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Aquaculture and early life stages of the Hawaiian Potter's angelfish (*Centropyge potteri*)

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Abstract

The Potter's angelfish (*Centropyge potteri*), a Hawaiian endemic reef fish, has long been prized in the global aquarium trade, with approximately 9000 individuals collected annually from the wild before Hawai'i's 2021 ban on commercial aquarium fish collection. This study marks the first detailed description of aquaculture techniques with replicated results for the species, providing foundational data on larval growth and survival critical for advancing culture techniques for *C. potteri* and other reef fishes. By iteratively refining feeding protocols, survival rates to settlement improved from 0% to 3.4%, largely by addressing acute mortality points during feed transitions. Egg and larval development closely resembled other *Centropyge* species, with flexion between 14 and 21 dph, and settlement behaviors observed at ~60 dph. Spawning in captivity followed lunar cycles, with broodstock producing viable eggs nightly from December 2022 to May 2024. Larval rearing trials compared three feeding protocols along with the effects of egg stocking densities and live algae densities. Early development revealed critical vulnerabilities during feed transitions, particularly with newly hatched *Artemia* and dry feeds, which were mitigated by delayed introductions. Continuous live algae (*Tisochrysis lutea*) and adult *Parvocalanus* copepods through

settlement were essential for success, while larger tanks (1000 L) improved survival by minimizing environmental stress compared to smaller tanks (200 L). The revised feeding regime supported the highest survival rates with algae densities of 75,000–150,000 cells/mL. The results emphasize the importance of diet composition and timing, environmental stability, and tank management for successful larval rearing. Future research should prioritize pathogenic bacteria control in live feeds and refining feed transitions to further improve aquaculture efficiency. This study aligns with efforts to transition the industry toward sustainable aquaculture practices, reducing pressures on wild populations and supporting reef conservation while offering a scalable model for aquaculture of other high-value reef fish species.

KEYWORDS

Centropyge potteri, Hawaiian endemic, larval fish development, larval rearing, marine ornamental aquaculture, marine ornamentals, potter's angelfish

1 | INTRODUCTION

1.1 | Marine ornamental aquaculture and the aquarium trade

The marine aquarium trade is a global industry valued at an estimated US $2.15 billion annually at retail, with approximately 30 million marine ornamental fish harvested from coral reefs each year (Biondo & Burki, 2020; Watson et al., 2023). Unlike freshwater aquarium fish, which are predominantly captive-raised, the marine trade relies heavily on wild collection from ecologically sensitive coral reefs (Olivier, 2001; Olivotto et al., 2011; Palmtag, 2017; Wabnitz et al., 2003). High post-collection mortality rates—ranging from 50% to 70% due to handling stress, disease, and shipping—further highlight the need for sustainable solutions (Biondo & Burki, 2020; Wabnitz et al., 2003).

Hawai'i has historically been a significant exporter of marine ornamental fish, particularly Yellow tang (*Zebrasoma flavescens*) and Potter's angelfish (*Centropyge potteri*), with annual harvests exceeding 300,000 and 10,000 individuals, respectively (Teague et al., 2024; Walsh et al., 2004). The state's aquarium fishery remains a subject of ongoing debate among stakeholders, including aquarium fishers, hobbyists, marine scientists, Native Hawaiians (Kānaka Maoli), policymakers, and local communities. Research suggests that collection has minimal impact on fish populations, and advocates argue that the trade promotes conservation awareness while supporting livelihoods (DLNR 2015; 2020). However, critics emphasize the risks of overharvesting, particularly when profit motives outweigh ecological responsibility (Schaar & Cox, 2021). Without adequate regulation, the trade risks a "tragedy of the commons" scenario (Ostrom, 1990).

In response to these concerns, the State of Hawai'i has implemented various management measures. The establishment of Fish Replenishment Areas (FRAs) demonstrated improvements in aquarium fish abundance, notably for Yellow tang and Potter's angelfish (Tissot et al., 2004; Williams et al., 2009). However, ongoing legal and regulatory actions have significantly reshaped the fishery. A 2019 statewide moratorium allowed aquarium fishing without commercial aquarium permits as long as fine-mesh net equipment was not used and fishers possessed a commercial

marine license (CML). A November 2020 order by Hawai'i's First Circuit Court required environmental review for new or renewed CMLs used for aquarium fishing, allowing collection to continue only until existing CMLs expired. In January 2021, the same court issued an injunction on all commercial aquarium fishing in Hawai'i pending an industry-led environmental review (Schaar & Cox, 2021). These policy changes had direct impacts on market conditions for Hawaiian species and spurred investment into aquaculture as a sustainable alternative to wild collection (Glover, 2024). Plans for limited commercial collection permits with strict quotas for eight species are under consideration following a recent lifting of an injunction on the west coast of Hawai'i Island (DLNR, 2024; Kaupiko v. BLNR, 2024). As of November 2024, commercial aquarium collection remains closed, with aquaculture as the only legal source of iconic endemic and native species from Hawai'i including Yellow tang and Potter's angelfish. While Yellow tang may be collected elsewhere, such as Japan or Guam, the global market now largely relies on their captive-bred alternative as wild collected specimens are now less available and more expensive. However, market conditions are sensitive to changes in availability, and if limited collection from Hawai'i resumes, it could influence both the price and supply of wild-caught specimens.

Marine ornamental aquaculture (MOA) offers a sustainable alternative to wild collection, addressing ecological concerns while promoting reef conservation (Calado, 2017; Chen et al., 2020; Olivotto et al., 2011, 2017; Pouil et al., 2020). Aquacultured fish are accustomed to readily available prepared foods, exhibit significantly lower mortality rates than wild-caught specimens, and tend to have longer lifespans (Palmtag, 2017). A thorough understanding of the full life history of marine ornamental fishes is essential for successful aquaculture efforts, enabling effective rearing and offering valuable insights into species biology to aid fisheries management (Chen et al., 2020; Holt, 2003; Kough et al., 2013; Tlusty, 2002). Successful examples, such as the rearing of Yellow tang, demonstrate MOA's potential for commercial production of high-value pelagic spawners that now dominate the market over wild specimens (Callan et al., 2018; DiMaggio et al., 2017; Groover et al., 2020; Pereira-Davison & Callan, 2018). Despite these achievements, challenges persist, including the need for high-quality broodstock egg production, optimized live feed schedules for larvae, and species-specific nutritional strategies (Hiew, 2023; Moorhead & Zeng, 2010). Addressing these obstacles and scaling MOA for commercial production are critical steps toward its broader adoption in the marine aquarium trade.

1.2 | Dwarf angelfish

The dwarf angelfishes (genus: *Centropyge*) are among the most highly valued fish in the marine aquarium trade due to their vibrant colors and small size at maturity. Within the Pomacanthidae family, the genus *Centropyge* stands out as one of the most sought-after and extensively traded marine angelfishes, making up approximately 77% of all marine angelfish imported into the USA in 2011 (Baensch, 2017). The reproductive biology of *Centropyge* has been extensively studied, revealing analogous features among the various species (Baensch, 2017; Bauer Jr. & Bauer, 1981; Hioki et al., 1990; Moyer & Zaiser, 1984). These investigations revealed many shared characteristics within the genus, including reproductive strategy, behavioral patterns, timing and frequency of spawning, egg and early larval development, sexual dimorphism and dichromatism, as well as social structure and territory.

Despite the promising economic and environmental benefits of culturing *Centropyge* species as an alternative to wild collection, there is a notable lack of published literature covering their captive breeding, larval rearing, or growout techniques, leaving significant technical challenges unresolved. The majority of published information on *Centropyge* aquaculture is outlined in Baensch (2017). The larval rearing phase, particularly for reef species producing pelagic eggs, poses inherent complications due to the delicate nature of the small larvae with complex larval stages (Holt, 2003). *C. fisheri* was the first species of the *Centropyge* genus raised in captivity by Frank Baensch in 2001 using wild-collected zooplankton collected from Kaneohe Bay (Baensch, 2002, 2003). Baensch also had success raising the Blue Mauritius angelfish (*C. debelius*) in small numbers using size-strained wild plankton (Baensch & Tamaru, 2009). Olivotto et al. (2006) used cultured copepod nauplii and wild plankton to raise *C. flavissima*; however, no larvae were raised to the juvenile stage. Research endeavors at Oceanic Institute (Waimanalo, Hawaii, USA)

managed to address key bottlenecks in rearing the flame angelfish (*C. loricula*) through the discovery of suitable size prey items and copepod production technologies (Laidley et al., 2008). They also looked at dietary effects on broodstock egg production, quality, and larval development (Callan, 2007; Callan et al., 2012, 2014). Such developments contributed to our understanding of the rearing and early developmental stages of species within this genus. However, substantial obstacles persist in optimizing reproducible rearing protocols to achieve commercial-scale viability. High postflexion-stage larval mortality and extended settlement phases continue to stand out as key challenges confronting researchers presently (Baensch, 2017).

1.3 | Potter's angelfish

The Potter's angelfish (*Centropyge potteri*) are endemic to Hawai'i, found exclusively along the island chain where they inhabit shallow reef environments. They are a strikingly beautiful species of marine dwarf angelfish that is highly valuable and sought after by aquarium enthusiasts. Therefore, there has been growing interest in the captive breeding of this species as the only current option for the aquarium trade. They have a distinctive appearance, with a bright orange body that is marked with bold black and blue stripes and spots. The fins and tail are also edged in blue, creating a dramatic contrast with the orange body. Potter's angelfish are omnivorous, feeding primarily on algae and small reef invertebrates such as copepods and amphipods (Pyle & Myers, 2010; Randall, 1985). Like other *Centropyge*, *C. potteri* is a protogynous hermaphrodite with seasonal and lunar reproductive periodicity (Lutnesky, 1988, 1989, 1991, 1992). This species was observed in the wild spawning each evening the week before the full moon from December to May, which was corroborated with gonadal and hormonal assessments (Collier, 2001; Collier et al., 2003; Lobel, 1978).

Despite their popularity in the aquarium trade and extensive research conducted on their reproductive behavior in the wild, there remains a notable absence of publications exploring Potter's angelfish reproduction in captivity, strategies for larval rearing, and early life history. This endemic angelfish has been raised in small numbers by researchers at Oceanic Institute of Hawai'i Pacific University (OI) prior to this study, however, they proved difficult to culture in large numbers due to high larval mortality and have not been raised since 2016 (Montalvo, 2017). Montalvo raised Potter's angelfish from eggs collected at an aquarium and raised on a rearing protocol similar to that established for Yellow tang, but modified for mixed species culture (Callan et al., 2018).

This research sought to develop a tailored larval rearing protocol for *C. potteri* as a critical first step for the commercialization of this species. Rather than comparing protocols, the primary objective was to share what worked in our system-highlighting successful approaches and methodologies that others can build upon in future efforts. Captive-bred Potter's angelfish, having been conditioned to aquarium environments from an early age and accustomed to readily available prepared foods, offer a more resilient and easily adaptable alternative to wild-caught specimens, which are known for their difficulty in acclimating and finicky feeding habits. Furthermore, the captive breeding of Potter's angelfish has the potential to stimulate the development of new aquaculture techniques and technologies that can be applied to other species of marine fish, furthering marine finfish aquaculture knowledge more broadly while offering a sustainable alternative to wild collection (Pouil et al., 2020).

2 | MATERIALS AND METHODS

2.1 | Broodstock

Potter's angelfish broodstock were collected from nearshore locations around the island of O'ahu in July of 2022 under a Special Activities Permit issued by the Hawai'i Department of Land and Natural Resources. Collection activities were conducted in accordance with all relevant state regulations and ethical guidelines to ensure minimal impact on wild populations. Prior to collection, population assessments were performed to ensure that total take did not

exceed 20% of *C. potteri* in a given collection area. All handling and transport procedures followed best practices to minimize stress and ensure the welfare of collected individuals. Once at OI, the fish were quarantined and conditioned to a prepared diet. Quarantine consisted of thirty-minute 100 ppm H_2O_2 baths, five-minute freshwater dips, and tank transfers 2–3 times per week for 4 weeks. While H_2O_2 was found to cause viable *C. potteri* eggs to cease development, adult *C. potteri* tolerated the 30-minute baths without issue. Other forms of quarantine including copper, hyposalinity, and formalin are not recommended for adult *C. potteri* as this species was found to be highly sensitive to these treatments in our lab, exhibiting high stress reactions including high gill rate, loss of swim bladder control, and erratic swimming sometimes leading to death. After quarantine, each fish was assessed as male or female through behavior observation and sexual dichromatic characteristics including color, shape, and size, with males exhibiting more dominant and aggressive behavior with darker color patterns and larger bodies. Males ranged from 8 to 11 cm TL while females ranged from 6.5 to 9 cm TL.

Eight identical 1000 L fiberglass tanks were used to house broodstock in a covered outdoor lab. Each tank was fitted with an egg collector, given artificial structures, exposed to natural light and temperature, and run on flowthrough well water (Table 1) at 5–6 liters per minute (lpm), similar to the broodstock system outlined in Laidley et al. (2008). Well water at OI has a low pH, elevated total gas pressure, and high CO_2 levels—common traits of saltwater wells in this region. Water pumped directly from the well arrives to the tanks at 26–27°C depending on time of year. Additionally, it mixes with some groundwater, causing salinity to fluctuate between 31 and 33 PSU, whereas the ocean water it draws from remains nearly constant at 35 PSU (Callan, 2007). The water was run through a UV sterilizer and 50 μm filter bag prior to entering the tanks.

The fish were placed into tanks in groups of two to four individuals ranging from 1M:1F to 1M:3F. Groups were observed closely for aggression and any fish that was found to be kicked out of a group was moved to isolation to heal. This process resulted in the formation of six pairs (1 M:1F), one trio (1M:2F), and one quad (1M:3F) for a total of 19 fish, 8 males and 11 females, that spawned over the course of this study. Each group was fed 4–5 times daily including pellets (New Life Spectrum Algaemax, New Life International, Homestead, FL, USA), frozen raw items (Herbivore Frenzy, Larry's Reef Services, NC, USA; PE Frozen Mysis Shrimp, Piscine Energetics, Vernon, BC, Canada), and an omnivore gel (Mazuri Aquatic Gel Diet for Omnivorous Fish, Mazuri Exotic Animal Nutrition, St. Louis, USA). *C. potteri* broodstock were found to be highly prone to gill fluke infections with Monogenean flukes, genus *Dactylogyrus*, seen multiple times in gill clips at our lab; however, 5 ppm (3 h) or 10 ppm (90 min) Praziquantel treatments usually eradicate parasitic gill flukes with little stress to the fish. Our lab experienced one outbreak of a Praziquantel resistant variant of *Dactylogyrus*. Formaldehyde treatments (37 ppm for 45 minutes) were attempted but were extremely stressful on *C. potteri* and are therefore not recommended due to the effects discussed above; however, these treatments were successful in completely eradicating the flukes.

TABLE 1 Water and environmental parameters for Potter's angelfish broodstock and larval tanks.

Parameter	Broodstock tanks	Larval tanks (flowthrough)	Larval tanks (RAS)
pH	7.70–8.02	7.83–8.05	8.03–8.12
Temperature (°C)	24.9–28.0	25.1–27.6	25.1–27.6
Photoperiod (light:dark)	Natural (11:13 to 13.5:10.5)	12:12	12:12
Water source	Well Water Flowthrough	Well Water Flowthrough	Well Water RAS
Salinity (PSU)	31.08–32.50	31.08–32.50	31.81–36.85
Total gas pressure	97.6%–104.6%	97.2%–104.6%	94.5%–97.6%
Ammonia (NH_3)	Undetectable	Undetectable	<0.25 ppm
Nitrite (NO_2^-)	Undetectable	Undetectable	Undetectable
Nitrate (NO_3^-)	Undetectable	<5 ppm	<40 ppm
Dissolved oxygen (mg/L)	7.11–8.15	7.11–8.00	7.45–8.15

2.2 | Egg collection and stocking procedures

Eggs were collected and counted each morning to determine the total number of viable eggs produced from the previous night's spawn. Eggs were collected and combined from each Potter's angelfish broodstock tank prior to counting using a 200 micrometer mesh screen placed in the surface drains of the tanks, then rinsed into a clean 1 L beaker. The beaker was then moderately aerated for 2–3 min to mix the eggs homogeneously. While under aeration, 10 random 1 mL samples were taken and combined into a 50 mL beaker. The 10 mL subsample was then counted using a 10 mL zooplankton counting wheel under a stereomicroscope (Olympus SZ61, Olympus Scientific Solutions America, Waltham, MA, USA). Eggs were characterized as either inviable or viable, as outlined in Callan (2007) with the viable egg count extrapolated to determine the total number of viable eggs. Viable eggs were identified as clear, buoyant, and having a developed notochord, while inviable eggs were classified as any egg that did not meet those criteria. Egg sample counts were divided by the subsample volume (10 mL) and multiplied by the volume of the beaker (1000 mL) to estimate the total number of eggs per spawning event.

Eggs were then separated and stocked into larval rearing tanks. Viable eggs were separated after turning off aeration for 20 to 30 min, allowing them to float while inviable eggs sank. Viable eggs were carefully transferred to a clean 1 L beaker, transported to the hatchery, and gently added to either a 200 or 1000 L hatchery tank. No disinfection methods were employed as it had been observed previously at our lab that *C. potteri* eggs do not tolerate disinfection protocols using H_2O_2.

2.3 | Larval growth, development, and survival data collection and analysis

Photographs were captured to document larval growth and development and compare across feeding regimens. Larvae from each tank were periodically transferred to a Sedgewick Rafter counting slide using a pipette and placed under an Olympus SZ61 stereomicroscope. Photographs were taken of the larvae on an iPhone 14 Pro (Apple Inc., Cupertino, CA, USA) using Manual Camera 4 (Kenneth Kao, USA). Photographs of larvae were measured for body length (BL) and body depth (BD) using ImageJ (US National Institutes of Health, Bethesda, MD, USA). Body length was measured from the anterior-most point of the larvae to the end of the notochord and in later developmental stages to the mid-caudal peduncle, at the posterior end of the vertebral column just anterior to the hypural plates. Body depth was measured across the myomeres at the anus. Larvae that survived sampling were returned to their respective tank. Images were used to assess developmental progress, recording major developmental milestones. As tanks matured to 30 days old and beyond, the number of fish mortalities was counted and recorded daily to assess survival. All growth data was obtained during an initial, preliminary research project, prior to the current study. Linear regressions were performed only during this preliminary phase to evaluate body length and depth growth from the onset of flexion (14 dph) (Glover, 2024). These analyses were not replicated in the subsequent trials. The limited number of replicates in 200 L tanks during the preliminary study prevented statistical comparisons between protocols; however, findings from those early trials helped inform the rearing strategies applied in the current study. Due to limited replication and minimal growth data collected in the present study, our findings are presented as observational insights into effective and ineffective rearing approaches rather than statistically comparable treatments.

2.4 | Life feeds production

All live feeds and microalgae were maintained at OI over the course of this study. *Chaetoceros muelleri* and *Tisochrysis lutea* were kept in semicontinuous cultures and scaled up in 1250 L photobioreactors (Industrial Plankton, Victoria BC, Canada). *Parvocalanus crassirostris* copepods were continually cultured and size-fractionated to separate eggs and N1 nauplii from later stage nauplii. Nauplii were matured for 10 days using *C. muelleri* and *T. lutea*, with adults

strained and fed out or restocked. S-type rotifers, *Brachionus rotundiformis*, were produced daily and fed Nanno 3600 algae paste (Reed Mariculture, Campbell, CA, USA) and enriched with Algamac 3050 (Aquafauna Bio-Marine, Inc., Hawthorne, CA, USA) for 2 h prior to feeding. *Artemia franciscana* cysts (Great Salt Lake strain; INVE Aquaculture, Inc., Salt Lake City, UT, USA) were hydrated and nauplii separated from cysts using magnets. Newly hatched Instar I *Artemia* were kept chilled during the day while one-day-old Instar II *Artemia* were enriched overnight using Algamac 3050. Frozen foods refer to a mixture of frozen cyclopoid and calanoid copepods: ARPods 500 (Reed Mariculture, Campbell, CA, USA) and Calafin (Argent Aquaculture, Redmond, WA, USA).

2.5 | General larviculture procedures

Potter's angelfish larviculture methods were first refined through the evaluation of six feeding regimens as part of a preliminary study (Glover, 2024). Out of the six protocols evaluated, two (Protocols A and B) provided valuable data, laying the foundation for subsequent trials and refinements in this study (Table 2). Initial trials, conducted in 200 L hatchery tanks, revealed significant variation in larval survival and growth between feeding regimes. Insights from these trials informed subsequent experiments, including a notable 1000 L tank in May 2023 that yielded 61 juveniles from 15,000 eggs. This success, although not part of the formal project, validated Protocol B and demonstrated the feasibility of scaling these methods. Over the following year, iterative refinements led to the development of Protocol C, specifically tailored to *C. potteri* (Figure 1). The present study incorporates findings from Glover (2024), testing Protocol C in detail, including the use of algae densities of 150,000 and 75,000 cells/mL to increase efficiency.

Each tank was stocked with eggs and left with constant flow and light aeration via airstone until 3 days post hatch (dph), at which point algae and initial feeds were introduced as larvae transitioned to exogenous feeding.

TABLE 2 Summary of feeding regimens tested across larval rearing protocols. Protocols A and B were used during preliminary trials to inform the design of Protocol C. (A) A feeding regime optimized for rearing Yellow tang. (B) A modified protocol initially developed to raise eggs collected from public aquariums, with a switch to algae paste at day 32 and no adult copepods. (C) A revised protocol tested in the current study for *C. potteri*, using algae densities of 150,000 and 75,000 cells/mL.

			Age fed (dph)		
Food type	**Food size**	**Density fed**	**A**	**B**	**C**
No food	-	-	0–2	0–2	0–2
Algae (*T. lutea*)	-	75 k to 300 k cells/mL	3–40	3–31	3–63
Nanno 3600 (Paste)	-	0.02 mL/L	-	32–50	-
Copepod nauplii	38–75 μm	1–5/mL	3–16	3–19	3–16
Rotifers	100–210 μm	5–15/mL	10–24	3–24	3–24
Adult copepods	125–500 μm	0.1–0.2/mL	-	-	17–71
New hatched Artemia	400–500 μm	0.025–0.1/mL	15–29	19–29	18–31
Enriched Artemia	600–750 μm	0.025–0.3/mL	24–40	27–55	28–90
Otohime A1	75–150 μm	-	13–23	-	-
Otohime A2	150–250 μm	-	15–34	25–35	24–41
Otohime B1	250–360 μm	-	24–39	25–39	24–44
Otohime B2	360–650 μm	-	-	25–90	41–59
Otohime C1	580–840 μm	-	-	25–90	50–90
Frozen foods	500–800 μm	-	-	53–90	32–90

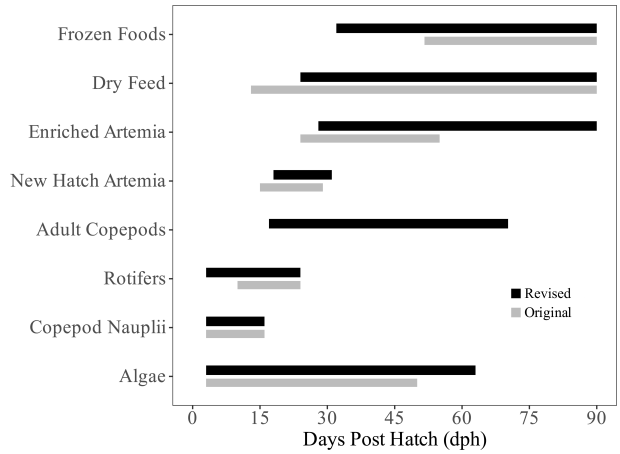

FIGURE 1 Outline of feeding schedules comparing the first protocol proposed and attempted to raise *C. potteri* (Protocol A) with a revised feeding regime found through iterative optimization (Protocol C).

200 L tanks were kept on a recirculating aquaculture system (RAS) filled from well water with a 12:12 h photoperiod. LED lights were scaled from 0% to 25% intensity over 30 min, Prime 16HD Reef lights (Aqua Illumination, Bethlehem, PA, USA) were mounted over 200 L tanks, while Thrive Agritech Infinity XE Linear LED Light Bars (Thrive Agritech, Los Angeles, CA, USA) were used over 1000 L tanks. In the 200 L tanks, the flow rate was held at a constant 1 lpm until 3 dph when it was raised to 1.2 lpm to reduce temperature fluctuations and allow prey items to flush out. The 1000 L tanks operated with either well water flowthrough or well water RAS, with flow maintained at 4 lpm through 3 dph. Post 3 dph, flow alternated between 0/6 lpm (day/night) until 20 dph and was adjusted to 3/6 lpm thereafter. All tanks were plumbed to receive either flowthrough well water or RAS well water, allowing flexibility in managing water quality. Tanks were switched from RAS to flowthrough well water if ammonia levels exceeded 0.1 ppm or other water quality issues arose. Standpipe mesh sizes were gradually raised from 250 to 1000 μm as the larvae grew and required larger prey items. Aeration was steadily increased as well. Each tank was also fitted with a small fan to agitate the surface water of the tank during the day. The well water used in these systems posed challenges due to its low pH, high silica content, and elevated total gas pressure. The RAS system mitigated these issues by off-gassing CO_2, raising pH, and alleviating gas pressure (Table 1). This system also included UV sterilization, mechanical filtration through 50 and 10 μm filter bags, and a biological bead filter.

2.6 | Tested protocols

Three larval rearing protocols—A, B, and C—were used over the course of this research effort. Protocols A and B were tested during preliminary trials, conducted prior to the present study, and served as the foundation for refining our approach to rearing *C. potteri*. Protocol C was developed and tested as part of the current study, incorporating insights from earlier efforts. Table 2 summarizes the key differences in feeding regimens and tank conditions across all three protocols. Protocol A was originally optimized for Yellow tang (*Zebrasoma flavescens*). Protocol B, designed for use with eggs sourced from public aquariums, included a switch to algae paste at day 32 and did not include adult copepods. Protocol C reflects a revised, species-specific approach tailored for *C. potteri*, utilizing microalgae densities of 150,000 and 75,000 cells/mL.

The algae *Tisochrysis lutea* was introduced starting at 3 dph in all protocols: twice daily to 300,000 cells/mL in Protocol A, once daily to 300,000 cells/mL in Protocol B, and once daily to 150,000 or 75,000 cells/mL in Protocol

C. Across all protocols, copepod nauplii were size-sorted between 38 and 40 μm using plankton sieves and fed twice daily to 5/mL, then weaned to 1/mL 5 days before feeding of nauplii ceased. Rotifers were enriched in Algamac 3050 for at least 2 h and fed at 15/mL in Protocol A from 10 to 24 dph. Protocols B and C started at 5/mL from 3 to 7 dph, raised to 10/mL from 8 to 11 dph, and kept at 15/mL after 12 dph.

Instar I *Artemia* nauplii were introduced at 0.025/mL twice daily starting at 15 dph in Protocol A, 19 dph in Protocol B, and 18 dph in Protocol C. Density and frequency of addition were incrementally increased through the duration of enriched *Artemia*. Enriched Instar II *Artemia* were added at 0.025/mL starting at 24, 27, and 28 dph for Protocols A, B, and C, respectively, and steadily increased to 0.25/mL. Protocol C was fed adult copepods at 0.05/mL twice per day. Consumer fish flakes (Garlic Marine Flakes, Omega One, Blacksburg, VA, USA) were fed after 50 dph in Protocol C. All dry feeds (Otohime and flake) were fed five times daily. Live algae was substituted with 20 mL of Nanno 3600 twice daily after 31 dph in Protocol B. No fish remained after 40 dph in Protocol A.

A formal nutritional evaluation of the diets used in this study was not conducted; however, all live feeds were carefully managed to ensure consistent nutritional quality. Rotifers and *Artemia* nauplii were enriched using Algamac 3050 prior to feeding to optimize essential fatty acid content. Feeding strategies were standardized based on prey density rather than absolute feed weight, with specific densities adjusted according to larval developmental stage to ensure consistent availability of appropriately sized prey. While differences in water content between live and dry feeds may introduce variability, the use of density-based feeding minimizes inconsistencies in nutrient availability across protocols.

3 | RESULTS

3.1 | Spawning

Courtship was observed in the evening 30 to 90 min before sunset. Both males and females experienced blanching with lighter nuptial coloration throughout their bodies and fins during courtship. Spawning began in August 2022, 1–2 months after collection from the wild, with 200–1000 eggs collected intermittently until December, at which point spawning became a nightly occurrence. Total eggs per spawn and viability quickly increased to over 3000 viable eggs most nights following December 2022. Peak egg production was seen in late winter or early spring and followed lunar periodicity with peak spawns near the full moon. Egg production exhibited a marked decline toward the end of summer, consistent with seasonal periodicity; however, nightly spawning events continued to occur (Figure 2). The

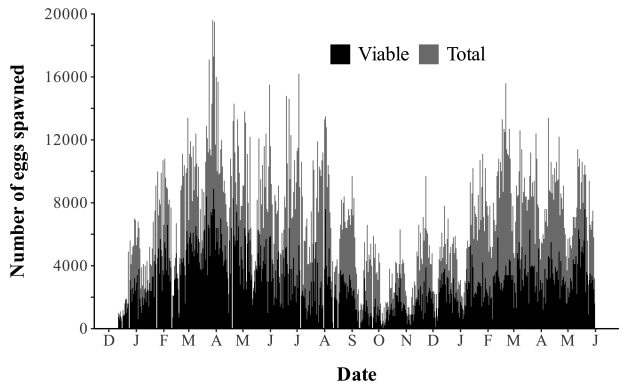

FIGURE 2 Daily egg output from six pairs (1M:1F), one trio (1M:2F), and one quad (1M:3F) of Potter's angelfish broodstock from December 2022 through May 2024.

average total number of eggs collected per day was 6728 (range 270–19,600) with an average fertility of 54.9%. Two spawns of 0% fertility were observed and one spawn of 100% egg viability. Average daily viable egg production was 2949 ± 1734.43 eggs, an average of 268 eggs per female or 368 eggs per group each night from December 2022 through May 2024. While egg production data were not collected for each individual group, all groups spawned consistently throughout the study.

3.2 | Early life history

A total of 20 eggs and 112 *C. potteri* larvae were sampled throughout this study, ranging from 0 to 140 days post hatch. The spherical eggs averaged 0.68 ± 0.01 mm in diameter and were clear and positively buoyant if viable, with a single oil globule averaging 0.11 mm in diameter (Figure 3a). Embryo development was found to be the same as that of other previously documented *Centropyge* species (Baensch & Tamaru, 2009; Bauer Jr. & Bauer, 1981; Hioki et al., 1990). As the eggs developed and hatched approximately 16 h after fertilization at 25.2–28.0°C, clusters of contracted melanophores were noticeable along the dorsal portion of the notochord and covering the oil globule immediately after hatching (Figure 3b). Larvae measured roughly 1.5 mm (notochord length) and had unpigmented eye spots without lenses and no discernable mouth or gut (Figure 4a). Larvae were still positively buoyant and had a large yolk sac that extended beyond the head and could only swim in short bursts (Figure 4b). Late 0 dph larvae (~24 h after fertilization) were much more elongate, with distinct unpigmented eye spots and the beginning development of an anal opening (Figure 4c) along with expanded finfolds and an anteriorly protruding yolk sac and oil globule (Figure 4d).

The yolk sac stage occurred from 1 to 3 dph with exogenous feeding beginning at 3 dph. 1–2 dph larvae exhibited three distinct extended melanophores: anterior along the oil globule, anterior to the anal opening, and on the dorsal finfold approximately ⅓ body length from the tail (Figure 3c). Larvae at 1 dph had used up most of their yolk sac, beginning to develop a deeper body, jaw, and gut (Figure 4e). 2 dph larvae saw the first growth of pectoral fins (Figure 4f) along with further development of unpigmented eyes, a jaw, and an anal opening (Figure 4g). At 3 dph, the larvae had begun preflexion and averaged 2.47 mm in body length and 0.16 mm in body depth with a developed jaw, pigmented eyes, pectoral fins, and significantly increased expanded melanophore concentration around the anus and central body now appearing orange when viewed from above (Figure 4h). The larvae had also formed a widened gut and were actively feeding, as seen by prey items in the digestive tract extending from the mouth to the anus (Figure 4i).

Larvae at 4–6 dph saw the development of significant orange pigmentation around the central and anterior zones of the body, appearing red or dark orange viewed from above. 5 dph larvae measured 2.56 mm BL, 0.22 mm BD, and had a widening of the gut and expanded jaw development (Figure 3d). Vascularization was observed at this time in the gut, jaw, body, and notochord areas in properly nourished larvae. 7–8 dph larvae exhibited deeper and more complex guts and the beginning of swim bladder inflation and head spination. As larvae reached 9 dph, the gut and notochord continued to deepen while orange pigmentation expanded posteriorly and increased in density (Figure 3e). The head became deeper, forming a short snout; the operculum had begun development, and larvae measured 3.15 mm BL and 0.39 mm BD. A notable color shift of the body was observed 10–14 dph, with larvae exhibiting a distinctive bright orange pigmentation across the whole body excluding the tail region.

Flexion occurred at 14–17 dph, marking the critical stage in which the notochord tip bends upward and the larvae's body becomes much deeper and more mature. Larvae averaged 3.75 mm BL and 0.75 mm BD at 14 dph (Figure 3f). Flexion was completed in approximately 5–6 days, with the last observation of flexion at 22 dph. During flexion, body depth more than doubled, and larvae became increasingly laterally compressed (Figure 3g). The swim bladder inflated significantly, the gills and operculum fully developed, head spination and spicules completely formed, and the whole body became vascularized. Pelvic and anal spines began to form as the caudal, dorsal, and anal fins developed rays (Figure 3h).

FIGURE 3 Photographs of representative *C. potteri* egg and larvae at (a) embryo ~16 hrs after fertilization, D = 0.68 mm, (b) 0 dph, BL = 1.49 mm, (c) 2 dph, BL = 2.47 mm, (d) 5 dph, BL = 2.56 mm, (e) 9 dph, BL = 3.15 mm, (f) 14 dph, BL = 3.94 mm, (g) 21 dph, BL = 4.53 mm, (h) 24 dph, BL = 4.73 mm, (i) 30 dph, BL = 5.93 mm, (j) 60 dph, BL = 13.30 mm, and (k) 140 dph, TL = ~40 mm.

Postflexion larvae measured 4.21 mm BL and 2.14 mm BD at 24 dph. Pectoral rays began to develop as the other fins continued to fully mature (Figure 5a). Head spines had grown but were small and sharp, while spicules covered the body (Figure 5b). Larvae fully developed dorsal, pelvic, and anal spines as they reached 30 dph, with a deep orange pigmentation across the entire body excluding the fins (Figure 3i). The average BL at 30 dph was 5.30 mm, while BD measured 2.98 mm.

After 30 dph, larvae developed a more vibrant shade of orange on the dorsal half of their body while the ventral half became silvery as scales slowly became noticeable (Figure 3j). From 30 to 60 dph, larvae grew steadily with no major points of development until settlement. Settlement behavior was observed starting at approximately 60 dph, with many larvae inhabiting fake coral structures placed at the bottom of the tank before the darkening of the soft anal and dorsal fins (Figure 5c). Larvae measured approximately 11–14 mm (standard length) at settlement. As larvae

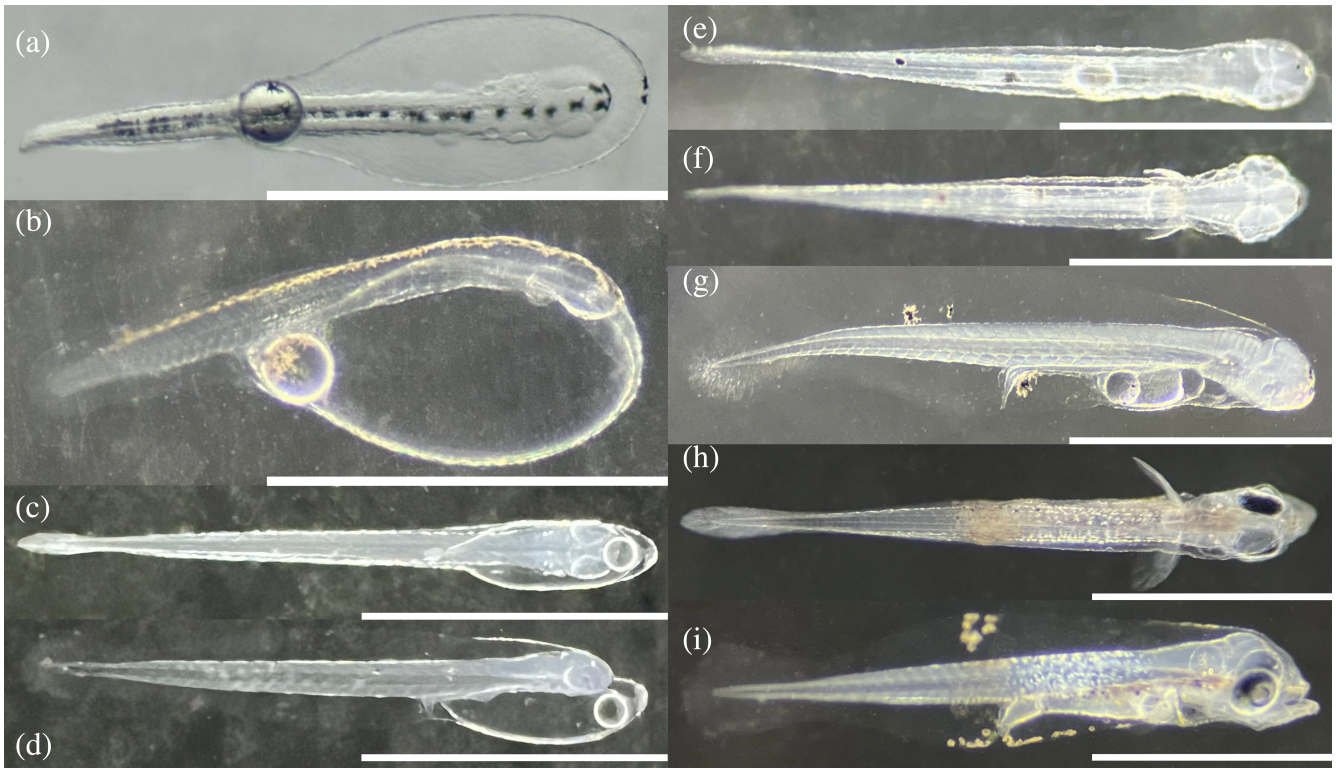

FIGURE 4 *Centropyge potteri* larvae 0 to 3 days post hatch. Scale bar = 1.0 mm. (a) 0 dph newly hatched ventral view, (b) 0 dph newly hatched lateral view, (c) late 0 dph ventral view, (d) late 0 dph lateral view, (e) late 1 dph dorsal view, (f) 2 dph dorsal view, (g) 2 dph lateral view, (h) 3 dph dorsal view, (i) 3 dph lateral view.

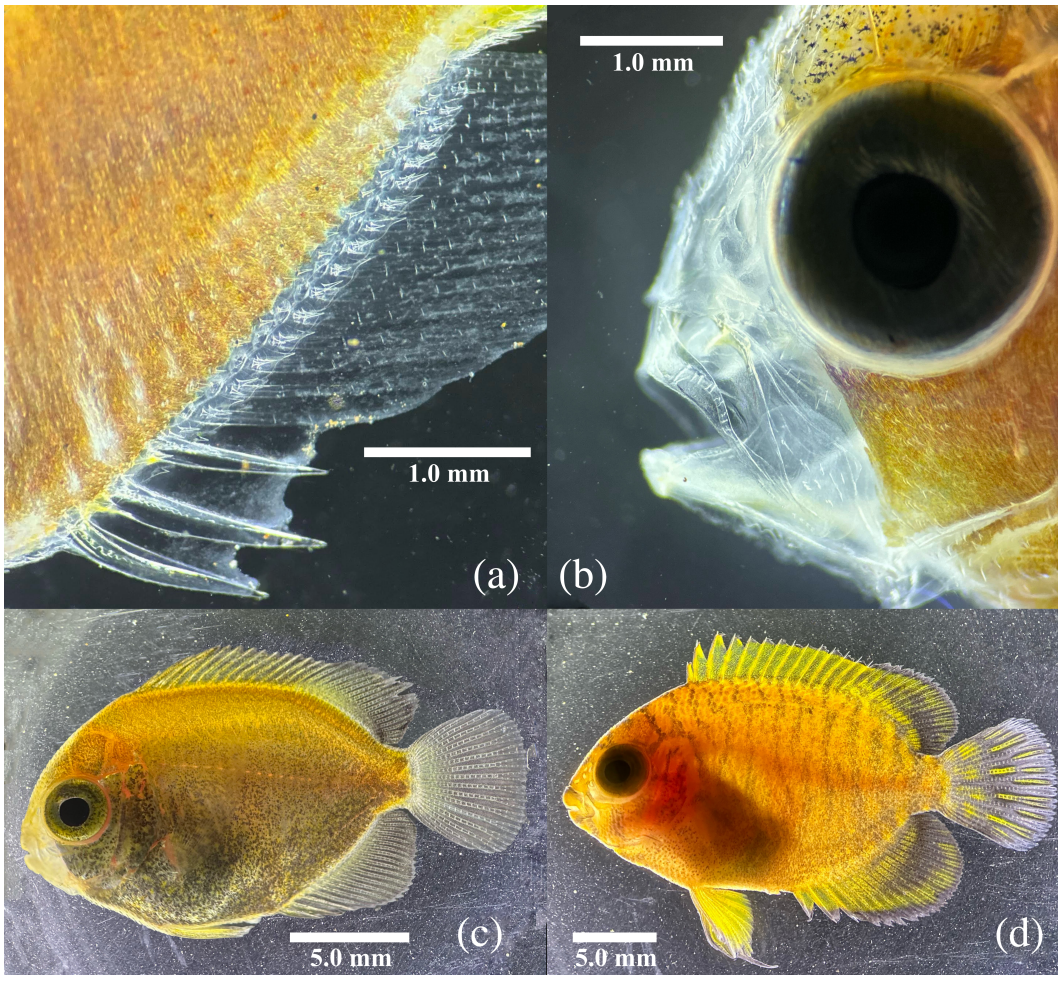

FIGURE 5 Photographs of *C. potteri* throughout this study. (a) Hairlike spines and pigmentation along the jaw and (b) anal fin at 30 dph, (c) a settled juvenile at 77 dph, and (d) a settled juvenile at 91 dph.

completed metamorphosis, juveniles exhibited an iridescent blue lining along the posterior edges of the dorsal, caudal, anal, and pelvic fins and spines (Figure 5d). This blue then expanded anteriorly, creating a maze-like pattern across the entire body excluding the pectoral and pelvic fins as the fish transitioned to its juvenile stage (Figure 3k).

After completing metamorphosis, juvenile *C. potteri* were robust, not experiencing any notable mortality events even after handling or shipping. The juveniles were grown out in the same tanks the larvae were cultured in, with added fake coral and PVC pipe for structure and fed Otohime B2/C1, enriched *Artemia*, flake foods, and Calafin. Juveniles were also seen picking at diatoms, algae, or other benthic organisms along the tank bottom and walls. This species was found to be very aggressive with conspecifics, with the most dominant fish in the tank becoming darker blue while the others remained mostly orange. This aggression hindered settlement behavior in higher density tanks but was mitigated by adding more structures for smaller individuals to hide in. Aggression was the main source of mortality after settlement, with the smallest and weakest siblings of the cohort being picked on. Head and Lateral Line Erosion (HLLE) was also seen developing quickly after juveniles completed metamorphosis. HLLE only developed on fish raised on flowthrough well water, and were moved to RAS water, which stopped tissue deterioration immediately.

3.3 | Effects on growth

Flexion onset and completion varied between protocols. In Protocol A, flexion began at 15 days post hatch (dph), with all larvae sampled already undergoing flexion. Flexion was completed by 19 dph for all larvae in this protocol. In contrast, flexion in Protocol B began slightly earlier, at 14 dph, but was completed later, with all sampled larvae achieving flexion by 22 dph. During the flexion period (approximately 14–21 dph), mean body length growth slowed while body depth increased rapidly, reflecting critical morphological changes. After flexion, both body length and depth resumed steady growth. Protocols A1 and C lack comparable data points for this period.

Linear regressions were performed on body length and depth growth from the onset of flexion (14 dph) to evaluate growth rate (Table 3). Growth rates were consistent across both protocols until approximately 14 dph, after which notable divergence occurred. Protocol A exhibited higher average growth rates of body length (0.227 mm/day) and body depth (0.195 mm/day) compared to protocol B, with average rates of 0.097 mm/day for body length and 0.132 mm/day for body depth. Protocol A demonstrated superior growth rates for both body length and depth, while Protocol B showed a plateau in growth around 30 dph. This stagnation coincided with the transition from live algae to algae paste (Nanno 3600) beginning at 32 dph.

3.4 | Effects on survival

Throughout the preliminary studies, several consistent points of mortality were observed. Mortality events (<25% of fish) frequently occurred during transitions to new feeds and during an acute hatchery-wide die-off. Notably, in preliminary trials using Protocol A, significant drops in survival were observed upon introducing newly hatched *Artemia* at 15 days post hatch (dph). These results prompted a shift toward protocols that exclusively utilized adult copepods instead of *Artemia*. Small-scale trials in our lab further supported this adjustment, finding that delaying the introduction of *Artemia* and dry feeds helped mitigate acute mortality events (Glover, 2024).

In contrast, Protocol B—also tested during the preliminary phase—did not experience any abrupt mortality events, but instead showed a gradual decline of 5 to 9 fish per day following the transition to algae paste on day 32. In March 2023, all active tanks in the hatchery were abruptly terminated due to a mortality event suspected to result from copper toxicity, likely due to rainwater intrusion into the RAS. All other water quality parameters (e.g., pH, O_2, NH_3, NO_3^-) remained within acceptable ranges as reported in Table 1. Despite this loss, survival data up to 32 dph were recorded for Protocols A and B (Table 3), with Protocol A reaching 0.12% survival and Protocol B reaching 0.39%.

Based on these preliminary findings, a refined approach was developed: delaying the introduction of newly hatched *Artemia*, using enriched *Artemia* and dry feeds (Otohime A1/A2), and maintaining consistent live algae densities improved overall outcomes. Protocol C, which incorporated these adjustments and used a stocking density of

TABLE 3 Survival and growth of Potter's angelfish raised on 3 feeding regimes tested. Protocol A1 was an attempt at co-culture with Yellow tang in 1000 L following protocol A until 37 dph stocked with 7200 *C. potteri* eggs and 28,200 *Z. flavescens* eggs. Twenty larvae were then transferred to a 200 L tank and raised using adult copepods. Protocol A had no fish remaining after 40 dph.

Protocol	Survival to 32 dph %	Survival to juvenile %	BL growth 14–38 dph (mm/day)	# of replicates	Tank volume (L)	Stocking density (eggs/L)
A	0.175	0	0.227	2	200	10
A1	0.385	0.097	-	1	1000/200	7.2
B	0.039–0.400	0–0.093	0.097	3	1000	7.5–16.1
C	-	0.021–3.417	-	8	1000	2.4–20.8

TABLE 4 Survival of *C. potteri* across eight trials in 2024 using protocol C tested using 1000 L tanks with stocking densities of 2.4–20.8 eggs/L and live algae densities of 75,000 or 150,000 cells/mL.

Stocking date	Survival to juvenile %	Stocking density (eggs/L)	Algae density (cells/mL)
1/29/2024	0.213	8.0	150,000
4/9/2024	0.124	17.8	150,000
5/7/2024	0.151	17.2	150,000
5/13/2024	0.011	20.8	150,000
5/20/2024	0.011	16.9	150,000
7/4/2024	0.598	4.4	150,000
7/22/2024	0.169	8.9	75,000
9/9/2024	3.417	2.4	75,000

2.4 eggs/L, achieved the highest observed survival rate of 3.42%. This protocol maintained 75,000 cells/mL of live algae from 3 to 63 dph. Six replicates at 150,000 cells/mL and two replicates at 75,000 cells/mL were conducted, with the lower algae density yielding higher average survival. Lower stocking densities also correlated with improved survival in our limited trials (Table 4). It must be noted that seven other 1000 L tanks were stocked in the first half of 2024, all of which had little to no success. These tanks included attempts at a mesocosm and trials of protocol B or C that experienced equipment malfunctions, water quality issues, or were inundated with small jellyfish.

4 | DISCUSSION

4.1 | Spawning

Courtship and spawning of captive *C. potteri* were consistent with patterns seen in the wild and in other *Centropyge* species, exhibiting lunar periodicity with peak egg production near full moons (Bauer Jr. & Bauer, 1981; Collier et al., 2003; Lobel, 1978). Broodstock began spawning 1–2 months post-capture and reached peak production during late winter and early spring. The male to female ratio did not appear to influence spawning frequency or fecundity; however, more research is needed to address optimal broodstock group makeup. Spawning frequency, viability, and the number of viable eggs increased substantially after December 2022, stabilizing at a daily average of 2949 viable eggs per spawn from December 2022 through May 2024. This consistent spawning output demonstrates that wild-caught broodstock can adapt successfully to captivity. The observed decline in spawning activity during the late summer months is likely related to environmental cues, including water temperature and photoperiod, suggesting that simulating natural winter season conditions in broodstock tanks may help sustain egg production year-round. Further research is needed to investigate the efficacy of holding *C. potteri* broodstock at constant cooler temperatures and if variation is needed for the fish to rest from spawning. Callan and Laidley (2010) found that water chemistry significantly influences the health, reproductive performance, and egg quality of *Centropyge* broodstock. Fecundity, fertilization rates, and egg viability were notably higher when broodstock pairs were maintained in sterilized (chlorinated and dechlorinated) ocean water compared to treated or untreated well water, which typically has a lower pH and higher gas pressure than natural seawater. These findings indicate that egg production and quality of *C. potteri* could have been suppressed in this study (Callan & Laidley, 2010). Differences in egg viability across spawns (0% to 100%) highlight the need for further studies to understand the factors influencing egg quality, such as diet, water quality, and stress on broodstock.

4.2 | Early life history

The early life history of *C. potteri* was found to closely align with that of other Centropyge species, but with notable distinctions in larval pigmentation. Eggs were clear and buoyant, measuring 0.68 mm in diameter with a single oil globule. Embryos developed similarly to other *Centropyge* species (Baensch & Tamaru, 2009; Bauer Jr. & Bauer, 1981; Hioki et al., 1990). Larvae hatched at approximately 1.5 mm body length (BL) and lacked functional eyes or digestive systems initially, relying on their yolk sac for nourishment. Pigmentation observed in early-stage larvae, including extended melanophores and orange pigmentation by 3 days post hatch (dph), serves as diagnostic markers for larval identification. Other preflexion and flexion stage *Centropyge* have been found to have red color in the water column, while *C. potteri* exhibits a distinct orange. By 30 dph, the dorsal area of postflexion larvae develops a deeper orange coloration distinguishing this species from others in its genus (Baensch, 2017).

The onset of flexion at 14–17 dph is also similar to other species of *Centropyge*. Postflexion larvae displayed lateral compression and continued pigmentation, with juveniles transitioning to their characteristic blue-lined coloration by settlement (60–90 dph). *C. potteri* begins settlement later than most *Centropyge*, but usually completes this stage by 90 dph, similar to *C. loricula* (Baensch, 2017). However, there were large differences in duration to complete metamorphosis seen within tanks, with smaller individuals taking up to 30 days longer to develop juvenile coloration compared to the first in its cohort. The detailed descriptions of larval development provide a valuable reference for future larviculture efforts, outlining the identification and monitoring of key developmental milestones.

Growout of *C. potteri* faced the exact challenges outlined in Baensch (2017) with aggression and HLLE as the two main issues. Fortunately, these were mitigated easily through increased structure, removing dominant individuals, and moving fish to RAS water.

4.3 | Effects on growth

Flexion occurred over 14–22 dph, aligning with observations in other dwarf angelfishes (Baensch, 2017). Notably, protocol A demonstrated an accelerated flexion completion by 19 dph, suggesting that enhanced growth rates can reduce the duration of vulnerable larval stages. During flexion, body depth growth rates increased sharply while body length remained relatively constant. This likely reflects energy allocation toward air bladder inflation and the development of a deeper, more laterally compressed body, characteristics typical of postflexion angelfish larvae (Baensch, 2017).

Interestingly, while growth and survival are often thought to correlate positively, this study found no significant relationship between the two. Protocol A, in particular, contradicted the "bigger is better" hypothesis (Litvak & Leggett, 1992). While larvae in Protocol A exhibited the highest growth rates, survival rates were notably low. This anomaly may stem from Protocol A's delayed introduction of rotifers, which were not offered until 10 dph. The resulting high preflexion mortality left a small number of surviving larvae, which faced little competition for prey. Consequently, these larvae grew larger on average compared to those in other protocols, inflating growth rates relative to protocol B. A stagnation in larval growth was seen in protocol B after switching from live algae to algae paste (Nanno 3600), suggesting a possible link between changes in environment and stress causing growth inhibition.

Nutritional factors also likely contributed to Protocol A's results. The primary feed, *Artemia*, offers lower nutritional value compared to copepods. While *Artemia* was enriched with essential amino acids and high concentrations of ascorbic acid, the efficacy of enrichment can be variable. Cultured copepods, in contrast, provide a more comprehensive nutritional profile including major nutrients, HUFA's, DHA, EHA, and phospholipids while also being less pathogenic, supporting both growth and survival (Altaff & Vijayaraj, 2021). The absence of adult copepods in Protocol B likely accounts for the lower growth rates observed in that group, highlighting the critical role of copepods in the nutrition of postflexion larvae. Furthermore, feeding adult copepods in combination with enriched *Artemia* appears to maximize larval growth and survival, as each provides distinct nutritional benefits. While growth rates

varied significantly across protocols, there was no consistent correlation between growth and survival. This suggests that additional factors, such as feed quality, timing, and competition dynamics, play key roles in determining larval outcomes. These findings emphasize the importance of tailored feeding strategies, particularly the inclusion of copepods, to optimize both growth and survival in *C. potteri* larviculture.

4.4 | Effects on survival

Mortality events (>25% of fish) were noted when introducing newly hatched and enriched *Artemia*. Larvae often exhibited stringy feces containing visible *Artemia* exoskeletons, suggesting difficulties in fully digesting this feed or internal bacterial issues. While the ability to digest *Artemia* may depend on development, gut microbiota, or other underlying mechanisms, individual variation in digestion was observed, indicating potential genetic or developmental differences. Delaying the introduction of Instar I *Artemia* to 19 dph and enriched *Artemia* to 27 dph significantly reduced mortality, underscoring the importance of developmental readiness for successful digestion.

Mortality events were also observed when introducing dry feeds (Otohime A1 and A2) before flexion completion. The exact cause is unknown, but preflexion larvae may either lack the ability to digest these feeds or be sensitive to their chemical or physical properties. While a significant majority of fish still experience mortality during the early larval stage, consistent but small drops of postflexion larvae exert a notable influence on final production numbers. DiMaggio et al. (2017) reported comparable mortality rates, both in terms of daily occurrence and timing, while attempting to culture Pacific blue tang. Similar mortality timing was found in other *Centropyge* as well (Baensch, 2017). This consistent pattern raises concerns about the vulnerability of postflexion-stage fish and subsequent metamorphosis. Given the apparent stress during this phase, further exploration is essential to identify strategies for mitigating stressors and reducing associated mortality.

A notable stress response was observed when live algae was replaced with concentrated algae paste (Nanno 3600) in Protocol B. Larvae exhibited erratic swimming at the surface known as "fluttering". Fluttering has been seen in many species raised in our lab, usually following abrupt changes in light intensity or other environmental variations. Water surface agitation by a fan seems to reduce this stress response. Despite efforts to conserve live algae, algae paste negatively impacted larval health, affirming the superiority of live algae in maintaining stable tank conditions. While similar stress reactions to algae paste have been observed across multiple species at OI, the underlying mechanism remains unclear.

Tank conditions also played a large role in survival. Larvae reared in 1000 L tanks exhibited higher survival rates than those in 200 L tanks, likely due to increased depth, improved water circulation, and reduced temperature fluctuations. This trend, previously observed for other species at OI, was reflected in the survival rates of *C. potteri*, with 0.097% survival in 1000 L compared to 0% in 200 L tanks under identical feeding conditions. No larvae were successfully reared to settlement in 200 L tanks alone. However, some success was observed when larvae were initially reared in 1000 L tanks and later transferred to 200 L at 37 dph, further supporting the advantage of larger tank environments for early larval development.

Stocking density also influenced survival, with lower densities yielding higher survival rates. While the study assumed an ideal density of 20–40 eggs/L based on previously established protocols, densities of 2.4–10 eggs/L were more successful overall. More research is needed to determine an ideal stocking density for Potter's angelfish. Protocol C, utilizing 150,000 cells/mL of *T. lutea* twice daily, achieved similar or higher survival rates compared to 300,000 cells/mL, significantly reducing resource requirements. Preliminary trials at 75,000 cells/mL indicate even higher survival; however, more replicates are needed to confirm this trend.

Water source did not appear to correlate with survival rates, indicating feeding regime and environmental consistency are more important to larviculture success. Recent research at OI suggests diet plays a larger role in seeding larvae gut microbiota than rearing water and may act as a vector for pathogenic bacteria, such as *Vibrio*, as seen in larval Yellow tang (*Z. flavescens*) (Deck, 2024). Live feeds like rotifers and *Artemia* are potential carriers of

Vibrio or other opportunistic bacteria, which could contribute to the observed mortality during feed transitions. Future efforts to eliminate *Vibrio* from live feeds may offer valuable insights into reducing larval mortality in Potter's angelfish.

While the water source did not influence survival, juveniles reared in well water experienced rapid HLLE after completing metamorphosis, which worsened during growout if not moved to RAS water. Coloration problems were previously observed in *C. loricula* raised in well water at OI, possibly from HLLE (Laidley et al., 2008). Conversely, fish reared in RAS water showed no signs of HLLE. Potential causes include bacteria, vitamin D3 deficiency, low pH and alkalinity, and high silica and gas pressure in well water, warranting further investigation. Fish with HLLE that were moved to RAS water showed no further tissue deterioration and slowly healed as they grew.

Below are the best practices for raising Potter's angelfish based on our findings:

- Broodstock water parameters: 25.0–26.5 °C; undetectable NH_3 and NO_3^-; stable pH ~8.2; winter photoperiod of 11L:13 D; consistent salinity between 32 and 35 PSU.
- Larviculture water parameters: RAS at 26.5–27.0°C; undetectable NH_3 and NO_3^-; stable pH ~8.2; 12L:12 D photoperiod; consistent salinity between 32 and 35 PSU.
- Continuous use of live *Tisochrysis lutea* from 3 dph until settlement is recommended over algae paste.
- S-type rotifers, *Brachionus rotundiformis*, and *Parvocalanus crassirostris* copepod nauplii are suitable first feeds at 3 dph.
- Copepod nauplii fed twice daily to 5/mL, then weaned to 1/mL 5 days before feeding of nauplii ceased at 16 dph.
- Adult copepods are critical for flexion and postflexion stage larvae (17 dph and older).
- Feeding adult copepods along with *Artemia* nauplii maximizes larval growth and survival overall, given *Artemia* are not introduced too early (before ~18 dph).
- Larger rearing tanks (1000 L) suggested for greater water depth, decreased temperature variance, and improved water circulation.
- Algae densities of 75,000–150,000 cells/mL yielded the highest survival.

This study represents a significant milestone in the aquaculture of Potter's angelfish providing the first detailed documentation of their larval development and species-specific rearing methods. By refining feeding protocols and identifying optimal feed protocols and live food organisms, this research lays the foundation for advancing larviculture efforts of *C. potteri* and other reef fish species. The study demonstrated that larval growth, development, and survival are highly influenced by the timing of suitable feed allocation. While this research achieved the first successful cohorts of Potter's angelfish, further studies are needed to optimize stocking densities and address postflexion-stage mortality to enhance survival rates and commercial scalability. Nonetheless, these results highlight the potential for sustainable aquaculture of *C. potteri* and other Hawaiian reef species, transitioning the aquarium industry away from wild collection and reducing reliance on wild populations. By contributing to the broader development of sustainable practices within the global aquarium trade, this work supports reef conservation efforts and provides a model for the aquaculture of other high-value ornamental fish species.

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CONFLICT OF INTEREST STATEMENT

The authors have no conflicts of interest to disclose.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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