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Development of In-Captivity Breeding and Larval Rearing Technology for Coral Reef Fish in Taiwan

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ABSTRACT

Coral reef fish populations are declining both in Taiwan and around the world due to the impacts of long-term overfishing and recent climate change. To overcome these issues, in-captivity breeding of coral reef fish can provide an alternative method for the conservation of wild coral reef fish populations. A total of 43 species of coral reef fish have been captive-bred for education, research, and exhibition purposes in Taiwan. This achievement can be attributed to: (1) successful broodstock management, including broodstock collection and cultivation, maturation and spawning, and egg collection and incubation; (2) establishment of live feed preparation techniques for larval feeding; and (3) complete larval rearing using green-water and inorganic fertilization methods. Taiwan's progress in captive breeding and larviculture techniques for coral reef fish species has led to significant breakthroughs in the captive breeding of various marine food/ornamental fish. These achievements have not only contributed to the sustainability of the marine aquarium trade, but also enhanced our understanding of the reproductive biology and ecology of these fascinating species. This study intends to offer a comprehensive review on the achievements of coral reef fish breeding and larval rearing and provide relevant bibliographic resources. It is also hoped that the technical details provided here can assist those who are interested in the conservation of coral reef fish through in-captivity breeding and larval rearing efforts.

Keywords: Coral reef fish, natural spawning, larviculture, ornamental fish trade.

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1 INTRODUCTION

Aquaculture worldwide has grown significantly during the past half century. From a production of below 1 million tonnes (live weight equivalent) in the early 1950s, production in 2020 was reported to have risen to 122.6 million tonnes, with a 2.7% annual growth rate and a value of US$ 281.5 billion (FAO, 2022). The marine finfish aquaculture industry in Taiwan has been focusing on the widely accepted consumed food fish such as milkfish (*Chanos chanos*), sea breams (*Rhabdosargus sarba, Pagrus major* and *Acanthopagrus* spp.), snappers (*Lutjanus* spp.), cobia (*Rachycentron canadum*) and groupers (*Epinephelus* spp.) for more than three decades (Leu, 1994, 1997; Leu & Chou, 1996; Liao et al., 2001, 2004; Leu et al., 2003, 2005; Leu & Liou, 2013). However, some problems still exist in mariculture that need to be addressed and solved in order to increase production. The main issues concerned are: overexploitation of aquafarms, saltwater intrusion in nearby agricultural lands, land subsidence, damage of the coastal landscape, and outbreaks of fish diseases (Liao & Chao, 2007).

Although coral reefs cover less than 1% of the ocean's area, they are recognized as the most biologically diverse and productive ecosystems on Earth. Coral reefs support over 4,000 species of fish, 800 species of reef-building corals, and thousands of invertebrate species, including cnidarians, sponges, mollusks, crustaceans, and echinoderms (Burke et al., 2011). Coral reef fisheries provide food and economic revenue that support coastal communities comprising millions of people worldwide (Loper et al., 2008). In recent decades, human activities have had numerous negative impacts on coral reefs, including the deposition of agricultural and domestic wastewater sediment, eutrophication, overfishing, and global climate change (Moritz et al., 2018). Among these, increasing fishing yields to meet the demands of the aquarium trade is an important factor contributing to the decline of fish species that reside inside coral reefs.

The majority of marine ornamental fish are coral reef species and are primarily found in coral reef environments located near the equatorial regions of the world's three major oceans. These fish are mainly sourced from the wild. However, unscrupulous traders continue to deploy destructive fishing methods, posing an increasing threat to coral reef areas worldwide. One example is the use of cyanide to stun and capture high-value fish species. Numerous studies have documented the high toxicity of cyanide, which often indiscriminately harms coral reef fish and invertebrates without targeting specific species (Mak et al., 2005). The use of toxic cyanide for capturing these coral reef fish indeed has severe consequences, and reef-building corals exposed to cyanide face the threat of bleaching (Cervino et al., 2003). Despite this, the practice of using cyanide for fishing to supply the aquarium trade remains prevalent in Southeast Asia (Pomeroy et al., 2008). In addition to the use of cyanide, dynamite fishing is also used to capture large size coral fish for consumption; however, the practice itself inadvertently resulted in collateral damages to those considered as ornamental fish.

It is estimated that over 80% of marine ornamental fish, from the time of capture to living in home aquaria, perish due to poisoning, poor capture techniques, handling, transportation, husbandry practices, and diseases (Sadovy & Vincent 2002; Wabnitz et al., 2003; Leu et al., 2019). Due to the low survival rate of these captured organisms throughout the entire industry supply chain, coupled with the current demand for these organisms in the aquarium trade, there is an urgent need to find a sustainable approach for the marine ornamental trade. The current volume of trade in marine ornamental fish cannot be accurately calculated, but the annual estimated trade volume ranges from 14 to 30 million individuals (Thornhill, 2012). With the continuous rise in demand, improving the breeding techniques of coral reef fish and developing new aquarium species to mitigate the threats to coral reefs are considered the top priority solutions for protecting coral reef fish species. While countries such as Indonesia, Vietnam, Palau, and Australia are actively engaged in the specific breeding and cultivation of certain species, they only represent less than 1% and 0.01%, respectively, in terms of species composition or quantity compared to wild capture. Although approximately 90% of freshwater ornamental fish species are

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derived from artificial breeding and cultivation, only 1-10% of marine ornamental fish are successfully reproduced in captivity and become commercially viable (Moorhead & Zeng 2010; Olivotto et al., 2011).

Aquaculture for the aquarium hobbyist trade, however, is a rapidly growing sector of the industry (Tlusty, 2002) and there are directed efforts and increasing pressures within the ornamental trade to develop reliable and sustainable hatchery procedures for the captive breeding of many reef fish species (Chen et al., 2020; Pouil et al., 2020). The value of the world ornamental imports has increased significantly too, from US$ 50 million to US$ 300 million over the past two decades (Olivier, 2003; Palmtag, 2017). The aims of this review are to highlight the current achievements and challenges, and to elucidate new research directions for captive breeding and larval rearing of coral reef fish in Taiwan.

2 PRESENT STATUS OF THE EXPLOITATION AND CONSERVATION OF CORAL REEF FISH RESOURCES IN TAIWAN

Taiwan is situated within the subtropical and tropical climate zones, surrounded by both an ocean and a strait. The distribution of coral reefs at both the northern and southern ends contributes to a high diversity of marine life. The estimated number of coral reef fish species in Taiwan is conservatively placed at over 2,000, accounting for more than two-thirds of the total marine fish species in waters of Taiwan (Shao, 2023). Taiwan boasts a diverse range of marine fish species, rivaling even the Great Barrier Reef in Australia, with the exception of a few tropical regions like the Philippines. The variety of species here constitutes about one-sixth of the approximately 12,000 species of marine fish worldwide. By contrast, there are only about 150 species of freshwater fish native to Taiwan, accounting for just one-fiftieth of the world's 800 species of freshwater fish. Therefore, the coral fish species in Taiwan are indeed quite abundant. Proper conservation and utilization of this indigenous ecological resource are imperative. Approximately 60% to 70% of them are considered rare or uncommon, which means that their numbers are quite limited, and they are sometimes only sporadically encountered in nature. It is no surprise that the most colorful and attractive coral reef fish are the most often captured and kept as pets in aquaria, leading to a phenomenon of reverse selection. In other words, the more beautiful and adorable a fish is, the higher its price, and the more it is captured, which eventually leads to depleted available resources. Conversely, many less colorful and less favored fish species have seen their populations increased. If the capture of coral reef fish could be restricted or prohibited, it might provide a chance for those rare and precious fish species to recover.

Furthermore, one of the main challenges in identifying and protecting endangered coral reef fish species is the lack of comprehensive data on marine fish populations. The appearance of some fish is not only seasonal, but also annual. This is a major reason why ichthyologists are often cautious about designating rare marine fish species. As for the number of fish species that are endemic to Taiwan (endemic species), it is also relatively low. So far, there are only a few species discovered in Taiwan that are regarded as endemic, likely not exceeding 20 species (Shao, 2023). Species such as the long-snout cowfish (*Thysanophrys longirostris*), Taiwan garden eel (*Gorgasia taiwanensis*), Taiwan pikeblenny (*Neoclinus nudus*), and some new species of cardinalfish should be particularly cherished and protected.

However, the population of coral reef fish in Taiwan is steadily declining. This can be attributed primarily to the rapid socio-economic development in recent years, which has led to a disregard for ecological conservation and balance (Wu & Dai, 2007). This, in turn, has resulted in severe pollution and degradation of the coastal environment today. Additionally, the majority of Taiwanese enjoy seafood, which includes many coral reef fish; moreover, destructive fishing methods have further contributed to the demise of coral reef fish species.

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3 DEVELOPMENT OF THE CORAL REEF FISH AQUACULTURE INDUSTRY AND BUSINESS IN TAIWAN

3.1 In-captivity coral fish breeding program at the National Museum of Marine Biology & Aquarium (NMMBA)

According to surveys conducted by the Council of Agriculture, Executive Yuan, and the Taiwan Institute of Economic Research, Taiwan currently has the ability to successfully breed and stably supply over 30 species of marine ornamental fish to both domestic and international markets. Among these, 15 species of anemonefish and 11 species of damselfish are the main representatives. However, they only account for 2% of the total domestic ornamental fish production and 3% of the total production value (Hou et al., 2013). Overall, there is still ample room for development in the research and cultivation of marine ornamental fish.

Since its establishment in the year 2000, the National Museum of Marine Biology and Aquarium (NMMBA) in Southern Taiwan has responded to the demands of marine education, research, and exhibition. Using advanced life support systems and artificial habitats, the museum nurtures a diverse array of marine organisms. Similar to zoos and aquaria worldwide, it requires a balanced approach of capturing a certain number of wild animals from natural environments to supplement losses due to disease, predation, and aging. This practice ensures the quality and content of exhibitions. Under the attentive care of the museum staff, these marine creatures that were originally part of wild populations are now displaying their natural instincts. They were capable of reproducing and being nurtured in artificial conditions, thereby avoiding the need for wild capture and even contributing to ecological habitat restoration. The cultivation and restoration of coral reef fish species are two of the NMMBA's main areas of study. The private coral reef fish aquaculture community also has high expectations for the museum's development of breeding techniques, from which they can learn to enhance their productions. From an academic perspective, the restoration of coral reef fish species is an urgent and challenging research topic in marine resource conservation.

Consequently, to counter the gradual decline of coral reef fish species, the NMMBA has not only intensified its ecological conservation research on Taiwanese coral reef fish, but also, since 2003, established the "Coral Reef Species Breeding and Aquaculture Team." This team actively conducts research and development on the cultivation and breeding techniques for several high-value or rare coral reef fish species in Taiwan. Notably, the team achieved the "world's first successful artificial breeding record" by successfully inducing natural spawning of 24 coral reef fish species in controlled environments, raising the larvae into juveniles (Table 1).

3.2 Coral reef fish bred in captivity at the NMMBA

A total of 24 coral reef fish species that have been successfully bred in captivity at the NMMBA include: the palette surgeonfish (*Paracanthurus hepatus*), dwarf hawkfish (*Cirrhitichthys falco*), longfin batfish (*Platax teira*), yellow prawn-goby (*Cryptocentrus cinctus*), ornate goby (*Istigobius ornatus*), painted sweetlips (*Diagramma pictum*), Indian Ocean oriental sweetlips (*Plectorhynchus vittatus*), auzrio tuskfish (*Choerodon azurio*), bluestreak cleaner wrasse (*Labroides dimidiatus*), mangrove red snapper (*Lutjanus argentimaculatus*), blacktail snapper (*Lutjanus fulvus*), five-lined snapper (*Lutjanus quinquelineatus*), Russell's snapper (*Lutjanus russellii*), silver moony (*Monodactylus argenteus*), pearlscale angelfish (*Centropyge vrolikii*), bluespotted angelfish (*Chaetodontoplus caeruleopunctatus*), black-velvet angelfish (*Chaetodontoplus melanosome*),

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Queensland yellowtail angelfish (*Chaetodontoplus meredithi*), bluestriped angelfish (*Chaetodontoplus septentrionalis*), semicircle angelfish (*Pomacanthus semicirculatus*), saddleback clownfish (*Amphiprion polymnus)*, malabar grouper (*Epinephelus malabaricus*), bluestripe pipefish (*Doryrhamphus excisus*), and ringed pipefish (*Dunckerocampus dactyliophorus*) (Leu et al., 2003, 2005, 2007, 2009, 2010, 2012, 2015a, 2015b, 2015c, 2018, 2022; Lee et al., 2010; Yang et al., 2011; Chen, et al., 2013; Leu & Liou, 2013; Wang et al., 2014a, 2014b; Tseng & Leu, 2016; Tu et al., 2017; Leu & Leu, 2018; Chiu et al., 2019, in press; Lin et al., 2019; Chiu & Leu, 2021; Wang et al., 2022a, 2022b; Chen & Leu, 2023; Xu et al., 2023). These technological innovations and breakthroughs contribute to researchers' understanding of the reproductive physiology, maturity, spawning, and embryonic and larval biology of coral reef fish. This achievement has laid a crucial foundation for the sustainable development of the marine fish breeding industry in Taiwan.

3.3 Crucial factors for the successful in-captivity breeding of coral reef fish

The long-term goals of the NMMBA breeding program are to develop aquaculture techniques for raising reef fish in captivity to increase our understanding of their ecological requirements, to preserve rare and endangered species, and to reduce harvesting pressure on natural populations. It was observed that controlling the concentration and ratio of different nutrients in the seawater enhanced the production of phytoplankton of desirable size, which in turn produced zooplankton of desirable size that eventually enhanced the survival rate of newly hatched reef fish larvae. The impact of water quality on the survival of the larvae was constantly monitored. Water samples collected were analyzed immediately for parameters including temperature, salinity, pH, dissolved oxygen, nutrients (as nitrite, nitrate, and phosphate), ammonium, and turbidity. These data provided basic information on the overall water quality and nutrient concentrations of the cultivation system, which in turn allowed the establishment of the database of the most favorable cultivation conditions for continuous operation.

There are numerous critical processes in early life of fish where deficiencies are limiting factors in captive rearing. These include spawning in captivity, embryo development, and the transition from endogenous to exogenous feeding. Recent research studies have resolved some of the problems related to egg quality, proper embryo development, and hatching by developing suitable diets and technologies. Major problems that still remain are the optimization of feeding schedules and environmental conditions for successful larval rearing.

Earlier research has shown that inorganic nutrients added to the water can significantly enhance successful larviculture in freshwater (Qin & Culver, 1992; Culver et al., 1994; Qin et al., 1995; Tew et al., 2006; Jacob & Culver, 2010). Recent advancements have extended this technique to generate abundant populations of small ciliates and choanoflagellates, suitable for the initial feeding of marine fish larvae with small mouth openings (Tew et al., 2013, 2016; Kuo et al., 2021). This approach involves introducing inorganic nitrogen and phosphorus into the pond or tank to cultivate a mixture of natural diets (comprising various phytoplankton and zooplankton) that mimics the fish's native environment (see Figure 1). The objective is to rear potential prey of diverse sizes that aligns with the changes of mouth gape sizes and nutritional needs of fish larvae during their critical early life stages.

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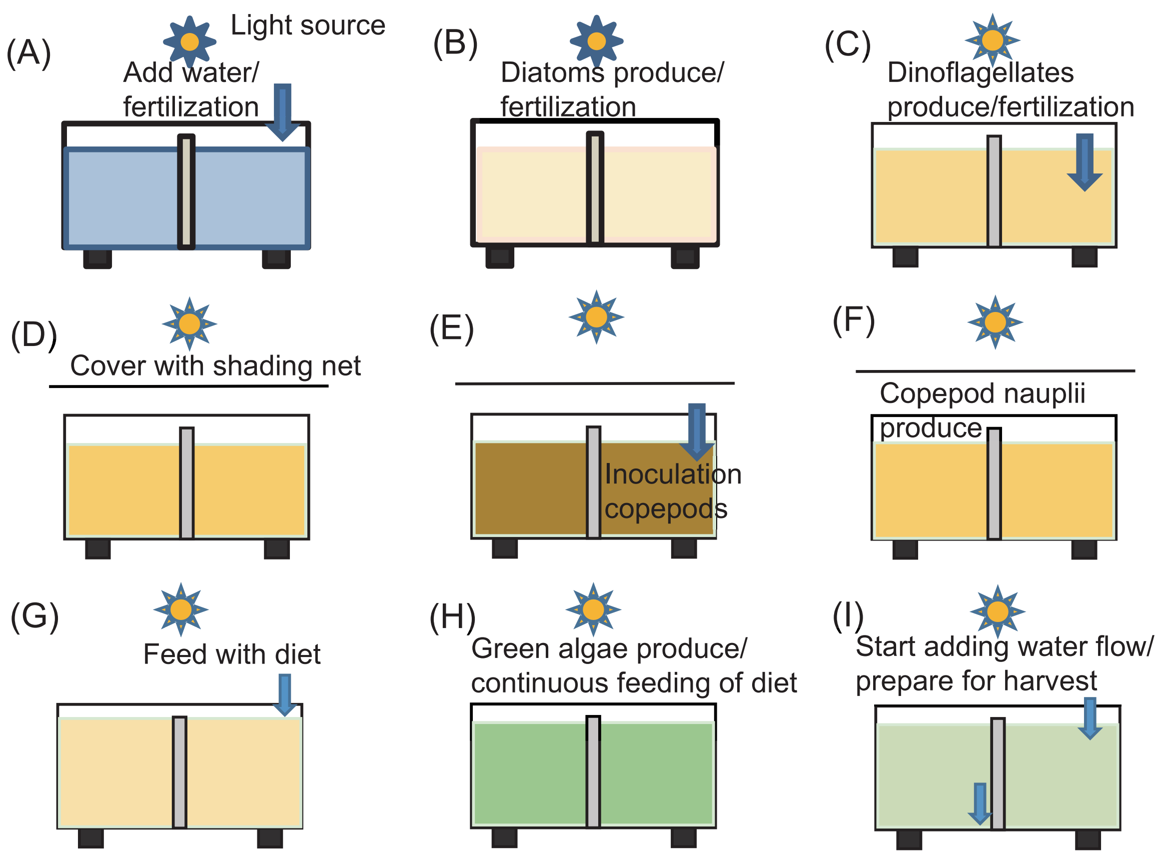

Figure 1. The operational processes of the inorganic nutrient salt enrichment method are as follows: (A) Adding natural seawater to the full water level that was filtered with a 300 μm mesh size scree, adjust aeration, and apply fertilization (N:P = 700:100); (B) cultivate a variety of diatoms first, maintaining a nitrogen-to-phosphorus ratio of 700:100; (C) after the production of dinoflagellates, introduce fertilized eggs according to algal density; (D) after introducing the fertilized eggs, cover with a shading net and continue controlling the nitrogen-to-phosphorus ratio in the water; (E) diatoms, dinoflagellates, and protozoa will proliferate extensively in the water. Copepods are introduced, and nutrient control is ceased; (F) copepod nauplii are produced in the water that served as initial food for the larvae; (G) when the larvae reach 10-14 dph, remove the shading net, and commence artificial feeding with rotifers and copepods; (H) diatoms recede, replaced by green algae to maintain the water's food density; (I) after about 2-3 months, the juveniles have settled and water can be gradually replenished in preparation for harvesting.

Leu et al. (2022) collected *L. dimidiatus* eggs that were produced naturally inside the aquarium display tanks at the NMMBA. The larvae were reared using the inorganic nutrient salt fertilization method and compared with the traditional feeding of rotifers. By controlling nutrient salt ratios and increasing inorganic nitrogen concentration, different types of phytoplankton were cultured to selectively cultivate various zooplankton species as live food organisms, aiming to enhance the survival probability of coral reef fish larvae. The results demonstrated that the inorganic nutrient salt enrichment method indeed helped the larvae successful transition through the initial stage of mouth opening for feeding. The outcomes of this study demonstrated that the know-how gained enabled commercial fish breeders to achieve sustainabilitygoals while significantly

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reducing the pressure to capture coral reef fish from the wild (Figure 2). However, the dynamics of phytoplankton suggested that the addition of nitrogen and phosphorus is necessary to maintain its growth, yet further examination is essential to determine the optimal frequency of nutrient salt enrichments for the best results. Given that high mortality within the first week of marine fish larviculture is very common (Moorhead & Zeng, 2010; Olivotto et al., 2011, 2017b; Chen et al., 2020), the inorganic salt enrichment method may be a viable alternative to other methods such as the green-water method (see Chen & Zeng, 2021) in marine fish larviculture.

Figure 2. Group of captive bred *Labroides dimidiatus* subadults reared with the inorganic salt enrichment method developed at the National Museum Biology and Aquarium.

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Table 1. Summary of captive breeding and larval rearing of coral reef fish in Taiwan.

Species	Source	Egg Production	Fecundity (eggs or larvae)	Egg Diameter (mm)	TL at Newly Hatched (mm)	Yolk-sac Absorbed (dph)	TL at Yolk-sac Absorbed (mm)	Larval Rearing Temp. (℃)	Larvicul-ture Method	Larval Duration (dph or dab)	TL at Juvenile (mm)
Acanthuridae *Paracanthurus hepatus*	Chen, et al. (2013); Ho et al. (2013); Leu et al. (2015c)	NS	–	0.68–0.70	1.52–1.58	3	2.19–2.21	28.0 ± 1.0	GW; IOF	55	15.85–22.40
Blenniidae *Omobranchus fasciolatoceps*	Chiu et al. (2021)	NS	–	0.92–1.05	3.20 ± 0.03	0	3.20 ± 0.03	29.0–30.5	GW	32	11.36 ± 0.48
Salarias fasciatus	Cheng et al. (2018a)	NS	15,000	0.91 ± 0.06	3.46 ± 0.30	2	3.68 ± 0.13	27.0 ± 1.0	GW	–	–
Carangidae *Gnathanodon speciosus*	Ho et al. (2011)	NS	–	0.91 ± 0.06	2.73 ± 0.10	3	2.81 ± 0.06	28.0 ± 0.5	GW	30	13.76 ± 1.48
Centriscidae *Aeoliscus strigatus*	Ho et al. (2010)	NS	–	1.10 ± 0.06	2.62 ± 0.12	2	3.0	24.0–27.0	GW	–	–
Cirrhitidae *Cirrhitichthys falco*	Chiu & Leu (2021)	NS	482,844	0.73–0.78	1.88–2.24	2	2.77–3.10	26.0–28.0	GW	–	–
Ephippidae *Platax teira*	Leu et al. (2018)	NS	–	1.17–1.32	2.81 ± 0.24	3	3.41 ± 0.11	25.7–27.8	GW	25	23.80 ± 1.98
Gobiidae *Amblygobius phalaena*	Chiu et al. (2023b)	NS	1,110,912	(1.21–1.44) × (0.40–0.47)	1.91–2.03	2	2.09–2.25	27.0 ± 0.9	GW	30	9.95–13.47
Cryptocentrus cinctus	Tseng & Leu (2016)	NS	9,725 ± 235	(1.37–1.58) × (0.62–0.73)	2.25–2.36	2	2.42–2.86	26.0–28.0	GW	22	8.78–10.25
Istigobius ornatus	Chiu et al. (2019)	NS	7,992	(1.31–1.54) × (0.46–0.50)	1.78–2.28	1	1.97–2.61	27.5 ± 0.5	GW	30	7.78–7.81
Mugilogobius cavifrons	Chiu et al. (2023a)	NS	38,900	(1.16–1.39) × (0.50–0.57)	1.89–2.37	2	2.32–2.59	22.9–27.0	GW	25	6.28–11.75
Haemulidae *Diagramma pictum*	Chiu et al. (in press)	NS	140,111	0.75–0.85	1.66–2.14	2–3	2.38–2.73	28.2–29.5	GW	25	24.8–30.8
Plectorhynchus cinctus	Chang (1997)	AF	–	0.78–0.82	1.6	2–3	2.8–2.9	26–29	GW	24	16.0
P. vittatus	Leu et al. (2012)	NS	204,550	0.76–0.83	1.82–2.00	3	2.69–2.95	23.0–28.9	GW	25	8.45–10.54
Labridae *Choerodon azurio*	Xu et al. (2023)	NS	–	1.03–1.14	2.58–3.43	3	2.46–3.80	27.8 ± 1.5	GW	38	10.9–13.4
Labroides dimidiatus	Leu et al. (2022)	NS	215,972	0.62–0.73	1.72–1.90	3	2.11–2.39	26.0–29.0	IOF	41	11.09–14.10
Lutjanidae *Lutjanus argentimaculatus*	Leu et al. (2003)	NS	2,350,000	0.74–0.81	1.62–1.94	2–3	3.1	25.8–28.7	GW	30	10.5–17.2
L. fulvus	Leu & Leu (2018)	NS	3,711,900	0.69–0.73	1.63–2.19	3	2.34–2.89	26.0–29.0	GW; IOF	26	13.6–15.9

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L. quinquelineatus	Leu et al. (2007)	NS	–	0.70–0.84	1.81–2.28	3	2.54–3.10	27.7–29.6	GW	30	12.9–16.8
L. russellii	Leu & Liou (2013)	NS	–	0.71–0.84	1.66–2.04	3	2.39–2.95	26.6–29.8	GW	30	10.67–16.75
Monodactylidae *Monodactylus argenteus*	Lee et al. (2010)	NS	–	0.90–0.97	2.50–2.55	2	2.53–2.93	25.9 ± 0.7	GW	35	10.56–14.87
Pomacanthidae *Centropyge vrolikii*	Wang et al. (2022a)	NS	–	0.67–0.71	1.77–2.04	2	2.20–2.56	26–27	GW; IOF	30	8.20–13.95
Chaetodontoplus caeruleopunctatus	Wang et al. (2022b)	NS	–	0.84–0.87	2.14–2.71	2	2.78–3.15	26–27	GW; IOF	30	13.16–15.71
C. melanosoma	Chen & Leu (2023)	NS	296,000	0.79–0.85	2.34 ± 0.09	2	2.94 ± 0.13	22.9–28.0	GW; IOF	–	–
C. meredithi	Lin et al. (2019)	NS	450,820	0.82–0.90	2.86–3.00	3	2.95–3.19	27.0 ± 1.0	GW; IOF	48	16.74–20.44
C. septentrionalis	Leu et al. (2010, 2015a)	NS	119,000	0.80–0.88	1.97–2.20	3	2.78–3.15	26.2–28.7	GW	52	16.51
Pomacanthus semicirculatus	Leu et al. (2009); Wang et al. (2014a, b)	NS	267,000	0.88–0.91	1.69–1.80	2	2.89–3.13	27.0 ± 1.5	GW; IOF	36	18.10–20.96
Pomacentridae *Amphiprion biaculeatus*	Jiang et al. (2012)	NS	52,000	(1.81 ± 0.09) × (0.79 ± 0.03)	3.64 ± 0.31	1	3.94 ± 0.14	24–28	GW	31	9.88 ± 1.48
A. clarkii	Cheng et al. (2019)	NS	–	(2.53 ± 0.08) × (0.94 ± 0.04)	4.93 ± 0.06	1	5.24 ± 0.10	27.3 ± 1	GW	30	22.20 ± 3.31
A. frenatus	Ho et al. (2009)	NS	31,500	(2.70 ± 0.12) × (0.96 ± 0.03)	4.48 ± 0.14	1	4.75	24–30	GW	25	12.15
A. ocellaris	Ho et al. (2007)	NS	16,800	(2.32 ± 0.10 ± 0.08) × (0.95 ± 0.07)	4.35 ± 0.14	1	4.37	24–28	GW	26	16.02
A. ocellaris var.	Cheng et al. (2015)	NS	19,550	(2.70 ± 0.12) × (0. 96 ± 0.03)	4.31 ± 0.13	1	4.32 ± 0.13	28–31	GW	25	13.02 ± 1.78
A. perideraion	Ho et al. (2006)	NS	11,000	(1.68–2.18) × (0.78–0.85)	3.20–3.80	1	–	24.5–32.0	GW	26	12.56
A. polymnus	Chen et al. (2003); Yang et al. (2011)	NS	32,300	(2.41 ± 0.08) × (0.86 ± 0.04)	3.50–4.48	1	4.04–4.47	25.6 ± 1.1	GW	28	19.32–30.95
Chrysiptera cyanea	Cheng et al. (2008)	NS	–	(1.19 ± 0.03) × (0.53 ± 0.02)	2.80 ± 0.20	1	2.87	25–28	GW	30	15.0

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C. glauca	Tsai et al. (2012)	NS	–	(1.18 ± 0.05) × (0.60 ± 0.01)	2.64 ± 0.22	1	2.67	25–28	GW	30	13.50
Neoglyphidodon melas	Cheng et al. (2018b)	NS	40,300	(1.58 ± 0.06) × (0.71 ± 0.02)	2.86 ± 0.12	0	2.86 ± 0.12	26–28	GW	25	13.00
Serranidae *Epinephelus bruneus*	Chiu et al. (2020)	AF	166,500	0.88–0.95	1.77 ± 0.02	3	2.90 ± 0.02	23.8 ± 1.2	GW	33	17.69 ± 0.09
E. lanceolatus	Ho et al. (1997)	NS	2,400,000	0.80–0.89	1.65 ± 0.12	–	–	–	GW	–	–
E. malabaricus	Leu et al. (2005)	NS	11,800,000	0.87–0.93	1.88–1.98	3	2.63–2.85	26.1–26.8	GW	30	17.69–23.81
E. tukula	Yeh et al. (2003)	NS	300,000	0.9	2.7	3	–	27–30	GW	33	29.3
Syngnathidae *Doryrhamphus excisus*	Tu et al. (2017)	NS	2,862	0.92–1.10	4.88–5.06	–	–	25.0–26.0	GW	4	6.90–7.40
Dunckerocampus dactyliophorus	Leu et al. (2015b)	NS	2,269	1.33-1.61	6.54–6.95	–	–	24.0–26.0	GW	3	8.60–8.97

NS: natural spawning; AF: artificial fertilization; TL: total length; dph: days post hatching; dab: day after birth; GW: green-water; IOF: inorganic fertilization; –: no data.

3.4 Successful breeding programs through collaborations

In addition, collaborations among research institutions, government agencies, and the private sector have played a crucial role in advancing these artificial breeding techniques. The exchanges of knowledge, expertise, and resources have accelerated progresses and facilitated the successful cultivation of once-difficult-to-breed species (Table 1). These species included: the blenny (*Omobranchus fasciolatoceps*), jewelled blenny (*Salarias fasciatus*), razorfish (*Aeoliscus strigatus*), golden trevally (*Gnathanodon speciosus*), *P. hepatus,* whitebarred goby (*Amblygobius phalaena*), mangrove goby (*Mugilogobius cavifrons*), crescent sweetlips (*Plectorhynchus cinctus*), spinecheek anemonefish (*Amphiprion biaculeatus, Premnas biaculeatus* was a senior synonym), yellowtail clownfish (*Amphiprion clarkia*), tomato clownfish (*Amphiprion frenatus*), clown anemonefish (*Amphiprion ocellaris*), black and white ocellaris clownfish (*Amphiprion ocellaris var.*), pink anemonefish (*Amphiprion perideraion*), *A. polymnus,* sapphire devil (*Chrysiptera cyanea*), grey demoiselle (*Chrysiptera glauca*), bowtie damselfish (*Neoglyphidodon melas*), longtooth grouper (*Epinephelus bruneus, Epinephelus moara* was a senior synonym), giant grouper (*Epinephelus lanceolatus*), and potato grouper (*Epinephelus tukula*) (Chang, 1997; Ho et al., 1997, 2006, 2007, 2009, 2010, 2011, 2013; Yeh et al., 2003; Chen et al., 2003; Cheng et al., 2008, 2015, 2018a, 2018b, 2019; Jiang et al., 2012; Tsai et al., 2012; Chiu et al., 2020, 2021, 2023a, 2023b). Most of the aforementioned fish species were successfully cultivated into juveniles using the conventional green-water larviculture method.

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4 OVERVIEW OF CAPTIVE BREEDING AND LARVAL REARING TECHNIQUES FOR CORAL REEF FISH

The life history of most coral reef fish is usually divided into three stages: larvae, juveniles, and adults. To successfully cultivate marine fish, it is necessary to rear them from fertilized eggs to adults. Generally, many scientists assume that coral reef fish can be induced to spawn in artificial environments and subsequently reared. Based on this assumption, the aquaculture techniques developed in the laboratory should be easily transferred to commercial-scale production (Holt et al., 2017), and yet the reality is far from this as outlined in the following section.

4.1 Bottlenecks in the development of captive breeding techniques for coral reef fish

Currently, aquaculture is widely regarded as the most promising approach for sustainable fisheries. If we can breed coral reef fish species that have been heavily harvested, we can effectively reduce the pressure on fishing coral reef fish. Aquaculture not only provides alternating methods of producing captive bred coral reef fish, it also provides valuable information on the life history of these organisms, such as maturation size and spawning age. This in turn enhances our understanding how these organisms respond to the impacts of human activities.

In recent years, the use of closed aquaculture systems to supply coral reef fish has gained increasing attention. The development of sustainable breeding techniques producing coral reef fish has become an essential element in reducing the pressure on wild populations. Because they are raised in closed systems, these coral reef fish can potentially exhibit greater robustness and higher survival rates in artificial captive environments. Production of coral reef fish through breeding can meet market demands; however, in reality, many challenges still exist in the majority of coral reef fish breeding efforts. For instance, there is a lack of critical information regarding their early life history, which becomes a limiting factor in artificial breeding. These critical processes include brookstock spawning (including gender determination and reproductive capacity development), embryo development (closely related to parental fish nutritional requirements, nurturing, and genetics), hatching (related to reproductive strategies), and the transitional period from yolk absorption to the initiation of external feeding for juvenile fish (Olivotto et al., 2017a).

4.2 Broodstock management and spawning

Careful selection of breeding broodstock is crucial at the outset, as high-quality broodstocks are fundamental for the successful rearing of larvae and juveniles. Once these criteria are met, captive bred individuals should replace those caught in the wild. This is because they are hardier, better adapted to captive environments, and can be reared from a young age, thriving in aquaria for extended periods without the stress of transportation.

Different fish species employ various reproductive strategies, so understanding their life history is crucial for successful breeding. The development of reproductive capacity relies on the integration of multiple internal and external cues, which provide us with essential insights into the reproductive behavior of fish. These cues include factors such as whether they have reached the appropriate reproductive size or energy state (metabolic factors), whether they are in optimal reproductive conditions (environmental factors), and whether suitable mates are available for reproduction (social factors). Attempting to breed specific fish species in artificial environments places significant emphasis on being able to discern their genders. For some fish, their gender is determined during development and cannot be changed; they lack the ability to undergo sex change. Forming reproductive pairs is often achieved through trial and error or by identifying specific characteristics and reproductive organs.

While the majority of fish are dioecious (having distinct sexes), some fish possess the ability to change their sex (hermaphrodites). Hermaphroditism refers to a biological entity having both testes and ovaries and being able to function as either gender during reproductive activities. Physiological adaptations often prevent self-fertilization. This trait is observed in a minority of coral reef fish species, such as those from the Serranidae family. Sequential hermaphroditism is a more dominant trait. In the early and late stages of their life history, these fish are controlled by their gender, influencing their behavior. However, they can change their sex when necessary. Hermaphroditism can be classified into protandry (initially male, then female) and protogyny (initially female, then male). An example of typical protandry is seen in clownfish, where social behavior determines gender. In clownfish colonies, a hierarchical system is present: the largest individual is female, the second largest is male, and other fish lack reproductive functionality (Yang et al., 2011). Examples of typical protogyny are groupers and angelfish (Yeh et al., 2003; Leu et al., 2010).

Other than the gender of the fish, the functioning of hypothalamic-pituitary-gonadal axis is crucial for the proper initiation of a reproductive cycle. Environmental conditions, including light cycle, temperature and food availability, play vital roles in sexual maturation. By adopting timing lighting and heating systems, we can adjust the light cycle and water temperature, mimicking natural spawning conditions like seasonal changes in daylight duration and temperature to induce fish spawning. Generally, high temperature (28°C) and long photoperiods (14 L/10 D, meaning 14 hours light/10 hours dark) can effectively induce spawning in demersal spawners. Other light combinations (13 L/11 D and 12 L/12 D) at 26°C have shown good spawning induction effects in *Elacatinus figaro* (Meirelles et al., 2009; Shei et al., 2010). For pelagic spawners (fish that produce pelagic eggs), seasonal changes (winter 22°C 10 L/14 D, spring 24°C 12 L/12 D, and summer 27-28°C 14 L/10 D) are necessary for spawning induction, although spawning behavior mostly occurs in the summer (Holt & Riley, 2001).

There are two different ways by which marine fish lay eggs: demersal and pelagic. Fish with demersal eggs typically lay adhesive eggs in clusters on solid surfaces or within caves. This method requires parental care, with the parent fish guarding and tending to the fertilized eggs until they hatch. During the spawning period, the female fish require sufficient feeding to maintain ovarian quality. When kept in aquaria, it's important to create appropriate water flow to ensure ample oxygen for the fertilized eggs.

Fish that produce pelagic eggs release their eggs into the water column and display complex courtship patterns. Compared to fish with demersal eggs, those with pelagic eggs typically have smaller egg sizes but higher egg quantities. Spawning behavior often occurs during dusk when predation pressure is lower and predators usually start seeking shelter at this time, and nocturnal predators are less active during this period.

4.3 Embryonic development and hatching

Based on different reproductive strategies, pelagic eggs hatch within hours to a day, while demersal eggs tend to have a longer hatching time. All fish embryos are protected by chorion during embryonic development to ensure proper metabolic processes during the early stage of ontogenesis. Prior to hatching, the secretion of hatching enzymes is needed in order to ensure that the breakdown of chorion is timely regulated. The hatching processes involve not only enzymatic actions, but also mechanical actions including embryo twisting in order to break up the chorion layer (Inhoaya et al., 1997). In several species of demersal eggs, successful hatching might be influenced by ambient light conditions, as their eggs mostly hatch at night. Hatching regulated by light could carry significant ecological significance, as this strategy significantly reduces the chances of predation on the newly hatched larvae.

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During the hatching process, both newly hatched larvae and early-stage larvae (prolarvae) are highly sensitive to any drastic physical or chemical environmental changes. Larvae from demersal eggs develop within the egg until they possess pigmented eyes and fins. They have functional jaws and pigmented eyes during this period, and the yolk sac is nearly depleted. Their mouths and digestive tracts open and become functional. However, larvae from pelagic eggs are extremely tiny and remain in the prolarval stage upon hatching. They lack pigmented eyes, a digestive system, or a functional mouth. At this stage, they still retain a large yolk sac and go through a 48-hour developmental period in the water column. After this phase, the prolarvae continue to develop into postlarvae with strong mobility, pigmented eyes, and a functional digestive system. Prolarvae at the initial stage are susceptible to predation by predators.

4.4 Fish larval rearing and live feed culture

Larvae and prolarvae are both highly delicate during the initial stages of a fish's life history. Various larval rearing systems have been designed to create environments rich in food resources and low in predators. Using 20-600 liters tanks, miniature ecosystems called microcosms have been successfully established to rear several fish species with demersal eggs, including pomacentrids (clownfish and damselfish), gobies (*Amblygobius, Istigobius,* and *Mugilogobius* spp.), dottybacks (*Pseudochromis* spp.) and some blennies (*Omobranchus* spp.) (Olivotto et al., 2006; Chiu et al., 2019, 2021, 2023a, 2023b; Chen & Zeng, 2021; Cañedo-Orihuela et al., 2023). The tanks are covered with black panels on the sides to reduce light exposure, and the tank bottom is covered with white panels for ease of cleaning. The water within the tanks circulates 10 times a day through a drip filtration system. For prolarvae, placing smaller 50-liter mesh cages within larger tanks (600-7,000 liters) has proven to be successful. This setup provides a confined space where both the prolarvae and their food can encounter, enhancing food consumption rates, offering shelter, and reducing potential physical damage. Additionally, temperature control and aeration are regulated from outside the larger tanks. The water within the smaller rearing spaces is clean, warm, and has a salinity similar to natural seawater. All conditions align with the natural survival requirements of the larvae.

The main bottleneck in the rearing of coral reef fish larvae is the transition from endogenous to exogenous nutrition (Holt, 2003). Additionally, since these larvae inhabit the open ocean, it is necessary to simulate such an environment. The tropical marine environment is warm, with a calm water flow, and has an appropriate density of live feed for the larvae to feed on. Many field studies report that oceanic larvae mainly feed on copepods, protozoans, and benthic organism larvae (Munk, 1997; Østergaard et al., 2005). The selection of proper live feed for larval feeding is based on several parameters, such as size, morphology, nutritional value, stock density, and growth rate (Pan et al., 2022).

Recent research suggests that feeding marine zooplankton to the larvae and examining their digestive contents reveals a substantial intake of copepod eggs and nauplii. Unfortunately, copepods are difficult to culture continuously. Most marine fish species are cultured using rotifers (*Brachionus* spp.) and brine shrimp (*Artemia* spp.). Despite their practical advantages in production, these are not the most suitable food for larvae as they do not mimic the natural diet of the larvae. For extremely small larvae like butterflyfish, angelfish, and groupers, these prey items are quite large, and their slow movement patterns are insufficient to trigger the larval feeding response (Leu et al., 2005, 2010, 2015a). Furthermore, their fatty acid composition does not match the nutritional needs of marine fish larvae. For instance, research on *Pseudochromis flavivertex* larvae demonstrated the importance of nutrient-rich rotifers and brine shrimp (Olivotto et al., 2006). Larvae were divided into experimental groups and fed enriched and non-enriched prey items. Larvae fed non-enriched rotifers survived only up to 7 days, while those fed enriched rotifers and brine shrimp had the highest survival rate (39% grew into juveniles). Moreover, the larvae fed enriched prey items showed faster growth rates and earlier completion of metamorphosis. This result clearly emphasizes the necessity of nutrient enrichment for successful rearing of *P. flavivertex* larvae. In the cultivation of *A. ocellaris* larvae, unusual pigment deposition and the reasons behind partial or complete loss of white bands (miss-banding) have been explored (Avella et al., 2007). Clownfish with missing white bands are sold at lower prices in aquaculture facilities. This interesting yet pressing issue has been addressed by using highly unsaturated fatty acids (HUFAs) to enrich the prey items. This not only positively affects larval growth, but also reduces the occurrence of missing bands. While other solutions like adding carotenoids to the diet or manipulating light intensity and backgrounds can be used to mitigate the issue, the absence of vibrant coloration in juveniles and adults diminishes the value of the product (Yasir & Kim, 2009a, 2009b).

Therefore, identifying suitable live feed to replace the deficiencies of rotifers and brine shrimp and enhance the diversity, growth, and survival of reared larvae remains a pressing issue for larval fish rearing. Copepod adults, copepod nauplii, and cladocerans are natural prey for larvae in their environment. They are often found in the digestive tracts of larvae and can be fed individually or mixed with rotifers and brine shrimp. The benefits of using copepods in larval rearing are as follows: copepods span a wide range of sizes between nauplii and adults, have diverse movement patterns, and are rich in highly unsaturated fatty acids (HUFAs) (Pan et al., 2018). Among these fatty acids, eicosapentaenoic acid (EPA, 20:5 n-3) and docosahexaenoic acid (DHA, 22:6 n-3) have been proven crucial for larval survival and growth. These fatty acids are essential components of larval diets and their absence would be detrimental to larval health, leading to lower growth rates, reduced feeding rates, anemia, and increased mortality (Sargent et al., 1997, 1999).

Currently, copepods are recognized as one of the most effective alternative food sources for many larval rearing systems (Figure 3). In intensive larval rearing, commonly used copepods include calanoid copepods such as *Acartia* spp., *Eurytemora* spp., *Parvocalanus* spp., and *Centropages typicus*, as well as harpacticoid copepods such as *Euterpina acutifrons*, *Tisbe* spp., and *Trigriopus japonicus.* Harpacticoid copepods are easy to culture at high densities, but during the cultivation process, individuals often prefer to stay near the walls of the rearing containers rather than in the water column. Because of this behavior, using harpacticoid copepods as a direct food source for fish larvae is not very effective; they are usually used as supplementary feed in addition to traditional rotifers and brine shrimp. Among copepods, calanoid copepods are the most effective for larval rearing, as they are rich in HUFAs and remain planktonic throughout their life history, making them an ideal food source for marine fish larvae. The nauplii stage of these copepods is typically small in size, making them readily accepted by fish larvae with small mouths during their initial feeding. However, unfortunately, there are still several challenges in the continuous cultivation of calanoid copepods. For example, they require low stocking densities in large tanks and need to be fed various types of microalgae.

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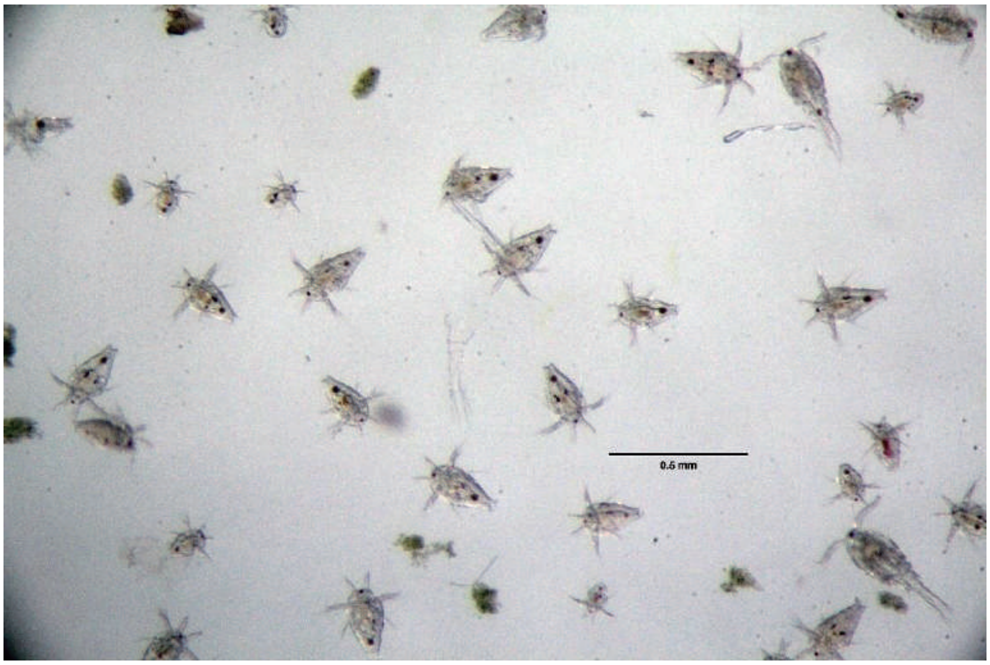

Figure 3. Readily available for commercial production, copepod nauplii are recognized as highly desirable early-stage live feed for marine fish larvae due to their appropriate size and rich content of HUFAs. They significantly enhance the techniques for rearing coral reef fish larvae.

Copepods are an ideal food source for smaller marine fish larvae, making them a good choice for feeding marine angelfish, a group of colorful coral reef fish that are highly sought after in the ornamental fish market (Ajiboye et al., 2011). The main challenge in breeding marine angelfish lies in their initial feeding stage, as tiny larvae cannot consume rotifers and there is a lack of other readily available food sources. In a study involving *C. septentrionalis*, researchers used harpacticoid copepods or a mixture of ciliates (*Euplotes* sp.) and rotifers as larval food. Under temperature conditions of 26.2–28.7°C, they were able to cultivate the larvae for up to 30 days after hatching, achieving a maximum survival rate of 8.9% (Leu et al., 2015a) (Figure 4).

A similar experiment was conducted during the breeding of *P. semicirculatus.* Different food sources were tested by feeding larvae individually or in different proportions. The highest survival rate was achieved by feeding a mixture of 30% dinoflagellates (*Gonyaulax* sp.) + 35% nanoflagellates (*Nannochloropsis* sp.) + 35% rotifers (*Brachionus rotundiformis*) to the larvae (Leu et al., 2009). Future research in this area requires a more thorough understanding of the reproductive cycle of these fish species.

Furthermore, the small-scale cultivation technique of *Calanopia, Centropages,* and *Oithon* copepods had successfully been applied to the captive breeding of some challenging fish species, such as *C. vrolikii* and *C. azurio* (Wang et al., 2022a; Xu et al., 2023). Maintaining a sufficient number of large copepod nauplii until the fish larvae are ready to consume newly hatched *Artemia* nauplii has become increasingly difficult. As a result, scientists are still working on extending the larval period to improve survival rates. Studies have shown that two copepod species, *Parvocalanus* copepods and *Pseudodiaptomus* copepods, outperform the commonly cultured *Acartia tonsa* copepods in terms of production quantity, size, and survival rates (Rhyne et al. 2009).

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Figure 4. Newly settled bluestriped angelfish (*Chaetodontoplus septentrionalis*) being transferred to a juvenile grow-out tank.

Furthermore, rearing larvae in "green-water," which is made by adding live microalgae or diluted microalgae paste into the culture water, is a common technique used in marine fish larviculture (Figure 5). Incorporating a variety of algae species in the water of rearing tanks enhances the visual foraging capabilities of fish and contributes to maintaining high water quality. The algae can also serve as a direct nutritional source for the organisms. Numerous studies (Naas et al., 1992; Fushimi, 2001; Lee & Ostrowski, 2001; Liao et al., 2001; Shields, 2001; Palmer et al., 2007; van der Meeren et al., 2007; Stuart & Drawbridge, 2011) have reported that the application of green-water has positive effects on various aspects of marine fish larval development. Specifically, it has been shown to enhance larval survival, growth, and feeding response (Cobcroft et al., 2012; Naas et al., 1992; Baensch, 2017; Degidio et al., 2018; Pereira-Davison & Callan, 2018; Leu et al., 2005, 2010, 2015a, 2018; Chiu et al., 2019). Using green-water also offers the added advantage of helping to reduce the incidence of abnormal behaviors like "walling" or the "head-butting syndrome," wherein larvae persistently swim against the tank walls (Cobcroft et al., 2012; Madhu et al., 2016). These behaviors may lead to possible jaw deformities and impede larval nutrition by restricting their ability to forage effectively, ultimately impacting their overall well-being (Cobcroft & Battaglene, 2009).

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Nevertheless, it is crucial to acknowledge that the assortment of microalgae species and their concentrations employed in generating green-water can vary significantly among different studies, and the precise protocols for managing this process are frequently not clearly defined (Chen et al., 2020). In addition, microalgae consumption could potentially influence the gut microbiome composition and immunomodulatory responses in aquatic species that are fed with microalgae (Sagaram et al., 2021). Currently, only a limited number of studies have undertaken endeavors to determine the optimal use of green-water for rearing reef fish larvae, primarily through short-term experiments (Degidio et al., 2018; Groover et al., 2021; Pereira-Davison & Callan, 2018; Chen & Zhen, 2021), leaving a significant gap in our understanding of this area.

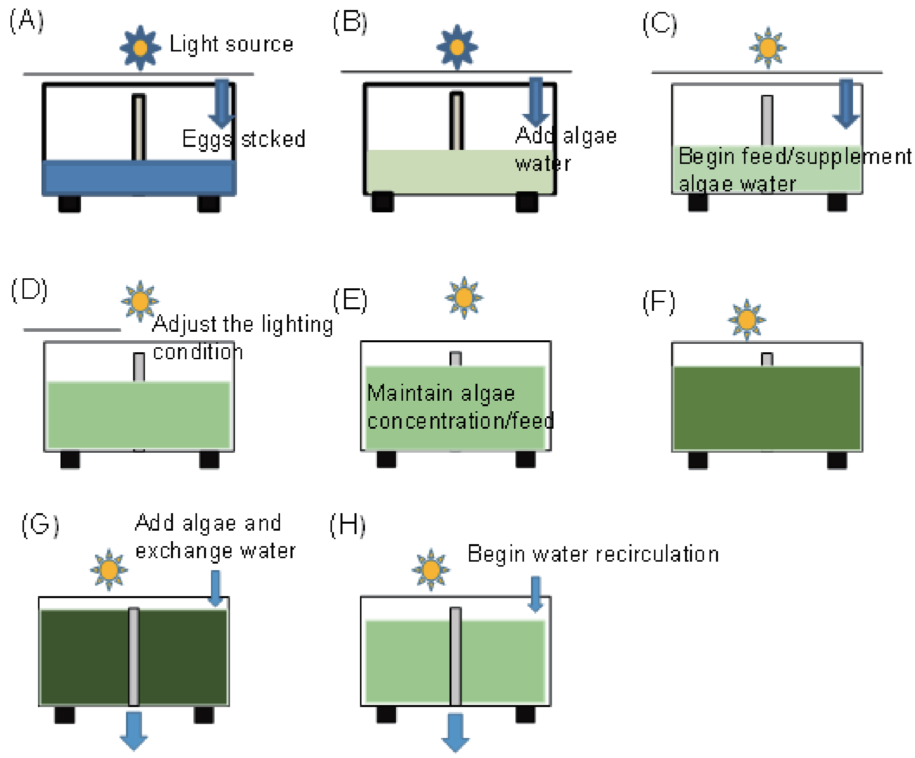

Figure 5. The operational procedures for the green-water larviculture method are as follows: (A) Add natural seawater filtered with a 300 μm mesh size screen, introduce fertilized eggs, adjust aeration, and provide shaded light by covering the tank with a shading net until the first day post hatching (0-1 dph). (B) On 1 dph, add algae water until the water appears pale green, but do not feed. (C) From 2-3 dph, as the fish larval eyes develop, feed nauplii of copepods, brine shrimp, and wild zooplankton; maintain algae concentration and supplement algae water to sustain the color while increasing water level. (D) From 4-7 dph, maintain the concentration of algae water and feed; adjust the lighting condition as water color becomes darker and water depth increases. (E) From 7-10 dph, maintain algae concentration and allow feeding only on copepods and wild zooplankton. (F) From 10-25 dph, maintain algae concentration and let larvae feed on copepods and rotifers, then adjust feed density with the increasing water level; if algae color lightens, supplement with algae accordingly, and consider partial water exchange. (G) Beyond 25 dph, maintain algae concentration and let larvae feed on copepods and rotifers; use a dual-tube system to simultaneously add algae water and exchange water, ensuring good water quality in the outdoor algae pond. (H) After 50 dph, if the juveniles begin consuming adult copepods or an artificial diet, initiate water recirculation, cease the addition of algae supplements, and gradually reduce algae water density.

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5 FUTURE PROSPECTS AND CHALLENGES OF CORAL REEF FISH AQUACULTURE

The main bottleneck in the cultivation of coral reef fish larvae is the transition from endogenous nutrition (yolk) to exogenous nutrition (food) and how to simulate the natural environmental factors for these fish. By examining the contents of the digestive tract of larval fish, it can be observed that they heavily ingest copepod eggs and nauplii. Unfortunately, copepods are difficult to culture continuously, and most marine fish are fed with rotifers (*Brachionus* spp.) and brine shrimp (*Artemia* spp.). Although rotifers and brine shrimp have practical advantages in production, they are not the most suitable prey organisms for larvae since they are not their natural food. Therefore, there is a significant need to develop and cultivate appropriately sized new species of planktonic organisms that can be cultured at high densities and possess suitable nutritional characteristics, in order to rear the early larvae of various coral reef fish species. Additionally, innovative rearing methods need to be explored for the nursery and grow-out culture systems and common diseases of coral reef fish.

Recently, a series of innovative aquaculture research and development technologies have been successively introduced. These include new and more efficient equipment, the use of small-scale or large-scale systems (referred to as micro-scale and meso-scale respectively), as well as live feed organisms for larval fish consumption, such as:

1. Revolutionary breakthrough technologies, including the use of inorganic salts enrichment methods to produce high-density small ciliates and flagellates for the rearing of larval fish with small mouths during their initial feeding phase (e.g., surgeonfish, wrasses).
2. Live copepods readily available for commercial production, which could significantly enhance the cultivation techniques of coral reef fish. Previously, the cultivation of these coral reef fish was hindered by inappropriate initial feed sizes.
3. The creation of new varieties (phenotypes) of coral reef fish through artificial breeding, opening up new avenues for coral reef fish aquaculture.
4. From an industrial and economic perspective, the development of recirculating aquaculture systems (RAS) has seen remarkable advancements in both small-scale and large-scale aquaculture systems in recent years.

Furthermore, there should be continuing efforts to enhance the interaction and connection among aquaculture scientists, marine aquarium enthusiasts, and businesses, in order to facilitate the effective applications of coral reef fish research results and technology to the market. In the future, the focus of coral reef fish aquaculture should also prioritize species with high market potential and unique life history characteristics, as these species will contribute significantly to the flourishing development of aquaculture and the transition from wild capture.

It is worth emphasizing that after years of research by scientists worldwide, one of the most emblematic fish in the marine aquarium trade, *P. hepatus* (commonly known as the Pacific blue tang)—famously depicted as 'Dory' in the movie *Finding Dory*—has been successfully bred in public aquaria and cultivated in artificial environments (Chen, et al., 2013; Ho et al., 2013; DiMaggio et al., 2017; Olivotto et al., 2017b; Sowaske et al., 2023). This significant achievement marks a crucial milestone in marine aquarium aquaculture. It allows us to envision that despite challenges posed by the overfishing of certain marine aquarium fish and persistent hurdles in aquaculture techniques, the industry will continue to witness significant breakthroughs in the aquaculture of coral reef fish species in the coming years. We hope that one day, through *Finding Dory*, we can promote the conservation of 'Dory' and other coral reef fish species, and achieve responsible harvesting, breeding, and sustainable aquaculture.

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6 CONCLUSIONS AND SUGGESTIONS

As the world's population continues to grow steadily, the significance of aquaculture will become increasingly evident in the coming century. To prevent the depletion of natural resources and alleviate market demand, the establishment and development of captive breeding and larval rearing techniques for coral fish are crucial for the conservation of wild fish populations. Many reef fish species can already naturally spawn in artificial environments, with the primary bottleneck being the availability of appropriate initial food items for newly hatched larvae. Copepod nauplii, copepod adults, and rotifers are considered good options for feeding marine ornamental fish larvae. The advantage of using copepods in larval rearing lies in their substantial size variation from nauplii to adults, their characteristic movement patterns, and their high content of essential fatty acids.

Nonetheless, challenges remain, especially in developing a closed culture system that mimics natural ecosystems to commercially produce marine fish larvae using copepods. Future research should focus on identifying suitable copepod species with shorter life cycles that can be reared at high densities. Additionally, selecting the suitable copepod species with regard to amino acids, proteins, pigments, and vitamins for larval growth and survival is vital. Ornamental fish aquaculture in Taiwan has evolved from simple field manual collection to successfully achieving the full cycle aquaculture of around 400 species (most of freshwater species), setting an unparalleled record globally. However, the number and variety of marine ornamental fish (i.e., coral reef fish) in cultivation remain relatively low. Both the government and private enterprises need to actively invest in research and development to enhance the industry's competitiveness. In this context, it is crucial to first act in favor of sustainable fishing methods (i.e., with proper stock management and avoiding habitat destruction) and the establishment of coral reef fish gene banks and marine protected areas (MPAs), and then promote captive-bred reef fish production (Pullin, 2000; Burke et al., 2011; Rhyne et al., 2014).

While the aquaculture industry in Taiwan has a history of over 300 years and possesses leading aquaculture techniques compared to other countries, the limited availability of water and land resources has led to the gradual deterioration of aquaculture environments. This has resulted in the spreading of diseases among fish, shrimp, and shellfish, causing the stagnation of the industry. These challenges highlight the necessity for Taiwan's aquaculture sector to incorporate new technologies to rejuvenate and ensure its sustainable development. One of the greatest distinctions of institutions like the NMMBA and other domestic research institutes lies in their ability to assemble interdisciplinary teams with diverse expertise. This approach harnesses the potential for innovation and exploration of new frontiers. For example, due to our comprehensive understanding of the complete life history of the aforementioned coral reef fish species in the research process (Figure 6), we can apply this crucial knowledge to collaborate with experts in various fields such as marine chemistry, fish physiology, and evolutionary genetics to enhance collaborations among scientists to tackle newly emerging issues such as global warming and ocean acidification (Ko et al., 2013; Tang et al., 2014a, 2014b; Tsai et al., 2018).

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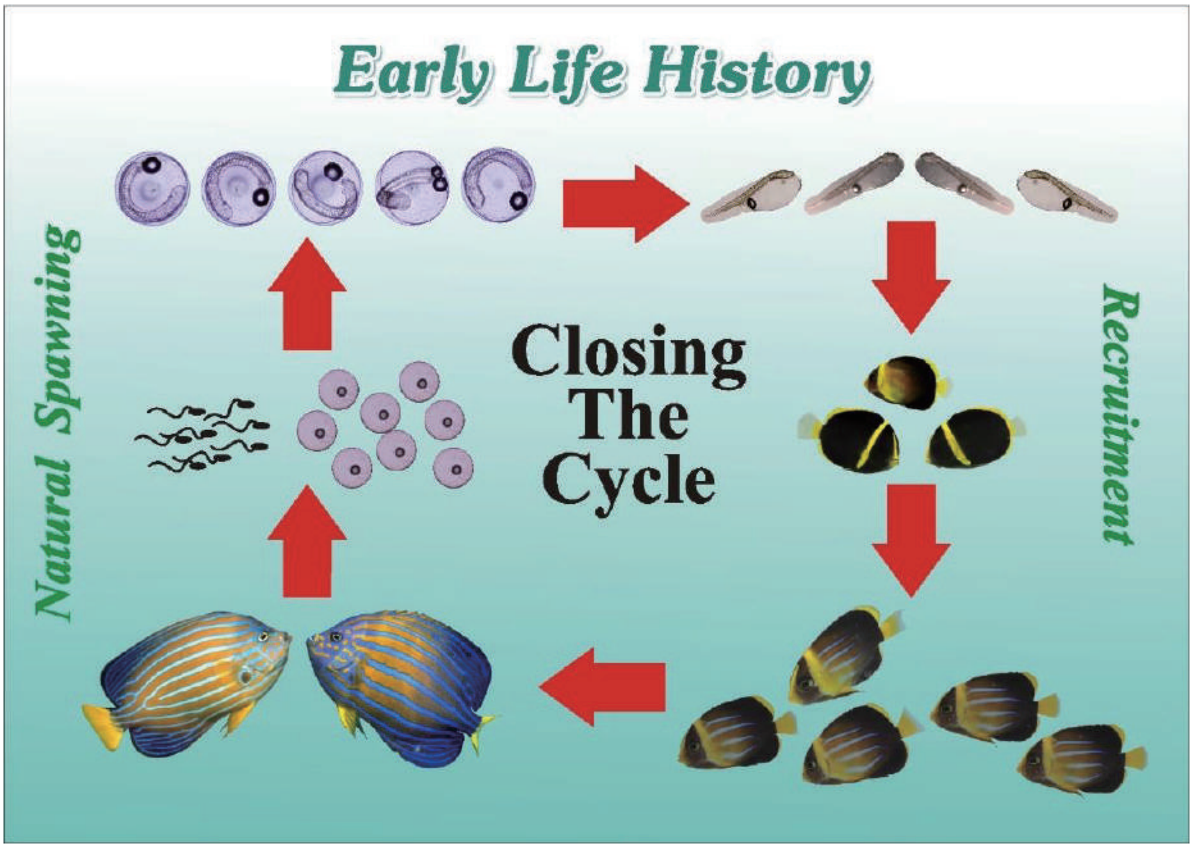

Figure 6. Schematic diagram of the life cycle of the bluestriped angelfish (*Chaetodontoplus septentrionalis*) in the coral reef.

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