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HUSBANDRY REPORT

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Husbandry conditions of spotted ratfish (*Hydrolagus colliei*, Chimaeriformes) in aquaria for successful embryonic development and long-term survival of juveniles

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Abstract

The spotted ratfish *Hydrolagus colliei* is the most common holocephalan species exhibited in aquaria worldwide for introducing deep-sea environments and raising awareness of their conservation. However, little is known about the biology of *H. colliei*. Current practices in aquaria allow long-term survival of sexually mature *H. colliei* specimens; however, this species struggles to complete a reproductive cycle in captivity mostly because embryos do not reach the hatchling stage. The aquarists of Planet Ocean Montpellier (POM, France) have bred *H. colliei* for 15 years and recorded parameters suitable for this species' successful embryonic and post-embryonic development. POM aquarists now regularly record egg-laying events of *H. colliei* and use four tanks to incubate eggs and raise neonates, late hatchlings, early and intermediate juveniles, subadults, and sexually mature specimens. In this work we provide the first long-term biometric data on *H. colliei* from the hatchling to the subadult stage. We also report the biotic and abiotic parameters sufficient to breed *H. colliei* in aquaria. We finally describe the methods used to facilitate individual monitoring of specimens along the ontogeny and several pathologies identified in this species, their putative causes, and the corresponding treatments. This work highlights the importance of ex situ research and points to the valuable outcomes of collaborative efforts between aquaria and academia in deciphering the biology of species whose study in the wild remains challenging.

KEYWORDS

aquarium, captivity, chondrichthyan, fish, ontogeny

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1 | INTRODUCTION

Chimaeriformes are cartilaginous fishes rarely observed in the wild, which implies that the biology and ecology of these species are mainly inferred from collection specimens or bycatch. Most Chimaeriformes inhabit great depths, and live catches are rare due to the capacity of fishing vessels and the ability of specimens to survive a rapid transition from great depths to the surface. Furthermore, maintaining captive chimeras in aquaria requires fully developed specimens to survive to capture, transport, and acclimatization, and collected eggs to undergo normal development in aquaria (Boisvert et al., 2015; Martins et al., 2018; Tozer & Dagit, 2004). The spotted ratfish (*Hydrolagus colliei*, Lay & Bennett, 1839) is a bottom-dwelling Chimaerid inhabiting the Northeastern Pacific coast from shallow to deep waters (0–1029 m; Weigmann, 2016), which allows live capture and survival of specimens. For this reason, *H. colliei* is the most common holocephalan displayed in aquaria worldwide for more than three decades. Adults display a high survival rate in aquaria; however, the complete reproductive cycle of *H. colliei*, including mating, egg laying, hatchling, and growth until sexual maturation, has not been achieved in captivity. Since 2014, a monitoring program has been initiated by the European Union of Aquarium Curators. Data from 13 aquaria worldwide were gathered on captive conditions of wild *H. colliei*. However, data about the reproductive cycle and the ontogeny of *H. colliei* are sporadic, especially regarding embryonic development and early ontogeny, because sexually mature specimens rarely mate in captivity, lay unfertilized eggs, or because the embryos do not reach the hatchling stage or die shortly after hatching. For more than a decade, the aquarium of Planet Ocean Montpellier (POM) has dedicated efforts to allow *H. colliei* to complete an entire reproductive cycle in captivity. The first hatchlings from captive breeding were observed in 2016 and regularly recorded since 2018 when aquarists reached suitable environmental parameters for the successful embryonic development of this species. Subsequent efforts aimed at setting up suitable environments for neonates to reach sexual maturity.

This work reports environmental parameter values for the breeding of *H. colliei*, as well as the life-history traits of this species, especially regarding the first ontogenetic stages. The pathologies observed in POM captive specimens, and the corresponding treatments are also detailed. We hope this work again proves the crucial need for collaboration between aquaria and academia to tackle challenging research questions on animals rarely studied in the wild (Feldheim et al., 2022; Harahush et al., 2007; Toledo et al., 2022). We expect long-term breeding of *H. colliei* to facilitate access to selected ontogenetic stages to update previous descriptive studies (Dean, 1906; Didier et al., 1998) on this topic and pave the way for future work on holocephalan development.

2 | HOUSING AND OBSERVATION METHODS

H. colliei specimens are maintained in four different tanks depending on their total length (TL) to avoid cannibalism on eggs (see Section 2.4 on Sexually mature specimens) and to provide water currents and volumes adapted to specimens of distinct sizes (Payne, 2012). This separation also facilitates individual monitoring and a suitable feeding process based on the age of specimens.

The photoperiod duration is 10 h (from 8:00 a.m. to 6:00 p.m.) for all specimens, but the light intensity differs between tanks (Figure 1). The light changes gradually in the morning and the afternoon to simulate sunrise and sunset. The photoperiod is synchronized with the working hours of aquarists to facilitate monitoring several times a day. The temperatures vary seasonally in the breeding, intermediate, and display tanks: 8°C from November to February, 10°C from March to April and September to October, and 12°C from May to August (Tozer & Dagit, 2004). The measured temperature can be 1°C more than expected in these tanks. The temperature in the incubation tank has the same seasonality as the other tanks but is 1°C lower. In the last 10 years, the water quality parameters of *H. colliei* tanks were 32–33 g/L for salinity, 7.8–8 for pH, 96% for dissolved oxygen levels, 0.04–0.17 mg/L for ammonium levels, 0.002–0.008 mg/L for nitrite levels, and 5–8 mg/L for nitrate levels. The bottom of the tanks is covered with light brown sand filter (granulometry: 0.8–1.25 mm) in incubation, breeding, and intermediate tanks, and with black sand filter (granulometry: 1–3 mm) in the display tank (Figure 1). *Nassarius* sp. was added in 2020 as a tank cleaner in all tanks, except in the incubation tank to avoid accidental suction of embryonic content after the opening of the egg capsule.

Neonates and early juveniles are weighed and measured at death. Equivalent values are taken from late juvenile and sexually mature specimens when anesthetized for a specific treatment (e.g., transfer between tanks, veterinary examination, and treatments; see Supporting Information S1: Material 1 for details on anesthesia). The body length of chimeras is often measured from the tip of the snout to the anterior edge of the vent (snout–vent length [SVL]; Didier & Rosenberger, 2002) mainly because the tip of the tail is often missing due to wounds caused by fishing methods or captive conditions, or decay of specimens. However, good preservation of captive specimens at POM allows the measure of TL from the tip of the snout to the tip of the tail (Clerkin et al., 2017; Didier & Rosenberger, 2002).

Each egg is identified with a unique combination of number and color code supported by a sectioned straw (Figure 2a). After hatching, the sex and unique color patterns (form and location of dots along the body) allow specimen identification (Figure 2b1,b2). However, these color patterns change along the ontogeny of a specimen and must be monitored carefully to avoid identification mistakes. Unique

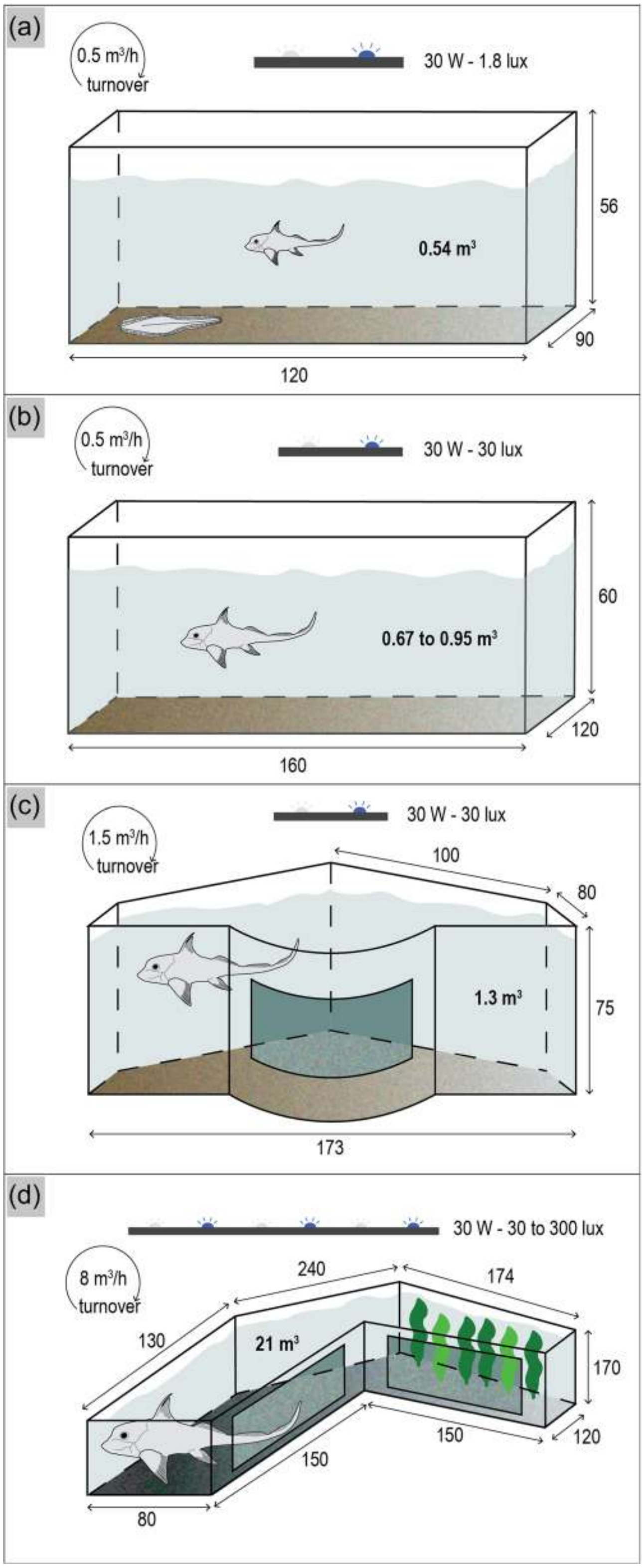

FIGURE 1 Abiotic conditions in the four tanks hosting *H. colliei* at POM. (a) Incubation tank; (b) breeding tank; (c) intermediate tank; (d) display tank. Tank volumes are in bold and tank dimensions are in cm. [Color figure can be viewed at wileyonlinelibrary.com]

identification of specimens is also based on TL when a few individuals share the same tank.

2.1 | Eggs and neonates

The eggs are maintained in the incubation tank (Table 1 and Figure 1a) as soon as they are laid or retrieved from the female. Neonates are kept in the same tank as eggs until they reach 6 months old (up to 17 cm TL, Table 1). The eggs are laid on the sand and are externally checked daily to remove the decayed ones. Once a month, the developing embryos are imaged using "candling" (Figure 3), a noninvasive method to observe the vitellus or the embryo by shining light through the egg case that has already been proven successful in elasmobranch species (Harahush et al., 2007; Musa et al., 2018, 2020). Hatchlings are fed twice a day with Dough paste (recipe adapted from Hoff [1996, p.70]; see Supporting Information S1: Material 2). Dough paste has many advantages: it has higher nutritional content than frozen food (see Hoff [1996, p.70] for detailed nutritional information) such as mysids, is very appetizing for fishes, and Dough paste sticks remain cohesive in seawater, which makes it easy to feed chimeras with pliers. In addition, hatchlings are provided with defrosted mysis, krill, and small pieces of smelt. They also feed on ophiurids, amphipods, and isopods, which were introduced in the tanks with seawater and rocks.

2.2 | Late hatchlings and early juveniles

Specimens in the breeding tank are 6 months to 1.5 years old (from 13.0 to 17.5 cm TL, Table 1 and Figure 1b). They are fed on Dough paste with pliers and small pieces of shrimp and smelt.

2.3 | Late juveniles

The intermediate tank houses late juveniles aged between 1.5 and 3 years (from 23.0 to 27.0 cm TL, Table 1 and Figure 1c). Specimens have access to a feeding box filled with Dough paste and are fed once a day with small pieces or whole smelt, mussel meat, and crushed shrimps (gills, pereiopods, and pleopods included) and crabs.

2.4 | Sexually mature specimens

Sexually mature specimens include one female and one male (Table 2) caught off Puget Sound. They have been at POM since 2016 (Table 2) in the biggest tank, also the only one on public display (Table 1 and Figure 1d). Specimens are fed primarily on whole smelts

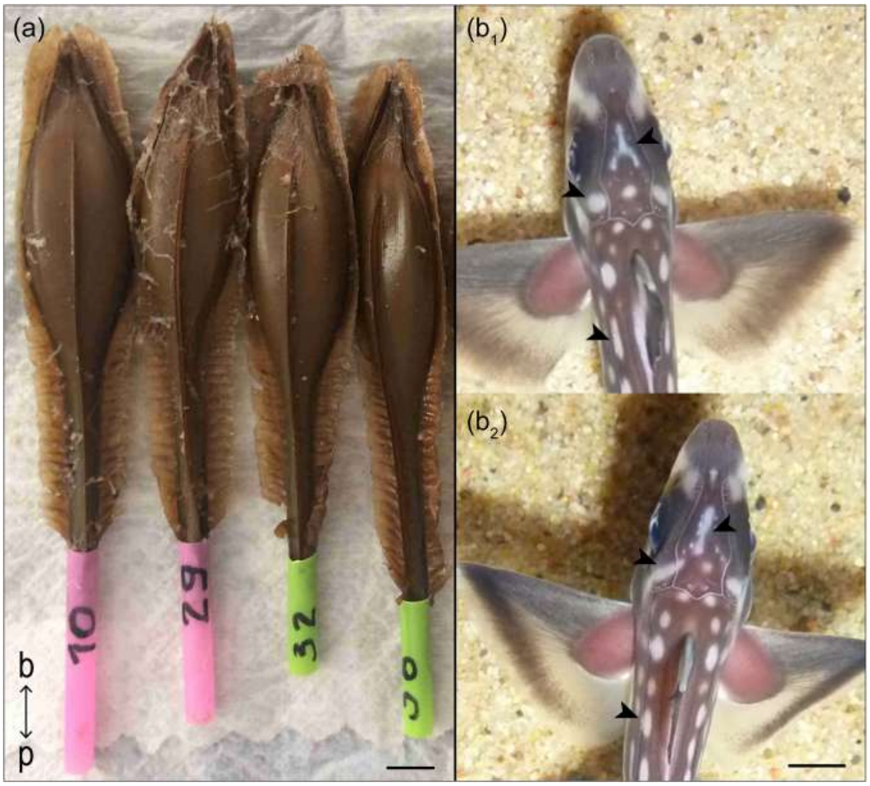

FIGURE 2 Identification method of eggs and hatched *H. colliei*. (a) A section of a colored and numbered straw is placed around the tip of the egg pedicle. b, egg beak; p, egg pedicle. (b1, b2) Specific color patterns (arrows) allow differentiating and identifying specimens of the same total length. The scale bar is 1 cm in (a) and 5 mm in (b). [Color figure can be viewed at wileyonlinelibrary.com]

TABLE 1 Age and biometrics of *H. colliei* specimens hosted in four tanks at POM.

Tank	Stage	Age	TL (cm)	Weight (g)
A. Incubation	Eggs and neonates	0–6 M	6.0–17.0	4.7–12.0
B. Breeding	Late hatchlings and early juveniles	6 M–1.5 Y	13.0–17.5	11.9–18.9
C. Intermediate	Medium juveniles	1.5–3 Y	23.0–27.0	62.0–106.0
D. Display	Late juveniles and sexually mature specimens	>3 Y	35–54	225.0–1110.0

Note: Overlapping TL and weight values are due to variable timing of biometric measurements in different specimens and intraspecific variability.

Abbreviations: M, months; POM, Planet Ocean Montpellier; TL, total length; Y, years.

or pieces of smelt, sprat, and sardines, as well as on crushed mussels and shrimps. The aquarists have observed and documented rare mating events because they spend, on average, 10 min a day for routine care of chimeras (Figure 4a).

Egg laying has often been observed because the eggs remain attached to the mature female through the cloaca for 24 h ("several hours" in the silver chimera *Chimaera phantasma* [Dean, 1906, p.25]) (Figure 4b). However, cannibalism on eggs has been noticed, as well as predation by tank mates once laid on the floor (Supporting Information S1: Material 3). For that reason, when possible, the eggs are gently and manually retrieved from the female and placed in the incubation tank. Since 2018, two boarfish (*Capros aper*) and more than 50 shiner perch (*Cymatogaster aggregata*) share the tank with the sexually mature spotted ratfish and no signs of aggression on adult ratfish or predation on their eggs have been reported.

3 | DEMONSTRATION OF EFFICACY

3.1 | Reproductive cycle of mature specimens

There is no evidence that the two late juveniles (AMN30 and AMN35, male and female, respectively; Table 2) are sexually mature because no mating event was observed and the female does not lay eggs. Assessment of the sexual maturity of specimens is beyond the scope of this report, because it would involve intrusive methods (e.g., blood analysis) that are stressful for the specimens. However, the sexual status of these specimens can be inferred from their size and compared to literature data. King and McPhie (2015) provide equations to convert between TL, precaudal length (PCL; Didier & Rosenberger, 2002), and SVL in *H. colliei* sampled from California and Washington's continental slope and shelf. Based on their TL, we estimate AMN30 to be >30 cm PCL and >17 cm SVL and AMN35 to be >23 cm PCL and >13 cm SVL. The PCL and SVL estimates for AMN30 fall within the range of subadult and sexually mature *H. colliei* because the smallest mature male reported by King and McPhie (2015) is 25 cm PCL and is 14.9 cm SVL in the work of Barnett et al. (2009). Based on the PCL and SVL estimates, AMN35 is farther from sexual maturity, as the smallest mature female is 37.9 cm PCL and 19 cm SVL in the study by King and McPhie (2015), and Barnett et al. (2009), respectively. On the other hand, AMN30 and AMN35 are 4.4

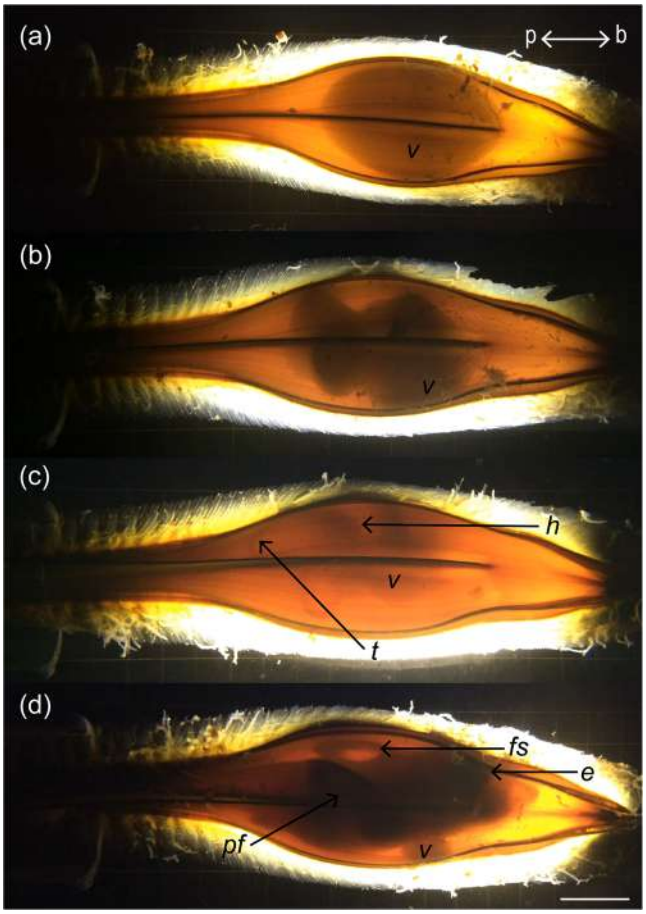

FIGURE 3 The monthly monitoring of *H. colliei* embryos is achieved with “candling”. (a) Freshly laid egg containing a rounded vitellus (1 day); (b) egg containing a subdivided vitellus (98 days); (c) early-term embryo with head and thin tail already visible (116 days); (d) late-term embryo, the vitellus is almost fully absorbed, the eye, pectoral fin, and dorsal fin spine are visible (395 days). b, egg beak; e, eye; fs, dorsal fin spine; h, head of the embryo; p, egg pedicle; pf, pectoral fin; t, tail of the embryo; v, vitellus. The scale bar is 1 cm. [Color figure can be viewed at wileyonlinelibrary.com]

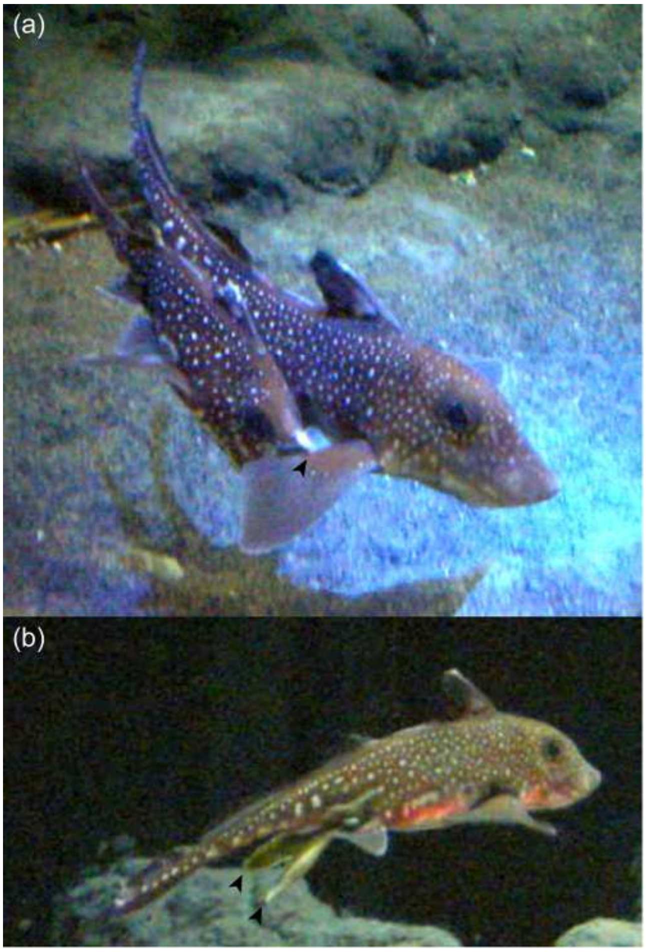

FIGURE 4 *H. colliei* specimens mate and lay eggs in captivity. (a) During mating, the male clasps the female's pectoral fin with its tenaculum (arrow) to facilitate clasper intromission; (b) eggs (arrows) are laid by pair every 2 weeks and remain attached to the female for a maximum of 24 h. [Color figure can be viewed at wileyonlinelibrary.com]

TABLE 2 Oldest *H. colliei* specimens at POM.

Specimen	Sex	Stage	Age (days)	TL (cm)	Weight (g)	Last biometry	Origin	At POM since
AMN13	Male	Mature	>2539	46	560	June 2021	Wild	September 15, 2016
AMN14	Female	Mature	>2539	54	1110	June 2021	Wild	September 15, 2016
AMN30	Male	Late juvenile	>1615	41	550	March 2023	CB	March 28, 2019
AMN35	Female	Late juvenile	>1554	30	400	March 2023	CB	May 28, 2019
AMN93	Female	Early juvenile	>326	15	59	July 2023	CB	October 7, 2022
AMN97	Male	Neonate	>245	17.2	46.2	July 2023	CB	December 27, 2022

Note: Wild specimens are attributed a minimum age corresponding to the number of days since their arrival at POM.

Abbreviations: CB, captive breeding; POM, Planet Ocean Montpellier; TL, total length.

and 4.3 years old, respectively. The youngest mature male and female from the study of King and McPhie (2015) are estimated to be 9 and 11 years old, respectively, which suggests that AMN30 and AMN35 will not reach sexual maturity before 5–7 years. However, in elasmobranchs, age and size at sexual maturity in different populations vary with environmental conditions (Capapé et al., 2014; Frisk & Miller, 2006; Lombardi-Carlson et al., 2003) and daily, abundant food supply might likely trigger earlier sexual maturity in captivity than in the wild (Howard, 2017).

Observations of rare mating events at POM are consistent with personal observations mentioned in the literature (Barnett et al., 2009; Didier & Rosenberger, 2002) that report a male approaching a female while swimming and clasping the female's pectoral fin with its tenaculum (Figure 4a) before clasper intromission into the female's cloaca. Mating events last a minimum of 10 min. As mating events are rarely observed, it is impossible to determine if a female mates with several males (when sharing the same tank) and, therefore, to attribute paternity of developing embryos. However, muscle tissue is regularly sampled from dead specimens and could be further used for DNA analyses to establish the pedigree of specimens born at the aquarium. Furthermore, the sexually mature female displays no mating scars (such as those described by Dean [1906]). However, these might be difficult to notice on a moving specimen and may also have disappeared when the specimen was anesthetized for healthcare. On average, the mature female lays eggs every 2 weeks (Figure 5). For comparison, the interval between spawning events in the elephant shark *Callorhinchus milii* is 7 to 10 days (Didier et al., 1998). The sexually mature *H. colliei* female most likely spawns during the whole year in constant environmental conditions. However, no eggs were found in the tank between October 7, 2021 and December 27, 2021 (Figure 5), which could indicate a pause (81 days, approximately five laying events) in the female's reproductive cycle. Yet, this pattern was observed in 2020 but not in 2021, when the female spawned every month, although less frequently between October and January than the rest of the year. In the past few years, several eggs have remained hidden from the aquarists while developing in the tank of mature specimens. Some were trapped in the pumping system and three reached the hatchling stage. The gap displayed in Figure 5 could, therefore, be an artifact due to eggs being undetected by the observers, and the recurrence of a seasonal spawning pattern will be confirmed moving forward.

3.2 | Embryonic development

The incubation duration of *H. colliei* eggs is 406 ± 34 days (12.2–14.5 months, $N = 8$). The interindividual variation might be attributed to slight temperature changes during the year (7–11°C). The duration of embryonic development in *H. colliei* at POM is longer than what was previously reported in the literature for this species in captivity ("not less than 9 months and possibly as long as twelve" at 10–15.5°C [Dean, 1906, p.27]) and for *C. milii* (143 ± 1.3 days [4.7 months] at 16–17.8°C [Boisvert et al., 2015]). There is evidence of a shorter incubation time as temperature increases in elasmobranchs, as exemplified in the brownbanded bambooshark *Chiloscyllium punctatum* (~19 days longer at 26°C than 30°C, Rosa et al., 2014), the small-spotted catshark *Scyliorhinus canicula* (~7 days longer at 15°C than 20°C, Musa et al., 2020), the big skate *Beringraja binoculata* (~47 days longer at 10°C than 13–15°C, Howard, 2017), and the small-eyed skate *Raja microocellata* (~27 days longer at 12.5°C than 14.5°C and ~21 days longer at 14.5°C than 16.5°C, Hume, 2019). Similarly, developmental time in *H. colliei* might fluctuate with temperature.

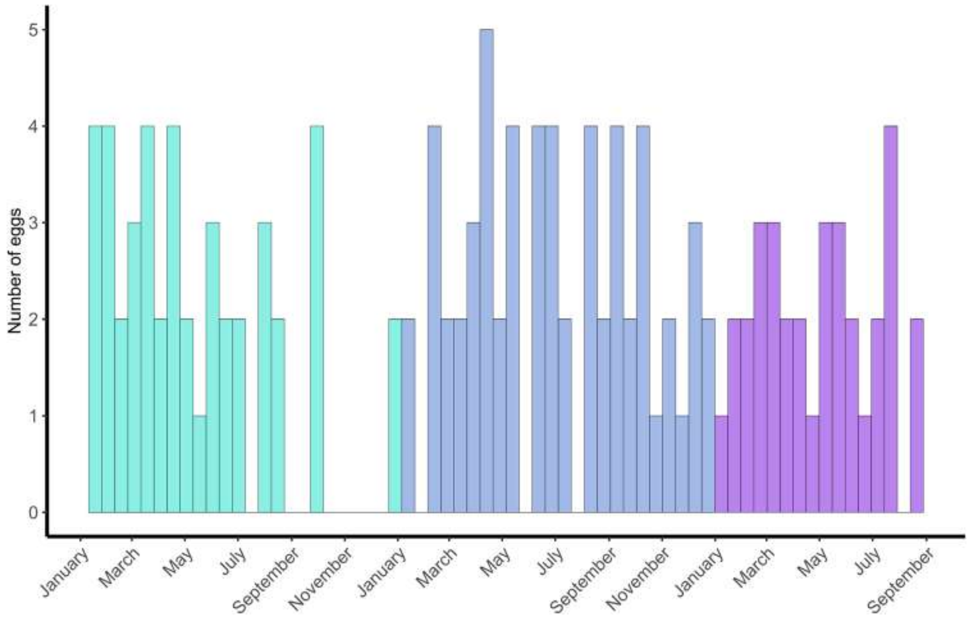

FIGURE 5 Egg laying frequency of captive *H. colliei*. Data from a >6 years old (>54 cm total length) female. One bar corresponds to 15 days. Years are of different colors: light blue, 2021; dark blue, 2022; violet, 2023. [Color figure can be viewed at wileyonlinelibrary.com]

Therefore, the longer incubation time reported in this study compared to Dean's (1906) work is consistent with the shorter developmental time in 3°C to 4.5°C warmer waters (Dean, 1906). Literature reports *H. colliei* inhabiting cold waters (~7 to ~14°C [Andrews & Quinn, 2012; Tozer & Dagit, 2004]) and a wide range of depths (from the surface to 1024 meters deep [Weigmann, 2016]), but the lacunary record of *H. colliei* nurseries makes it challenging to infer the natural temperature range(s) of developing embryos. Even if embryonic development is completed from ~7°C to 15.5°C (Dean, 1906; this study), embryonic mortality might fluctuate within this temperature range.

We report that about half of the eggs are either not fertilized (embryo not developing from the vitellus), decayed (whitish egg case [Payne, 2012]), or empty (wind eggs [Powter & Gladstone, 2008]). Although the reason for empty egg cases remains unknown, decaying eggs might be due to endogenous (e.g., congenital abnormalities) or exogenous (e.g., pathogens crossing the mucous plug) issues during embryogenesis, as this has already been suggested in oviparous sharks (Payne, 2012). Nevertheless, assessing the factors that stop the development remains challenging due to the rapid decomposition of the vitellus and, eventually, the embryo (Harahush et al., 2007; Payne, 2012).

Monthly monitoring of *H. colliei* embryonic development with "candling" provides this species' first pictures of yolk subdivision (Figure 3b, see also the drawings of Dean [1906]) and allows determining approximate developmental timing of the eyes, fins, and fin spine (Figure 3d). Imaging of precise embryonic details would require a more stressful procedure for the specimens (e.g., removal of the egg case external membrane, windowing of the egg case, removal of the embryo from the egg case) that would increase the mortality rate of embryos. However, our primary goal is to reach a high survival rate for *H. colliei* embryos before starting experimental procedures on the specimens. Therefore, further developmental investigations would only be conducted at POM once ≥50% of captive-bred specimens routinely reach the hatchling stage.

3.3 | Life-history traits of post-hatched specimens

At hatchling (1–3 days), *H. colliei* specimens weigh 5.7 ± 0.7 g ($N = 6$) and are 9.3 ± 0.7 cm in TL ($N = 7$). As specified in Section 2 on Housing and observation methods, access to such data at hatching is only available from specimens dying shortly after hatching, which limits the amount of data retrieved for this specific ontogenetic stage. For that reason and because of the rapid decay of specimens after death, the sex ratio at birth is uncertain (26.0% indeterminate, 44.8% females, and 29.2% males). The vitellus is already fully absorbed in neonates, although small external remnants of the yolk and yolk stalk could still be observed at hatching in some specimens. Neonates consume food provided by the aquarists 16.6 ± 10 days (minimum = 2, maximum = 37, $N = 10$) after hatching. Reasons for such variability in first food intake can be twofold. First, the amount of absorbed vitellus differs between specimens at hatching (qualitative size differences of vitellus inside the abdominal cavity, observations during necropsies), making them differently dependent on external food supply. Second, interindividual preferences of food sources could explain why some specimens feed on Dough paste shortly after hatching. In contrast, others may prefer to start feeding on the ophiurids and crustaceans living in the tank. This latter behavior has been observed in neonates, even before they start eating Dough paste.

Neonates undergo near-exponential and near-logarithmic increases in weight and body length, respectively (Figure 6a,b). Furthermore, posthatching weight increases rapidly during 1200 days, whereas growth rate decreases from the third year (Figure 6a,b). The rapid TL increase early in ontogeny is consistent with growth curves estimated with different methods in several elasmobranch (e.g., *C. punctatum* [Rossi et al., 2015], Port Jackson shark *Heterodontus portusjacksoni* [Tovar-Ávila et al., 2009], zebra shark *Stegostoma tigrinum* [Toledo et al., 2022], Alaska skate

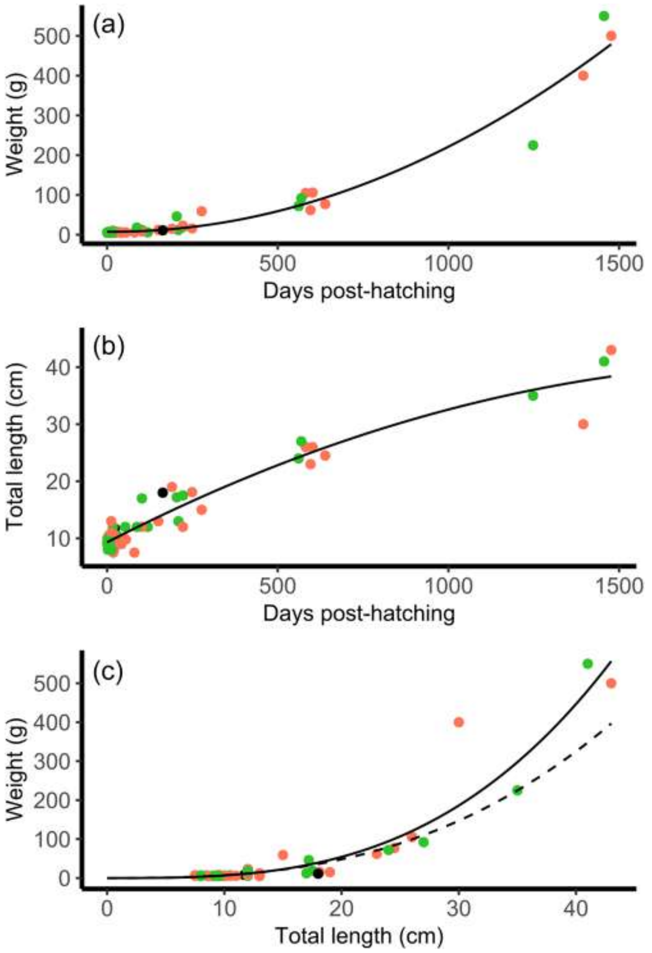

FIGURE 6 Body measurements during the ontogeny of *H. colliei*. (a) Weight; (b) total length; (c) length-weight relationship from this study (solid line; nonlinear regression, combined sexes; $R^2 = .903$) and from Barnett et al. (2009) after snout–vent length to total length conversion based on King and McPhie (2015) (dashed line; combined sexes). Black, indeterminate sex; green, male; orange, female. [Color figure can be viewed at wileyonlinelibrary.com]

Bathyraja parmifera [Matta & Gunderson, 2007]) and holocephalan (e.g., rabbit fish *Chimaera monstrosa* [Calis et al., 2005]) species.

The relationship between body weight and total body length in neonates and juveniles born in captivity is displayed in Figure 6c and described by the following equation:

$$W(g) = 0.005979 \times TL(cm)^{3.041984}$$

Barnett et al. (2009) provided a length-weight relationship for *H. colliei* from trawl and longline surveys (Figure 6c). We report that chimeras at POM tend to grow faster than these specimens in the wild, which is most likely due to more stable environmental conditions and abundant food supply in captivity.

3.4 | Pathologies

3.4.1 | Parasites

Necropsies of wild caught, sexually mature *H. colliei* maintained at POM often reveal tapeworms located in the spiral intestine, which are suspected to be *Gyrocotyle* based on their morphology and location within the host (Barčák et al., 2021; Karlsbakk et al., 2010; Simmons & Laurie, 1972). However, they are never found in captive-bred neonates and juveniles, indicating that this parasite cannot complete a developmental cycle in the chimera tanks at POM. Gyrocotylideans are widely spread among holocephalans but the identification of species among this group is a challenging task since it requires a fixation protocol that favors specimen relaxing and good preservation, and taxonomic expertise in this group (Barčák et al., 2021; Bray et al., 2020). Furthermore, we report that the parasitized specimens showed no necrosis or extended signs of inflammation around the flatworms' anchorings, and it seems unlikely that the parasites caused the death of the hosts. However, the specimens were treated with praziquantel (taken orally; 10 mg per kg once a week for 3 weeks) to limit the infestation. No tapeworms were reported in *H. colliei* following this treatment. Free coelomic parasites are also reported in the wild specimens and identified as the digenetic trematode *Otodistomum hydrolagi*, Schell, 1972 (Morrison et al., 2020; Schell, 1972). The captive spotted ratfish were not treated against this parasite (praziquantel injection was considered) because they might have undergone anaphylaxis after parasites' death. Parasitic copepods from the species *Acanthochondria epachthes*, Wilson, 1908 (formerly *Chondracanthus epachthes* Wilson, 1908 [Wilson, 1908]) were identified in the gill cavity of some *H. colliei* specimens. The chimeras were successfully treated by balneation with the chitin-synthesis inhibitors lufenuron or diflubenzuron at 0.1 ppm for 4 weeks.

3.4.2 | Morphology and physiology

Dental plates

The dental plates of some specimens displayed abnormal shapes (Figure 7a1,a2), likely resulting from irregular abrasion.

Chimaeriformes display three pairs of dental plates, two on the upper jaw and one on the mandible, that undergo continuous growth, unlike elasmobranch teeth that are permanently shed and replaced (Didier, 1995; Luer et al., 1990; Rasch et al., 2020). In the wild, a diet high in hard-bodied prey contributes to tooth plate abrasion in Chimaeriformes; however, gut contents of several species reveal a diet composed of both soft- and hard-bodied prey (e.g., shrimps, urchins, gastropods, fishes, nudibranchs, squids, and opisthobranchs), suggesting that chimeras are opportunistic feeders (Dean, 1906; Didier, 1995; Johnson & Horton, 1972). In the wild, *H. colliei* feeds on shrimps, gastropods, echinoderms, bivalves, and polychaete worms, and diet composition varies between populations (Johnson & Horton, 1972; Reum & Essington, 2008). In aquaria, *H. colliei* is fed from diverse sources, including Dough paste, squids, krill, fish, and shrimps. An increased proportion of hard food, such as crabs and mussels, has been introduced into the diet of captive *H. colliei* to reduce the uneven abrasion patterns of dental plates. However, macroscopic observations did not reveal a subsequent significant change in the dental plate shapes of the specimens, and tests with different hard food sources (e.g., urchins) remain to be done.

Fins

Several young specimens exhibited abrasions of the pectoral and pelvic fins (Figure 7b). The skin covering the edge margin and the tip of these fins was lost, and the ceratotrichia were no longer tight together within connective tissue but separated from each other (Figure 7b) and in direct contact with seawater. Injured fins were disinfected locally under anesthesia with povidone-iodine. The specimens were further treated by balneation with metronidazole and sulfadiazine-trimethoprim (5:1) to prevent infestation with protozoa and bacterial necrosis of the fins, respectively. Despite these treatments, death of the specimens occurred during the weeks following the emergence of the pathology. As Tozer and Dagit (2004) suggested, fin abrasion may be caused by specimens swimming too close to the tank wall. This pathology was mitigated after adding artificial aquarium kelps against tank walls (Figure 1d).

Goiter

A goiter was reported in several specimens (Figure 7c), presumably resulting from thyroid enlargement. Such pathology has already been observed in other captive chondrichthyans and was attributed to difficulties in iodide uptake by the thyroid epithelium (Stedman & Garner, 2018). This pathology was resolved 1 to 2 months after seawater was supplemented with potassium iodide at 0.08 ppm every month and no goiter was further observed.

3.4.3 | Behavior

Short after hatching and during the juvenile stage, some specimens displayed abnormal locomotory behavior consisting of vertical or horizontal spinning, primarily close to the water surface. This behavior could persist for weeks after hatching and alter the feeding

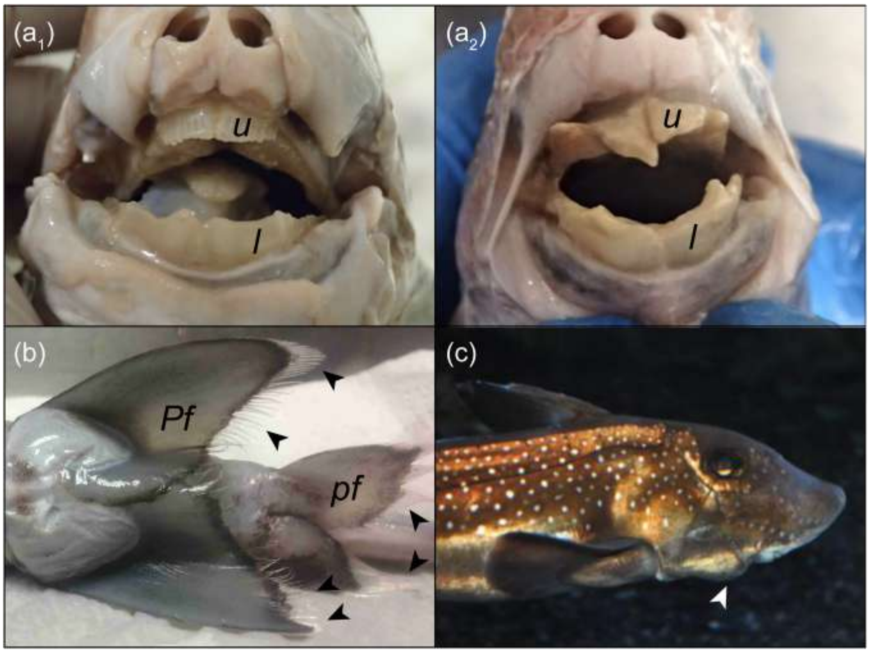

FIGURE 7 Pathologies exhibited by captive *H. colliei*. (a1) Frontal view of the snout showing evenly abrased dental plates; (a2) frontal view of the snout showing uneven abrasion of dental plates; (b) ventral view of a specimen showing raw fin rays (arrows) on pectoral and pelvic fins; (c) lateral view of a specimen exhibiting a goiter (arrow). l, lower plate; Pf, pectoral fin; pf, pelvic fin; u, upper dental plate. [Color figure can be viewed at wileyonlinelibrary.com]

behavior of specimens that died from starvation or exhaustion during early ontogeny. The large and prominent eyes of chimeras are thought to be highly sensitive to the low-light environments they inhabit (Garza-Gisholt et al., 2018; Lisney, 2010). However, species such as *C. milii* migrate from deep to shallow waters for mating and have a visual system adapted to a broad range of light brightnesses (e.g., presence of cones, low number of rods, high number of ganglion cells, absence of a tapetum lucidum) and to color vision (Garza-Gisholt et al., 2018). Furthermore, similarities between the visual system of *H. colliei* and *C. milii* have been highlighted (Garza-Gisholt et al., 2018), which are consistent with the vertical migrations these species operate (Andrews & Quinn, 2012; Last & Stevens, 2009). Nevertheless, *H. colliei* mostly migrates to shallow waters at night, probably to increase foraging success (Andrews & Quinn, 2012). This species may, therefore, not be adapted to the intense light brightness of regular aquarium tanks, and its visual and spatial orientation systems might struggle in these conditions. In several juveniles, the spinning behavior partially or entirely disappeared after decreasing the indirect illuminance of the incubation tank to 1.8 lux and changing light color sources into shades of white and blue. However, some juveniles still exhibit abnormal swimming movements, which endogenous neurological issues could induce.

4 | CONCLUSIONS

Achieving successful breeding of chimeras from eggs laid in captivity to sexual maturity of specimens has long remained a challenge. This work is an example of fruitful collaboration between aquaria and academia to determine optimal captive conditions of *H. colliei*. This work offers promising prospects for this species' conservation in aquaria and evo-devo study of chondrichthyans. Embryonic development of *H. colliei* could, therefore, be compared with the one of model elasmobranchs (*S. canicula* [Ballard et al., 1993; Grunow et al., 2022], *S. stellaris* [Musa et al., 2018], *C. punctatum* [Onimaru et al., 2018], and *Leucoraja ocellata* [Maxwell et al., 2008]) and to ancient descriptions of chimeras (Dean, 1906) to refine knowledge of diverse ontogenetic patterns in chondrichthyans. Furthermore, accessing holocephalan specimens of selected age would facilitate developmental studies on structures already investigated in elasmobranchs, such as the dermal denticles (Cooper et al., 2018, 2023; Rangel et al., 2016). We hope that morphological and anatomical cues provided by embryonic monitoring of chimeras will provide insights into the adaptations of cartilaginous fishes to deep-sea environments (Berio et al., 2021; Dean et al., 2015; Pears et al., 2020).

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in hcolliei_husbandry at https://github.com/fberio/hcolliei_husbandry.

ETHICS STATEMENT

Observations were performed on live specimens. All specimens were kept in the aquarium Planet Ocean Montpellier, France

(Permits 2005-1-3087 and 2093/104-2011) under the supervision of N. Hirel (National curator's licenses 2001-I-1011, 10-XIX-049, and 19-XIX-031). A veterinarian reviewed and approved protocols and procedures for handling and treating specimens.

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