Mating, birth, larval development and settlement of Bargibant's pygmy seahorse, *Hippocampus bargibanti* (Syngnathidae), in aquaria

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Abstract. Pygmy seahorses are among the most charismatic inhabitants of coral reefs. Their diminutive size, impressive coloration, and rarity make them attractive to recreational divers, underwater photographers and videographers. However, very little is known about their life history and ecology due to their relatively recent discovery and the difficulty of studying them in the wild. In May 2014, we collected a pair of adult *Hippocampus bargibanti* Whitley, 1970 in the Philippines. These were successfully transported to Steinhart Aquarium at the California Academy of Sciences for husbandry and life history research. The pair mated in their aquarium and gave birth to three broods of offspring. We raised a portion of the juveniles through settlement to a maximum of 120 days from birth, documenting for the first time the planktonic part of their life history, their selection of host corals and settlement, and plasticity within the ensuing color change. Despite our successes, we were unable to raise the juveniles beyond 21.2 mm height (coronet to tail tip), which was approximately 73% of the average height of the two wild-collected adults (28.8 mm). The methods described in our study facilitate additional research on the feeding and nutritional requirements of juvenile and adult pygmy seahorses, as well as the criteria used for host selection.
Key Words: captive breeding, conservation, coral reefs, fishes, reproduction.

Introduction. Pygmy seahorses, including *Hippocampus bargibanti*, are among the smallest known vertebrates. Standard lengths of adult specimens are in the range of 20 to 30 mm, with one species, *H. denise* Lourie & Randall 2003, being less than 15 mm SL (Lourie & Randall 2003). Recent discoveries have increased the number of true pygmy seahorses to at least seven known species (Lourie & Kuiter 2008). Most of these occur in the Coral Triangle region of the Indo-West Pacific: the center of marine shore fish biodiversity (Carpenter & Springer 2005), and also an area with the highest levels of anthropogenic stresses and degradation facing the oceans (Bryant et al 1998; Burke et al 2011). These remarkable, diminutive animals are uncommon, incredibly charismatic, and the subject of a great deal of attention from the recreational dive community and professional and amateur underwater photographers and videographers. A multi-tiered combination of threats to individual animals, their hosts, and their ecosystem may put pygmy seahorses at risk, but there is a general lack of knowledge of their biology and ecology due to the difficulty of finding, tracking and studying them in situ.

Pygmy seahorses exhibit complex homochromy - that is, the body color, shape and types of skin ornamentation of the various species closely mimics that of their hosts: usually octocorals (primarily gorgonians), but also hydroids, bryozoans, seagrasses and coralline algae (Zmarzly 1990; Lourie & Kuiter 2008; Reijnen et al 2011). This "protective", or "deceptive" resemblance, makes them nearly invisible among the corals' branches, and undoubtedly is the primary reason that they remained unknown until 1969, when a pair of specimens was discovered by Georges Bargibant on a *Muricella* coral collected for display at the aquarium in Nouméa, New Caledonia (Lourie & Randall 2003; Randall 2005). There are two color morphs: the first has a grey striated body and

red/pink bulbous tubercles, and the second has an orange striated body and golden-yellow tubercles (Lourie et al 2004). Both color morphs have striped tails.

H. bargibanti was the first species of pygmy seahorse to be discovered and described (Whitley 1969; Gomon 1997). It occurs throughout the coral triangle region at depths between 16 and 40 m, and can occasionally be found as shallow as 4.5 m (Kuiter 2001; Lourie et al 2004; Baine et al 2008; Smith 2010). They are among the most specialized pygmy seahorses in terms of host selection, living in an obligate association with at least five species of sea fans of the genus *Muricella* (Reijnen et al 2011). Individuals remain on their host corals for periods from 3 to 40 weeks, moving over the entire surface of the *Muricella* (Baine et al 2008). Transit between host corals has not been observed, so it is assumed that following settlement individuals remain on a single host coral for their entire adult lives (Baine et al 2008; Smith 2010). Despite having planktonic larvae, dispersal beyond the current geographic range is unlikely due to the high degree of host specialization (Teske et al 2004).

Exceptional and highly specialized paternal care strategy is closely associated with the evolution of pipefishes and seahorses, and a diversity of approaches exists within the family Syngnathidae (Herald 1959; Wilson et al 2001). *H. bargibanti* is sexually dimorphic; females have a circular genital pore with a raised rim, while males have a slit-shaped brood pouch opening in the post-anal region (Lourie & Randall 2003). Male *H. bargibanti*, like all known members of the genus, brood the young entirely within the trunk or abdomen (Lourie & Randall 2003; Lourie & Kuiter 2008). Reproduction in this species is assumed to occur year-round, with a gestation period of 11 to 14 days based on the very limited field observations of *H. bargibanti* and closely related species (Foster & Vincent 2004; Smith & Tibbets 2008).

Conservation threats. Unlike other, larger members of the genus, *H. bargibanti* is not regularly collected for the traditional Chinese medicine, curio or aquarium trades. However, they exist in the lowest population densities of any unexploited seahorse in an area that is the subject of tremendous anthropogenic stressors: climate change and ocean acidification, unsustainable fishing practices, sedimentation, coastal development and multiple forms of pollution including solid waste (e.g. plastics) and human sewage (Burke et al 2011; Smith 2010). Gorgonian skeletons are often processed and polished to resemble black coral, and then sold into the jewelry and curio trade (Cooper et al 2011). Data from the compilation of cases involving illegal transport and trade in corals by the Philippines' Bureau of Fisheries and Aquatic Resources (BFAR) shows that illegal harvest of sea fans is an unfortunate reality. More than 21,000 pieces, including *Muricella*, were contained in a single shipment apprehended by Manila authorities in May 2011. Threats to the conservation of *H. bargibanti* thus include the overall degradation of Indo-Pacific coral reefs, specific targeted collection of its host gorgonian, unintentional destruction of the host gorgonian by trash and abandoned fishing gear, and the negative effects of repeated harassment by SCUBA divers who touch, chase, or shine bright lights on pygmy seahorses in an attempt to obtain photos or video of this charismatic species (Smith 2010). This multi-tiered suite of threats has the potential to reduce the already low population densities and may cause localized extinctions in highly impacted areas.

Difficulty of scientific study. Knowledge of the life history, ecology and behavior of *H. bargibanti* is rudimentary, and mostly limited to what is present in species descriptions (Lourie & Kuiter 2008; Lourie & Randall 2003; Smith 2010). Most discoveries and species descriptions have occurred in the last few decades, so fundamental knowledge regarding this group is also extremely limited (Foster & Gomon 2009; Gomon & Kuiter 2009; Lourie & Kuiter 2008; Lourie & Randall 2003). Opportunities to study this species under controlled conditions in laboratory aquaria are complicated by the challenging requirement of maintaining both the host species and the pygmy seahorse (Chan & Delbeek 2004). In his dissertation, the most detailed study of the ecology of this group, Smith repeatedly notes that, because these animals are "unable to be successfully kept in captivity", major aspects of their biology simply cannot be studied (Smith 2010).

Our main objectives for this study were to document reproductive biology, brood size, feeding behavior, developmental changes, and lifespan by breeding and rearing *H. bargibanti* through settlement in the controlled environment of laboratory aquaria. These advances open the door for further research on reproductive behavior, the dynamics of host interaction, including juveniles' selection criteria of appropriate hosts, the genetic and environmental mechanisms for their camouflage, feeding behavior, nutritional needs, and other studies of these species, which are rare and charismatic ambassadors for coral reef ecosystems.

Material and Method. Our approach toward aquarium culture of *H. bargibanti* consisted of three sequential phases: (1) collection, transport and husbandry of the host gorgonians, (2) collection, transport and husbandry of the brood stock, and (3) larval rearing of the offspring from birth through settlement. The single prior work with this species in aquaria suggested that culture of *H. bargibanti* in the absence of host gorgonians was not successful, so our study began with investigations of long-term husbandry of *Muricella* spp. sea fans (Chan & Delbeek 2004).

Host gorgonian culture. In preparation for working with *H. bargibanti,* several small fragments of *Muricella* spp. sea fans were collected in the Philippines in May 2011. Only small cuttings (less than 8 cm by 3 cm) were taken from sea fans approximately 1 m^2 or larger, leaving the majority of the colony intact and on the reef. Cuttings were packed in 1.5 L plastic bags filled with fresh natural seawater and oxygen in a 2:1 ratio, securely packed in Styrofoam coolers with cardboard outer liners and shipped to Steinhart Aquarium in San Francisco, CA, USA, via air cargo (30 hours total transit time, where they were added to a mature 1,000-liter aquarium. This aquarium was maintained at a temperature of 23-26°C, a salinity of 34-35 ppt, and was lit by blue and white LED lamps (3W MR16) for eight hours a day. Propeller pumps (Ecotech Marine, Allentown PA, USA) produced water motion strong enough to slightly vibrate the branches of the corals. Regular aquarium maintenance included removal of hydroids, small brittle stars, and glass anemones (*Aiptasia* sp.) from the aquarium, which is necessary to prevent these pests from smothering or stinging the *Muricella*. Mucus was periodically blown off the corals with a turkey baster or small pump.

A diverse range of planktonic foods was offered (Table 1). These items were mixed together in a 4 L insulated beverage thermos, topped off with fresh seawater, and chilled with a reusable ice pack for the entire feeding period. A peristaltic pump (Rola-Chem Corporation, St. Paul, Minnesota, USA) delivered these foods to the aquarium every 10 minutes during the 8-hour daylight period (0900-1700 hours), resulting in a continuous low density of foods in the water column of the aquarium. Only the automatic feeding system was used and corals were not specifically target-fed.

Table 1

Planktonic feed used to culture *Muricella* corals. Each type of food is indicated by volume per 1,000 L aquarium system volume. All types are mixed and continuously added over a time period of 8-hours

Food type	*Volume per 1,000 L*
Oyster Feast © oyster ovarian tissue and eggs: Reef Nutrition, Campbell CA USA	40 mL
L-strain *Brachionus plicatilis* rotifers, preserved: Reed Mariculture, Campbell CA USA	40 mL
Artemia nauplii, newly hatched: GSL, Artemia International, Fairview TX USA	40 mL
Shellfish Diet © preserved microalgae: *Isochrysis* sp., *Pavlova* sp., *Tetraselmis* sp.; Reed Mariculture, Campbell CA USA	20 mL
Phyto Feast © preserved microalgae: *Pavlova* sp., *Isochrysis* sp., *Tetraselmis* sp., *Nannochloropsis* sp., *Synechococcus* sp.; Reef Nutrition, Campbell CA USA	20 mL
Cyclopeeze © copepods, frozen: Argent Laboratories, Redmond WA USA	10 mL

Seahorse broodstock. A pair of adult *H. bargibanti* (Figure 1) orange color morph was collected from 24.4 m depth at the dive site, "Dead Palm", Mabini, Batangas Province, Philippines (13.69569 N 120.88472 E). This work was conducted under a joint memorandum of agreement between the Philippines Department of Agriculture and National Fisheries Research and Development Institute (NFRDI) and the California Academy of Sciences (CAS), was approved by a gratuitous permit from the Bureau of Fisheries and Aquatic Resources (BFAR) as well as a permit from the Mayor of the local municipality. A pair of pygmy seahorses was collected by hand, at night, along with a small cutting of their host *Muricella* coral. They were immediately placed in a 1 L polyethylene jar and transported to a temporary aquarium holding system located directly on the beach at our field station. The following morning, the animals were packed in 1.5 L plastic bags filled with fresh natural seawater and oxygen in a 2:1 ratio, and transported by van to metro Manila, where they were repacked in 1.5 L plastic bags filled with fresh natural seawater and oxygen in a 2:1 ratio, securely packed in Styrofoam coolers with cardboard outer liners and shipped to Steinhart Aquarium in San Francisco, CA, USA, via air cargo (30 hours total transit time). Once they reached our facility, they were acclimated to aquarium water quality by slowly equalizing temperature and salinity, and then placed in a 60 L accessory aquarium connected to a 1,000 L mature exhibit of azooxanthellate stony corals and teleost fishes. They remained on a small cutting taken from their host *Muricella* coral during the entire collecting, packing, shipping, and acclimation period. Adults were fed the same variety of planktonic feeds as their host *Muricella*.

Figure 1. The pair of adult pygmy seahorses collected in the Philippines, shown on their host *Muricella* in the aquarium (original).

Larval culture and settlement. Immediately following birth, the entire brood of larval *H. bargibanti* was transferred to circular pseudo-kreisel tanks (30 cm diameter x 13 cm tall, 9 L). This type of tank is commonly used for the culture of planktonic organisms. The circular water flow keeps both the larvae and food concentrated in the center of the tank and prevents them from being removed by the filters. Fresh, filtered seawater was supplied to the pseudokreisel from a larger water system at 8-10 L/hr. Larval *H. bargibanti* were manually fed newly hatched *Artemia* nauplii (GSL, Artemia International, Fairview TX USA), a mix of live copepods (*Tisbe* sp., *Parvocalanus* sp., *Pseudodiaptomus* sp., AlgaGen LLC, Vero Beach FL USA), and live phytoplankton (*Isochrysis* sp., *Chaetoceros* sp., AlgaGen LLC, Vero Beach FL USA) four times a day during the daylight period (0900-1700 hours).

At 18 days after birth, small colonies of *Muricella plectana* and *M. paraplectana* (3-6 cm^2 surface area) were introduced to the tank to encourage settlement. When this was found to be unsuccessful, much larger (200-300 cm^2) colonies of *Muricella* were placed in the aquarium with the juveniles. A two-part feeding strategy was employed once the *H. bargibanti* settled on *Muricella* corals: (1) an automatic feeding system was used to feed the coral throughout the day as described above, and (2) additional "flood feeds" were done four times per day, at approximately two hour intervals from 8 am until 5 pm (Table 2). A "flood feed" is a high-density pulse of food added manually to the aquarium to overwhelm the gut of the *Muricella* polyps so that food accumulates on the polyp surface and tentacles, where it can be eaten by *H. bargibanti*.

Table 2

Formulation for flood feeds used to culture juveniles in an 8 L aquarium

Food type	Quantity	Frequency
Artemia nauplii, newly hatched: GSL, Artemia International, Fairview TX USA	10 mL	4x/day
Chaetoceros sp., live microalgae: AlgaGen LLC, Vero Beach FL USA	200 mL	4x/day
Isochrysis sp., live microalgae: AlgaGen LLC, Vero Beach FL USA	200 mL	4x/day
Pseudodiaptomus sp. live copepods: AlgaGen LLC, Vero Beach FL USA	10,000 adults	4x/day
Tisbe sp. live copepods: AlgaGen LLC, Vero Beach FL USA	10,000 adults	4x/day
Parvocalanus sp. live copepods: AlgaGen LLC, Vero Beach FL USA	10,000 adults	4x/day

Photo documentation of larval specimens presented some difficulty, given the limited number of larvae and the goal of keeping them alive. In order to reduce stress on the animals, all photographs were taken from outside of the culture aquaria; underwater cameras were not used in the culture aquaria. Photographs were taken with a minimum of invasive practices: no flash, and continuous supplemental lighting for no longer than ten minutes per session. Photo and video documentation was restricted to the early morning and late afternoon. With the exception of a small group of larvae from the first brood, animals were not removed from their rearing tank, so nearly all photo and video was captured opportunistically. Most photo and video work took advantage of cameras with low-light capabilities (Nikon D7000 and Nikon D800 digital SLRs). In order to take detailed measurements of size at birth, one individual was humanely euthanized with MS222 and photographed prior to preservation in 90% ethanol. In addition, a group of live seahorses from the first brood was photographed submerged in seawater in a shallow dish with a scale bar, giving us a sample size of five just-born larvae.

Mortalities that occurred during this research were preserved in both 90% ethanol and 10% formalin. Attempts were made to retrieve mortalities as soon as they were observed, but as these animals were not euthanized for documentation, the bodies were preserved in various states of autolysis. A growth sequence (Figure 2) was captured from the best-preserved specimens. These photographs were taken using the California Academy of Sciences' "Big Kahuna" imaging system (Visionary Digital, Palmyra, Virginia, USA). This system consists of a Canon 5D MkII digital camera mounted on the Visionary Digital P-51 Camlift camera carrier, controlled by proprietary software that moves the camera along the z-axis to create a stack of photographs from the subject's highest to lowest focal points. Lighting was provided by a Dynalight 2000 W flash pack and 2 flash heads. Images were captured using the Canon MPE-65 macro lens, sometimes with the addition of a Canon 1.4x extender lens. RAW images were captured using Adobe Lightroom 5 software, were color balanced and minimally sharpened, and then sent to Helicon Focus 6 stacking software for the composed image. Scale bars, final editing and

head length and height (tail-tip to coronet) measurements were made using Adobe Photoshop CS6 software analysis tools.

Figure 2. Growth series of *Hippocampus bargibanti* cultured in laboratory aquaria. Seahorses are shown (left to right) at birth, 9, 26, 28, and 124 days after birth. Scale bar indicates 1 mm (original).

Results and Discussion. This paper documents the first successful aquarium breeding and rearing (through settlement) of any pygmy seahorse species. To our knowledge, this species has only been kept in aquaria once before, with moderate levels of success (Chan & Delbeek 2004). This study shows that adult *H. bargibanti* and their host *Muricella* are difficult but not impossible to maintain in the controlled environment of laboratory aquaria.

Host Gorgonians. Host *Muricella*, being azooxanthellate, require significant and diverse feedings to thrive in aquaria. *Muricella* corals grew slowly and consistently under our husbandry regime. An advantage of our two-part feeding strategy was that the automatic feeding system kept the polyps open continuously throughout the day so that they were available to catch the flood feeds. Closed *Muricella* polyps may take as long as 30 minutes to open in response to food in the water.

Broodstock. The adult pair of *H. bargibanti* collected in the Philippines survived handling and shipping and began feeding immediately upon arrival in the facility on a variety of live foods. The male was 30.5 mm in height (measured coronet to uncurled tail-tip), and the female was 27.2 mm in height. Adults fed exclusively from the surface of their host coral: both from the area between the polyps and directly from polyp mouths. They were not observed feeding on plankton suspended in the water column. The two adult *H. bargibanti* collected lived 51 days (male specimen) and 61 days (female specimen) in aquaria. Lifespans for *H. bargibanti* in the wild are unknown, so they must be extrapolated from studies of other, similarly sized species. While many larger species of seahorses may live longer than a year, Strawn (1958) measured longevity in wild populations of the Atlantic dwarf seahorse, *H. zosterae,* and found that lifespans were rarely longer than a year, and that there was no evidence for individuals living two years. Baine et al (2008) monitored *H. bargibanti* over a 13-month period, tracking eight individuals – the longest for 40 weeks. Our aquarium observations are similar, with larger species of seahorses living three to five years, and smaller species living approximately one year. Thus, as the two specimens in this study were collected as reproductively active adults, it is quite possible that mortality occurred at the end of their natural lifespan.

However, a combination of declining brood size and poor longevity in aquaria suggests that we were not meeting the nutritional needs of a reproductively active pair of adult pygmy seahorses. Presumably the consistent decline in brood size was a direct result of inadequate adult nutrition. Prior studies with other seahorse species have shown a direct correlation between adult nutrition and brood size and health and survival of offspring (Palma et al 2012; Palma et al 2017). Adjustments to the feeding approach for the F1 generation, specifically "flood feeding" four times per day increased survival, growth and longevity, with juveniles living longer than the adults (120 days maximum lifespan for F1 generation). The "stalling out" of the juveniles' growth at approximately 73% of adult size also supports the hypothesis that the nutritional needs of adult and subadult pygmy seahorses were not being met. However, these theories need further testing with replicates and controls.

There are several potential explanations for our lack of success with adults and juveniles. First, the surface area of the host *Muricella* in aquaria was much smaller than the hosts that are selected in the wild. If pygmy seahorses feed exclusively on plankton trapped by their host coral, then their dietary intake may be directly proportional to the available surface area for feeding. The smaller host corals in aquaria would thus offer less overall nutrition than the larger hosts they would be living upon in the wild. Alternately, video of *Muricella* in the Philippines shows that there is a rich community of organisms living upon the coral, including a diversity of crustaceans and other potential prey items. It is possible that pygmy seahorses may prey upon a diverse assemblage of organisms that share their host coral; these types of prey items could not be provided in the aquarium environment. Another potential explanation is that complex social interactions and perceived competition between the juveniles occupied time that would have otherwise been spent feeding, and increased energy expenditures, resulting in stalled growth. All of these theories are supported by a free-swimming behavior observed in several specimens; they may have been attempting to find a more suitable host - either larger, with a more diverse community of commensal organisms, and/or with a less-competitive social structure. Further studies with controls and replicates are needed to evaluate these theories.

Birth and remating. Birth occurred about one hour prior to when the aquarium lights turned on. Remating occurred within 30 to 60 minutes of birth. Links to videos of pygmy seahorses giving birth and mating are provided in Annex 1. Mating includes a vertical swim, or copulatory rise, where the eggs are transferred from the female to the male, as in other species of syngnathid fishes. The mating pair leaves the gorgonian briefly for this behavior, and then returns to the branches of the coral once the egg transfer is complete. No observations were made during the first mating episode (25 May 2014). The second mating episode (8 June 2014) consisted of two copular rises; the first lasted 13 seconds, followed by 79 seconds where the pair remained on their host. They subsequently performed a second, longer copular rise that consisted of a nine second egg transfer, two seconds where they briefly separated, and a 34 second egg transfer. The final mating episode (22 June 2014) consisted of a single 29-second copulatory rise and egg transfer. Gestation was consistently 14 days, with subsequent births occurring exactly two weeks after each mating event.

Gestation period for the first brood is unknown as the adults mated prior to collection from the wild, but it is assumed to be two weeks. Brood sizes are summarized in Table 3. A final "birth" produced no viable offspring, but instead consisted of the male expelling 12 undeveloped eggs. Denise Tacket and Sara Lourie observed parturition in the wild in Lembeh Strait, Northern Sulawesi, where a single male gave birth to 34 planktonic juveniles (Lourie et al 2004). When combined with unpublished data from one observation of birth in the wild, the average brood size for the four known *H. bargibanti* birth events is 35.25 juveniles (S.D. 22.02). The three broods in our study were highly variable (max of 65 offspring, minimum of 12 offspring, average of 35.67 (S.D. 26.95). There was a decline in mating success with each subsequent birth: birth two was 46.2% of birth one; birth three was 40.0% of birth two and only 18.5% of the first birth.

Assuming pygmy seahorses produce 65 offspring every two weeks and live for one year, their potential reproductive output is more than 1,500 offspring in their short lifetime.

Brood sizes of the three pygmy seahorse births documented in our study

Date	Brood size
25 May 2014	65
8 June 2014	30
22 June 2014	12
6 July 2014	0 (12 undeveloped eggs expelled)

Larval development. Newly born *H. bargibanti* were between 6.16 and 6.56 mm in height (measured tip to coronet), with an average of 6.5 mm (S.D. 0.17 mm). Head length at birth ranged from 2.03 to 2.30 mm, with an average of 2.24 mm (S.D. 0.12 mm). Foster & Vincent (2004) cite Lourie's unpublished data measuring newborn *H. bargibanti* to be 2 mm SL. This is much smaller than the newborn larvae in our study. Seahorses were born with their tails tightly coiled against their bodies. During their planktonic phase, they continued to swim with their tales tightly coiled, using their median fins to stay in the water column and hunt for food. As in *Hippocampus kuda*, the tail plays no obvious locomotory role during the planktonic phase for *H. bargibanti*, and does not appear to be used until settlement (Choo & Liew 2006).

Growth proceeds over the 14 to 18 day planktonic larval stage, with the appearance of spines at the sites where tubercles develop. During the first 14 days, head length, expressed as a percentage of height, gently increases, while after settlement (approximately 18 days post-birth), it stabilizes and remains a relatively consistent percentage of body length. This trend continues at least through the first 34 days following birth. During this time, the tubular snout visibly shortens, as the animals change from planktonic to benthic feeding strategies (Figure 3). This is similar to allometric growth patterns observed in aquacultured *H. kuda*, where head length decreases and there are marked changes in the seahorses' feeding structures associated with an ontogenetic shift from pelagic to benthic habitats (Choo & Liew 2006).
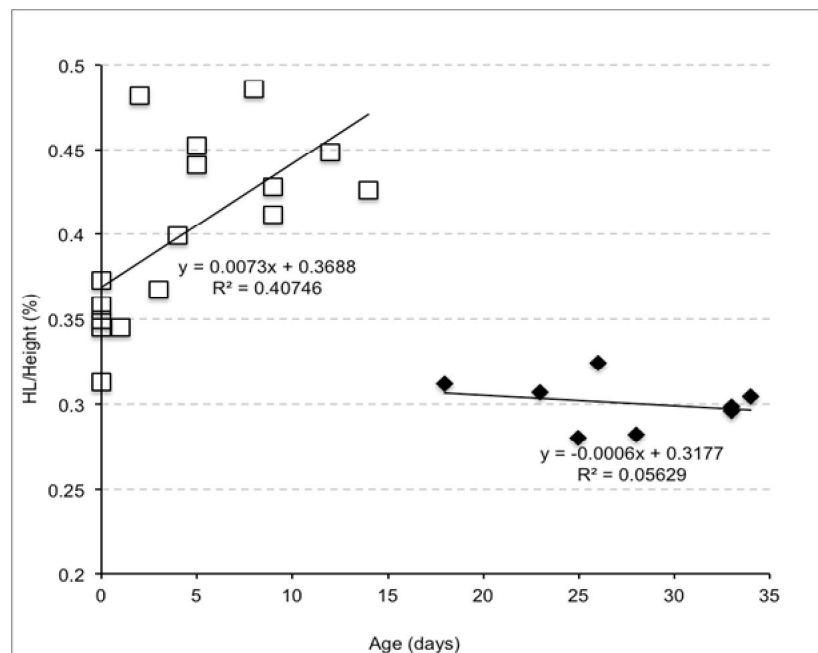

Figure 3. Head length (mm) expressed as a percentage of height (mm) over 35 days post-birth. Values are for individual seahorses, with multiple individuals of 0-day old (n = 5), 5-day old (n = 2), 9-day old (n = 2), and 33-days old (n = 2). White squares indicate larval seahorses pre-settlement (0-14 days), and black diamonds represent juveniles, post-settlement (18-34 days).

Settlement*.* The planktonic larval phase for *H. bargibanti* lasted approximately 18 days at a water temperature of 25°C. At 14 to 16 days, the larvae exhibited a lightening of the brown-black pigment on the head, and began leave the central area and swim assertively toward the bottom of the aquarium. Shading the sides of the aquarium halted this behavior; that is, the larvae appeared to be negatively phototactic. It is our belief that this behavior represents the programmed beginning of settlement behavior, where the larvae are leaving the plankton and moving toward the sea bottom, the habitat of their host *Muricella*. Juveniles settled on their *Muricella* hosts 18 to 24 days post-birth, at approximately 12.2 mm height. This is very similar to *H. denise,* where settlement occurs at 13 mm SL, and sexual maturity at 13.3 mm SL (Smith 2010).

Underwater photographers have documented recently settled juveniles, and photos are readily available on the Internet. Young *H. bargibanti* resemble adult color and morphology: enlarged tubercles, bulbous snout, patterned coloration on the body and striped tail (Lourie & Randall 2003). Juveniles are slightly darker than adults, with an underlying grey color on the body as they transition to their cryptic coloration. Individuals transitioned from a dark brown-black color with spines on their body, to a greyish body with pink tubercles over a period of 72 hours post-settlement (Figure 4).
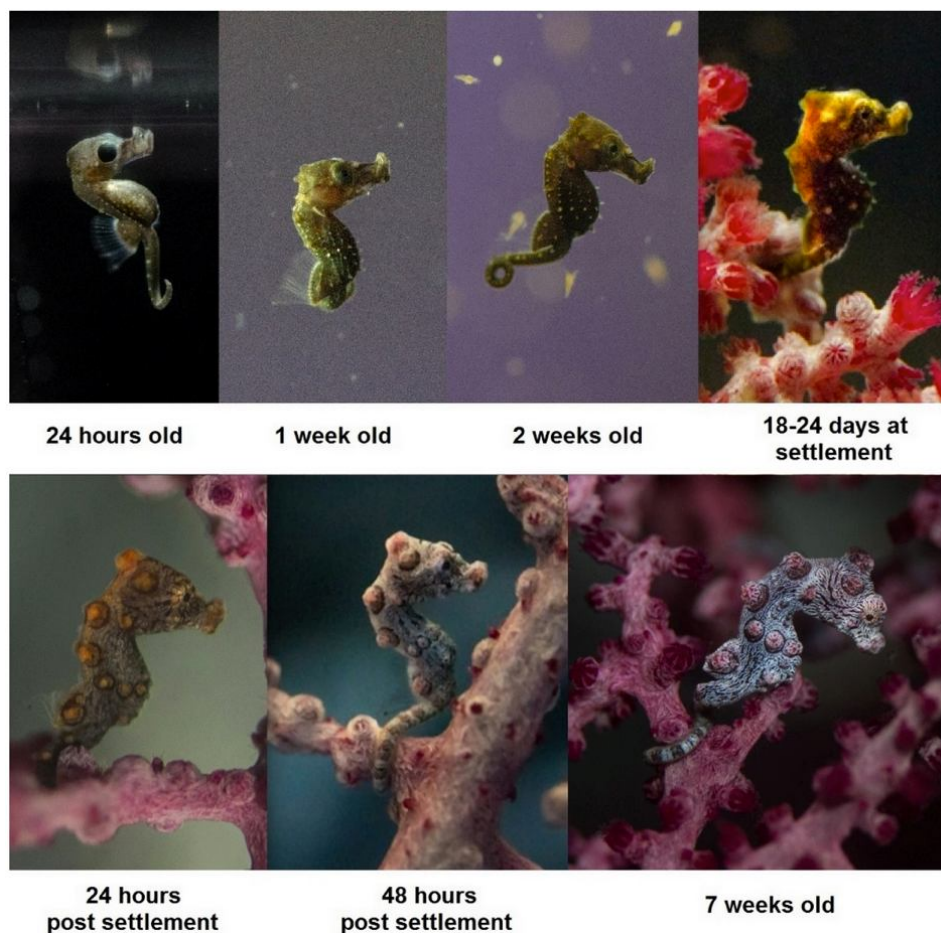

Figure 4. Color and morphological changes during the early life history of *H. bargibanti.* The transition to adult coloration occurred over 72 hours after seahorses chose settlement sites on host gorgonians in laboratory aquaria (original).

Inducing settlement was a significant bottleneck in closing the life cycle of *H. bargibanti*. We initially hypothesized that planktonic juveniles would settle based on chemical cues from their host corals. Small cuttings (3-6 cm^2 surface area) of both orange and pink *Muricella* spp. were placed in the culture tanks. Juveniles exhibited what we call "transitory settlement", where they temporarily attached to the coral, but released after less than ten minutes. True settlement and the associated change to cryptic coloration were not observed until much larger (200-300 cm^2) colonies of *Muricella* were placed in

the aquarium with the juveniles. This reinforces prior authors' suggestion that settlement, as a key component of the seahorses' life history, consists of a thorough evaluation of the host coral and a decision on whether or not it meets the animals' specific criteria (Smith 2010). Chemical cues may lead larval pygmy seahorses to their hosts, but perhaps host-selection is through visual exploration, based on criteria related to core areas necessary for feeding, mating and sleeping, and the creation of what Smith (2010) calls a "cognitive map" of their host. If adults have minimum core area sites and home ranges needed for major activities (e.g. sleeping, feeding, mating), then presumably the size of the gorgonian and the presence of appropriate protective core areas is a significant component of host selection by larvae. This would suggest a minimum size of *Muricella* needed to initiate settlement. Our data supports this hypothesis. The presence of *Muricella* in the culture tank is not enough to encourage settlement; the host coral needs to at the very least be of the right size in order for settlement to occur, and the shape and a diversity of core areas may also play a role, Further study with various sizes of host gorgonians and controls is needed in order to evaluate this hypothesis, and determine minimum host size/surface area required to initiate successful settlement.

The transition from a pelagic, planktonic larvae to a settled juvenile is marked by a relative shortening of the head and snout, expressed as a percentage of height (Figure 3), and an accompanying change in feeding strategy. Settled juveniles and adults appear to use the large surface area of the *Muricella* as a "net" to collect the plankton on which they feed. We observed individuals feeding on a combination of trapped particles, coral mucus, *Artemia* nauplii, and copepods directly from the tentacles of the open *Muricella* polyps, from the space between the polyps, and even inserting their snout into and feeding from within the polyps (video links are provided in Annex 1).

Of the 111 offspring born in aquaria, 13 were raised to settlement. The 11.7% success rate is extremely low, but understandable as this was the first attempt to raise this species in laboratory aquaria, where numerous bottlenecks impacted the survival of offspring. The greatest losses during the planktonic larval phase were due to our inability to provide proper hosts and induce settlement, and from a single episode where the culture aquarium overflowed due to clogged screens on the drains. Four of the offspring survived more than 100 days, transitioning to adult coloration and living exclusively on *Muricella* corals in aquaria (Figure 5). The last surviving individual was humanely euthanized at 120 days following birth in order to preserve a single aquarium-reared specimen for morphometrics, DNA sampling and photography. Juvenile *H. bargibanti* continued to grow slowly to about 20 mm height, and then ceased growing. The maximum size of an aquarium-reared juvenile was 22.2 mm height (120 days old), compared to the 30.5 mm height of the wild-collected male and 27.2 mm height of the wild-collected female (unknown ages).

Near the end of the study four individuals exhibited periods of free-swimming behavior: leaving the protection of their host coral and swimming in the water column for several minutes to several hours at a time. This was first noticed in a single female, who was the smallest of the four individuals being housed together. She began to leave the *Muricella* and swim in the water column 47 days after settlement (18 August 2014). This behavior was seen two days in a row and then was not observed again until 29 days later (16 September 2014) when it was seen three days in a row at the same time of day (1330 hours) for at least eight-minute intervals. The fish was already free swimming when the observation began, so exact times are not known. Two days later the fish was observed free swimming at 1000 hours, and continued free swimming for four hours, at which point she was transferred to a separate aquarium with its own resident population of host corals. She continued the free-swimming behavior in the second aquaria, absent interactions with other pygmy seahorses. She died at 110 days old with visible growths, infection or abrasions on the internal surface of the tail. Autolysis prevented thorough medical examination of these growths to identify them and determine if they were the cause of death. Three other individuals (one female, one male and one undetermined) also exhibited free-swimming behavior near the end of their lives, with similar circumstances to what was described for the smallest female. Extended periods of free-

swimming suggest that pygmy seahorses will abandon their hosts if they do not meet the basic needs for survival and reproductive success, whether due to social interactions, nutritional requirements, reproduction, and/or other factors.

Figure 5. A captive-reared juvenile *H. bargibanti* on its host *Muricella* coral (original).

Plasticity of camouflage. While the specific mechanism of pygmy seahorses' deceptive camouflage is unknown, color and morphological changes in teleosts are largely based upon neuronal and hormonal control (Sugimoto 2002). Other teleosts, most notably zebrafish and various cichlid species can adapt to light and dark backgrounds through the suppression or expression of melanophores (Henning et al 2013; Sugimoto 2002). Frogfishes (*Antennarius* spp.) exhibit incredible plasticity in regards to deceptive camouflage and mimicry of their surrounding habitat (Randall 2005). Lourie & Randall (2003) note that it is unknown whether individual *H. bargibanti* can change color or the development of their tubercles based upon the color of their hosts. The morphological color change of pygmy seahorses is gradual, and must be distinguished from physiological color change of organisms such as cephalopods, where specialized chromatophores allow for rapid color change in response to external stimuli (Henning et al 2013; Sugimoto 2002; Zmarzly et al 1990). In our experience, orange adult seahorses did not change color when hitched to pink *Muricella*, however this was over a relatively short period of time (51 to 61 days post-collection) and the pair had access to, and routinely used, both pink and orange *Muricella* in their aquarium (Figure 6). It is possible that *H. bargibanti* could change from one color morph to another, given time, however this would be highly unlikely in the wild, given that adults have not been observed moving from one host to another (Baine et al 2008). In aquaria, the pair of orange adults produced offspring that, upon settlement, were pink in color. This shows that, at least during the settlement phase, tubercle morphology and color are subject to neuronal, hormonal, and perhaps other mechanisms of control, and that juveniles mimic the color of their chosen host, rather than color being predetermined by parental genetics. Our study is the first to document this plasticity within the larval development and transition to juvenile coloration in any pygmy seahorse species.

Figure 6. An orange adult pygmy seahorse hitched to a pink *Muricella* coral (original).

Until this point, studies concerning behavior, social structure, growth rates, and feeding strategy, along with the dynamics of the selection and interaction with *Muricella* have been impossible for *H. bargibanti*. The techniques described in this paper allow future experimental work on the genetic mechanism and flexibility of protective coloration in *H. bargibanti*, as well as delineation of the criteria driving host-selection and social structure of seahorse groups on a single host. Further work needs to be conducted to determine feeding strategies for subadult and adult pygmy seahorses in order to accomplish sustainable captive breeding and to determine the duration of their natural lifespan. Prior studies have shown increased growth rates and survival of seahorse offspring when they are fed enriched or naturally high fatty acid foods, including copepods and copepod nauplii, compared to those raised on less nutritious foods (Chang & Southgate 2001; Payne & Rippingale 2000). Assuming the nutritional needs can be met, aquacultured *H. bargibanti* could be used for restoration efforts in the event of localized extinctions, or perhaps to enhance local populations to increase the socioeconomic value of dive related tourism. Due to the complex husbandry for both *H. bargibanti* and its host *Muricella*, this species is not likely to ever be a good candidate for the marine aquarium trade. Assuming sustainable production is possible, displays of captive-bred *H. bargibanti* in public aquaria have the potential to highlight the charismatic pygmy seahorses and help inspire advocacy for global conservation of coral reef ecosystems. However, we discourage repeated field collections due to the apparently low population densities and multiple tiers of existing conservation threats.

Conclusions. This study successfully documents that *H. bargibanti* and their host *Muricella* are difficult but not impossible to maintain in the controlled environment of laboratory aquaria. The presence of host corals is not enough to elicit settlement; juvenile seahorses select their hosts based on certain criteria. For the first time, it is reported that the development of camouflage in *H. bargibanti* is based on environmental cues, and does not necessarily reflect the parents' coloration. Pygmy seahorses will abandon their host sites if they do not meet their basic needs for survival and reproduction.

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The following video files of various seahorse behaviors are available online on the California Academy of Sciences' YouTube channel:

S1. *Hippocampus bargibanti* mating, free swimming, and resting on pink *Muricella.* https://youtu.be/VqIEvTNBU7o

S2. *Hippocampus bargibanti* mating, free swimming, and resting behind orange *Muricella*. Note the newly born seahorse swimming in background at 0:20. https://youtu.be/b7EBIkW8RcY

S3. Male *Hippocampus bargibanti* giving birth to a single offspring. https://youtu.be/XZ49fe1WNpI

S4. Larval *Hippocampus bargibanti* feeding on 24-hour old *Artemia*. https://youtu.be/p_bUlWN7xYA

S5. Adult, captive bred, *Hippocampus bargibanti*, feeding directly from a coral polyp. https://youtu.be/7hJ07yQ9K0U

S6. Adult, captive bred, *Hippocampus bargibanti*, feeding directly from a coral polyp. https://youtu.be/9I-puJRPRGM