growth trial following a temperature acclimation of 3 C every 48 h. Seahorses were fed *Artemia* at a ratio of 14% body weight d adjusted daily based on growth and mortality. No data were recorded at 26 C because of 100% mortality. All values represent the mean of four replicates per treatment  $\pm$  1 SE.

**Discussion**

This study reports for the first time an improved growth response of *H. abdominalis* cultured at a temperature of 23 C, while 26 C produced 100% mortality. Juveniles cultured at 23 C showed significantly improved growth compared with juveniles at 17 C. There were no differences in survival among the remaining

TABLE 2. Artemia ingestion during the experiment as strikes (mean  $\pm$  1 SE of four replicates per treatment) over a 3-min period recorded from one randomly selected fish tank in four different temperatures in a 6-wk growth trial.<sup>1</sup>

Temperature	Week 1			Week 3			Week 5		
	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3
17 C	9.5 $\pm$ 1.3	11.8 $\pm$ 1.8	13.0 $\pm$ 1.7	15.3 $\pm$ 5.6	9.8 $\pm$ 4.1	10.8 $\pm$ 4.8	16.8 $\pm$ 3.1	12.8 $\pm$ 4.6	19.0 $\pm$ 1.6
20 C	25.5 $\pm$ 5.2	38.3 $\pm$ 4.5	28.0 $\pm$ 7.2	35.8 $\pm$ 5.4	17.0 $\pm$ 0.9	32.0 $\pm$ 7.4	22.0 $\pm$ 6.3	17.0 $\pm$ 2.7	11.8 $\pm$ 2.5
23 C	28.3 $\pm$ 9.0	55.0 $\pm$ 6.7	32.0 $\pm$ 6.3	23.0 $\pm$ 3.9	20.0 $\pm$ 3.6	20.8 $\pm$ 4.3	27.0 $\pm$ 8.0	22.5 $\pm$ 6.3	23.3 $\pm$ 2.8
26 C	7.5 $\pm$ 3.0	7.0 $\pm$ 3.5	4.3 $\pm$ 2.5	<sup>2</sup>	<sup>2</sup>	<sup>2</sup>	<sup>2</sup>	<sup>2</sup>	<sup>2</sup>

<sup>1</sup>The use of superscripts has been omitted as there were no statistical differences among treatments (orthogonal ANOVA,  $P > 0.05$ ).

<sup>2</sup>No data because of 100% mortality.

treatments (17, 20, and 23 C). However, it would be expected that the temperature in which juveniles had the best growth results (23 C) presented the highest survival rate. Perhaps, the 15 min acclimation used in this study was too abrupt as seahorses were transferred from 17 to 20 and 23 C. As literature on the temperature effect on seahorse culture has provided limited information regarding temperature acclimation prior to trials (Woods 2001; Wong and Benzie 2003), the acclimation protocol used in this study was based on a preliminary short-term trial, in which because of its duration (8 d) no mortalities were observed. Further research is needed to determine the optimal temperature acclimation rate for *H. abdominalis*, with the use of an automatic controlled heater which could diminish temperature related stress by gradually transferring the fish from the reference temperature to the temperatures to be tested.

Overall, mortality occurred during the first 2 wk of the experiment and leveled out after that period. However, more than 30% mortality was present in the control treatment (17 C), which was the temperature experienced by the juveniles before they were transferred to the different levels tested. This fact suggests that not only the temperature changes caused this mortality. Post-handling stress (transport from Seahorse World Pty. Ltd. to the hatchery in the Aquaculture Centre at the University of Tasmania, Launceston) and poor adaptation skills may explain to some extent the decline in survival during the first week of the experiment. Alternatively, the mortalities could be the result of inherent early juvenile mortality of *H. abdominalis*, or 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).

The poorer growth recorded in juveniles cultured at 17 C was consistent with their significantly greater moisture content compared with seahorses at 23 C, which showed better

growth and a smaller amount of moisture as an indicator of good condition in early stage fish (Shackley et al. 1993). Although C:N ratios in samples of seahorses cultured at 23 C appeared to be higher compared with the other treatments, no significant differences were found. However, the overall C:N ratios were above 3.0, which indicate that the seahorses were not nutritionally stressed (Harris et al. 1986). Although there were no significant differences in *Artemia* ingestion among treatments, it was noted that in most observations, the seahorses at 17 C ingested less *Artemia* than those in the rest of the treatments, and that, contrarily, in most observations the seahorses in 23 C ingested more *Artemia* than the rest of the treatments. The *Artemia* ingestion recording technique used in this study was used in a previous study on *H. abdominalis* (Martinez-Cardenas and Purser 2007). In that study, the seahorse's ingestion presented high variability among treatments with no clear patterns observed over time. The tendency recorded in this study might explain to some extent the differences in growth between seahorses cultured at 17 C and those cultured at 23 C. Therefore, in further research the use of a more accurate ingestion recording technique is needed to determine if better ingestion relates to better growth.

Although, a temperature range of 17–18 C appears to be considered a standard for *H. abdominalis* culture (Seahorse World Pty. Ltd., pers. comm.) and experimentation (Thomson 1999; Adams et al. 2001; Wardley 2001; Florent 2003; Shapawi and Purser 2003; Woods 2003a; Ouyang 2005; Martinez-Cardenas et al. 2008), it may not be optimal for early juveniles of this species as it caused the poorer growth and the greater amount of moisture of the seahorses cultured at 17 C, compared with those cultured in 20 and 23 C. This may suggest that early stages of *H. abdominalis* are better adapted to temperatures above 17 C than late juveniles. The more successful adaptation of the seahorses cultured at 20 and 23 C in this study may produce a more efficient metabolism and assimilation efficiency (therefore less moisture). This could be related to the fact that *H. abdominalis* is considered pelagic during the

first month of life (Gomon and Neira 1998), inhabiting close to the surface where water temperature is warmer compared with the colder temperatures at the bottom, associated with adults and late juveniles. In addition, despite *H. abdominalis* having been reported to have the capacity to breed year-round (Poortenaar et al. 2004), breeding peaks have been recorded during summer, suggesting that this species may also be better adapted as early juvenile to temperatures above 17 C.

Juvenile *H. abdominalis* cultured at 26 C suffered 100% mortality, which indicates that this temperature is outside their tolerance range; at least after the acclimation period used in this study. In general, exposure of teleosts to an extreme temperature can alter the function of the cardiovascular system, nerves, proteins, and enzymes, especially in juveniles of sensitive species (Tucker 1998). Woods (2003c) reported the presence of *H. abdominalis* in temperatures as high as 24 C in the wild. This is consistent with the findings of this study in which *H. abdominalis* appeared to have an upper thermal tolerance for survival at some point between 23 and 26 C. Therefore, further research is needed to determine the critical upper temperature limit for *H. abdominalis*.

The findings of this study are consistent with Woods (2003c), who found improved growth in late juveniles (80 mm, 0.7 g) of *H. abdominalis* with increasing temperature, although the highest temperature used in that study was 21 C, and a maximum tolerance temperature was not determined. Similarly, Wong and Benzie (2003) reported an increase in length and wet weight of *Hippocampus whitei* when cultured at 26 C, a temperature level associated to the geographical distribution of that species but lethal for early juveniles of *H. abdominalis*. Comparisons with other seahorse 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 considered a temperature of 17 C to provide a close reference to the temperature

of 18 C used in *H. abdominalis* commercial culture (Seahorse World Pty. Ltd., pers. comm.) and a high temperature of 26 C to assess its suitability for the tropical aquarium market. On the basis of our results, it can be concluded that under the experimental protocol used, *H. abdominalis* cannot be considered for the tropical aquarium trade. However, it would be advantageous to examine the viability of the large-scale rearing of *H. abdominalis* at 23 C after longer temperature acclimation protocols, which could optimize commercial culture, through improved growth rates.

Prolonged culture over subsequent generations at elevated temperatures such as 23 C could potentially lead to temperature tolerance through selective breeding. However, the effect of elevated temperature on courtship, mating, and pouch incubation is unknown. In addition, this study was based on constant temperatures as found in intensive recirculation systems in aquaculture in contrast to fluctuating seawater temperatures at the commercial seahorse farm at Beauty Point in Tasmania.

From this study, it can be concluded that the use of temperatures of 20 and 23 C lead to an improvement in growth in early juvenile *H. abdominalis*, but that a temperature of 26 C is too high for this species resulting in complete mortality. Commercial scale culture facilities such as Seahorse World Pty. Ltd. may find these results useful when managing seasonal salinity and temperature fluctuations at their site. Further research would be useful to understand the effect at a physiological level. Therefore, there is a need in future studies to test the effect of temperature fluctuations relevant to flow-through systems or cage systems on early juvenile seahorse rearing.

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