Disease Occurrence and Water-Quality Associations in Quarantined Sebae Clownfish (Amphiprion sebae)

Alrich Michael.M[1], Angelin Jebisha.D[2], Poolpandi.K[3], Serine Michael.M[4], J. Renuka[5], Raghul.K[6*]

[1]Research Department of Zoology, Scott Christian College- Affiliated to Manonmaniam Sundaranar University, Nagercoil-629003
[2]Department of Marine Science and Technology, Centre for Marine Science and Technology (CMST), Manonmaniam Sundaranar University
[3]Research Department of Zoology, Pope's College-Affiliated to Manonmaniam Sundaranar University Thoothukudi
[4]Research Department of Zoology, Vivekananda College- Affiliated to Manonmaniam Sundarnar University, Agastheeswaram
[5]Department of Microbiology, The American College, Madurai
[6*]Department of Marine Biotechnology, AMET Deemed to be University, Chennai-603112 &
Email: raghulrichards@gmail.com

Abstract:
The present study investigated the occurrence of major diseases in the Sebae clownfish, Amphiprion sebae, collected from the southern region of Palk Bay and maintained under quarantine conditions, with emphasis on the relationship between disease occurrence and selected physicochemical parameters of the culture water. Fish were maintained in recirculatory aquaculture systems and monitored for three months from January to March 2022. Water temperature, salinity and pH were assessed throughout the observation period, while clinical signs associated with bacterial, fungal and protozoan diseases were recorded. Temperature ranged from 26.83 to 31.60°C, salinity from 36.00 to 38.50 ppt and pH from 7.92 to 8.33. Marine velvet/white-spot-like symptoms, body haemorrhage and mouth fungal lesions were observed during the study. Correlation analysis indicated that bacterial disease showed a negative relationship with temperature ($r = -0.676$) and pH ($r = -0.652$), but a positive relationship with salinity ($r = 0.602$). Protozoan disease showed moderate positive associations with salinity ($r = 0.461$) and pH ($r = 0.322$), whereas fungal disease showed relatively weak correlations with the measured environmental parameters. The findings demonstrate that variations in water-quality conditions are associated with disease occurrence in captive A. sebae. Continuous monitoring of physicochemical parameters, quarantine, sanitation and early disease detection are therefore essential for maintaining the health and sustainable hatchery production of this valuable marine ornamental fish.

Key words: Amphiprion sebae; Sebae clownfish; Palk Bay; ornamental fish; disease occurrence; quarantine; water quality; temperature; salinity; pH.

Introduction

Marine ornamental fishes are highly valued in the aquarium trade because of their attractive coloration, distinctive behaviour and relatively small size. Among marine ornamental fishes, clownfishes belonging to the genera Amphiprion and Premnas are particularly important because of their popularity, adaptability to captive conditions and suitability for hatchery production. The development of marine ornamental fish aquaculture has been considered an important alternative to dependence on wild collection and may contribute to reducing pressure on coral reef fish populations (Pouil et al., 2020).

The Sebae clownfish, Amphiprion sebae (Bleeker, 1853), is an economically important marine ornamental fish distributed in the Indian Ocean and associated with sea anemones. Its attractive body coloration, relatively small size and suitability for captive breeding make it a desirable species for ornamental fish culture. Captive breeding studies have demonstrated that A. sebae can successfully spawn and complete embryonic development under controlled conditions, supporting its potential for hatchery-based production. However, successful ornamental fish culture depends not only on reproduction and larval rearing but also on maintaining good health and stable environmental conditions.

Disease is one of the major constraints affecting the survival, growth and commercial production of marine ornamental fishes. Fish maintained under captive conditions are exposed to several stressors, including fluctuations in water quality, handling, stocking density, nutritional imbalance and inadequate quarantine. Such factors can increase susceptibility to bacterial, viral, fungal and protozoan infections. Consequently, monitoring environmental conditions and early recognition of disease symptoms are essential components of ornamental fish health management (Fioravanti & Florio, 2017).

Viral diseases are also important; betanodavirus-associated viral nervous necrosis has been reported to cause mass mortality in hatchery-reared larvae of A. sebae, demonstrating the susceptibility of this species to serious infectious diseases (Binesh et al., 2013). Similarly, lymphocystis disease has been documented in other cultured clownfish species, indicating that viral infections can become important health problems in intensive ornamental fish culture.

Environmental factors such as temperature, salinity and pH have a major influence on fish physiology, immune function and pathogen development. Even when these parameters remain within the general tolerance range of marine fishes, variations may influence the severity and occurrence of infectious diseases. Therefore, simultaneous assessment of environmental parameters and disease symptoms is useful for understanding disease patterns in captive ornamental fishes. The present study was therefore undertaken to assess the occurrence of major diseases in Amphiprion sebae collected from the southern region of Palk Bay and maintained under quarantine conditions, with particular emphasis on the relationship between disease occurrence and selected physicochemical parameters of the culture water. Such information may contribute to improved health monitoring, disease prevention and sustainable hatchery production of A. sebae.

Materials and Methods:

1.1. Sampling stations:

Six sampling stations were fixed in southern part of Palk Bay for the collection of Amphiprion sebae clown fishes. All Sampling points were set by GPS randomly to cover southern region of Palk Bay. Sampling stations and its longitude and latitude were shown in the table 1.

Table 1. Sampling stations and its longitude and latitude

Sampling stations	Latitude	Longitude
1	9○26‟06.06” N	78○55‟17.45” E
2	9○25‟14.21” N	78○55‟52.70” E
3	9○25‟26.53” N	78○ 56‟24.54” E
4	9○ 26‟19.21” N	78○55‟49.44” E
5	9○ 27‟22.57” N	78◦55‟14.54” E
6	9○27‟06.09” N	78○54‟44.61” E

1.2. **Collection of Sebae clown fishes**

Amphiprion sebae is an anemone fish found in the Palk Bay, usually living in association with sea anemones. The body of A. sebae is blackish or dark brown with a yellow snout and belly. It has two broad white bars, with the mid-body bar angled backwards. The tail is yellow or orange. They have 10–11 dorsal spines, 2 anal spines, 14–17 dorsal soft rays and 13–14 anal soft rays. They can grow to 14 centimeters (5.5 inch). They are sequential hermaphrodites, changing from male to female, with a strict dominance hierarchy and only the largest fish being female.

The male starts courting the female a week before they spawn. The male initiates maintenance of the site chosen for habitation. The female enters the nest to lay her eggs. About 300 to 600 eggs are laid. The males usually guard the eggs, which hatch after six to eight days. The yolk sac of the larvae was small. Between the third and fourth day they fed, and their body shape changed. On days fifteen to eighteen in their life cycle they enter metamorphosis. When A. sebae eggs are recently fertilized, they are transparent (yellow/clear in colour) with oil droplets. Amphiprion sebae eggs mature, they become bright yellow to orange color, the size of the eggs can range from length between 1.7- 2.6 mm and width: 0.8-1.3 mm.

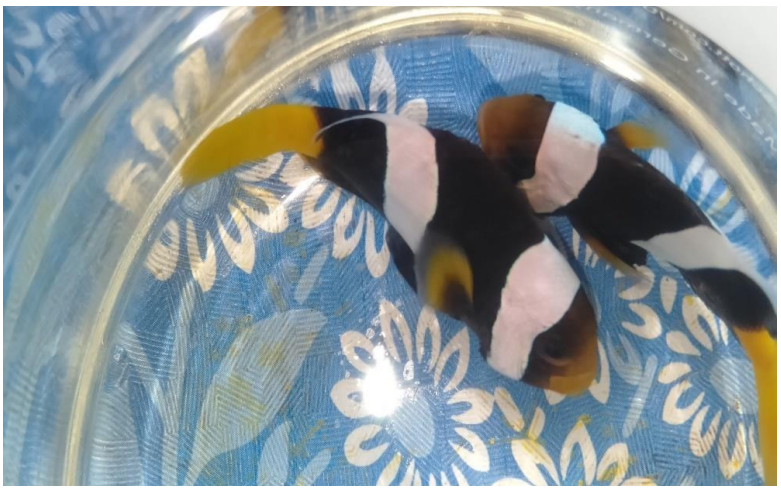


Fig.2 Amphiprion sebae clown fishes at hatchery

1.3. Disease assessment and its setup

In the present study, the clown fishes were grown in six glass tanks which were kept in rack for disease assessment study. All the glass tanks are uniform in size with 20 inch in length, 22inch in width and 12 inch in height. They are separated in the center by the divider glass (partitions) and two pairs are possible to maintain. All the glass tanks were

fitted with 2watt illumination. RAS (Recirculatory aquaculture system) is attached to all cultural tanks for normal maintenance of fishes.
Twice a day, the feed can be given to the fishes. Tiles are provided to hide and take rest of sick fishes.

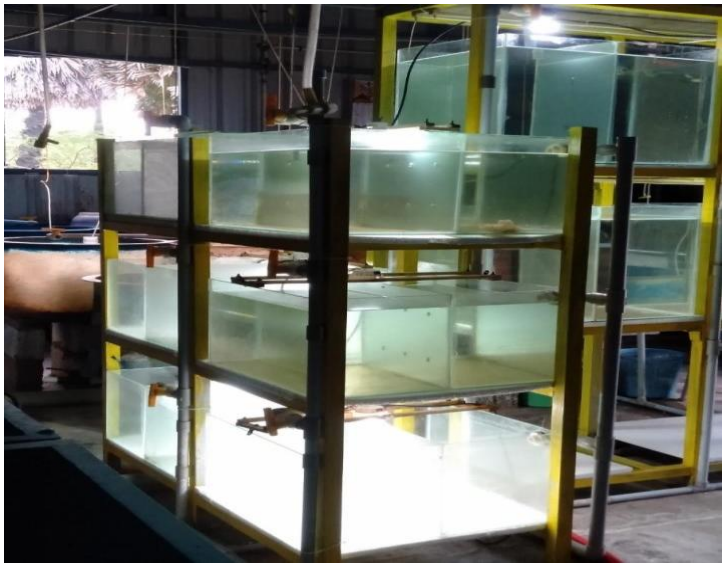


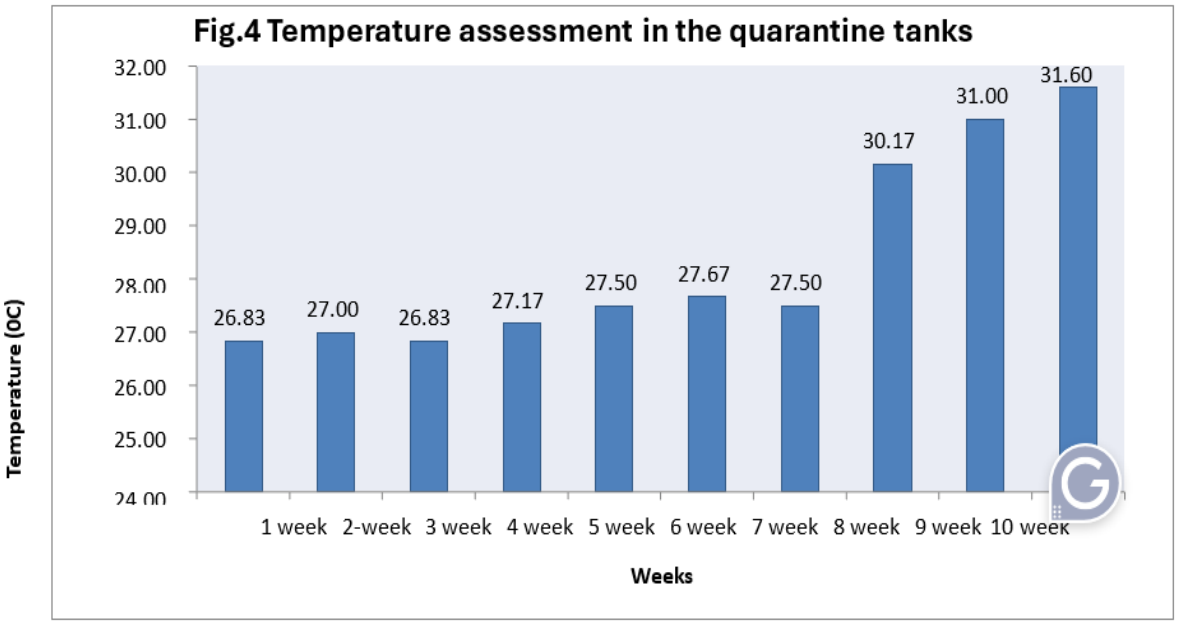
Fig. 3. Disease quarantine setup

1.4. **Physicochemical parameters**
Water samples were collected in the hatchery and analysed. The water temperature was measured using a standard centigrade thermometer. Water salinity was estimated using a salinity refractometer (Aatago - Japan). The water pH was measured using Weathertronics pH meter (Model 705).

Results and Discussion:
The present study has been executed for the period of three months (from January – March 2022). Physicochemical parameters were assessed in the quarantine tanks to find the influence on disease intensity.

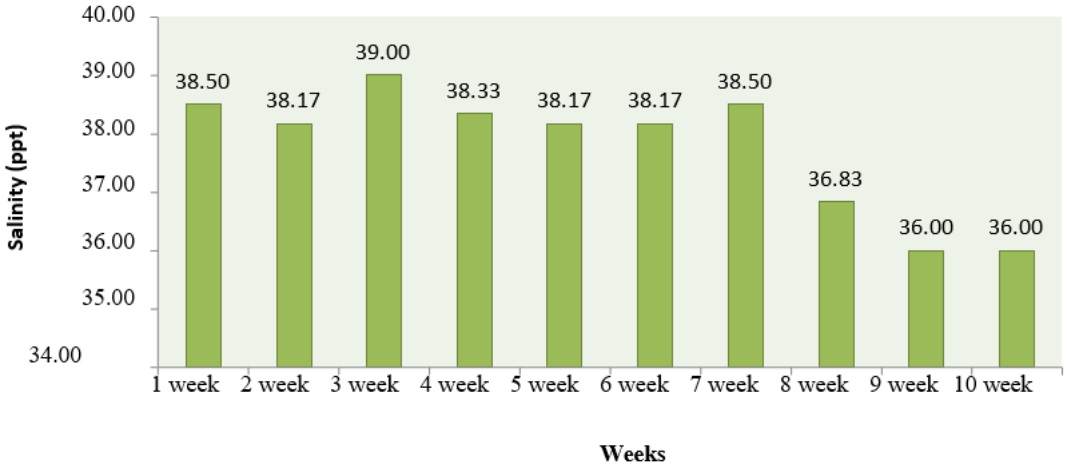
2.1. Physico-chemical Parameters

2.1.1. Temperature
Temperature is an important factor which has profound influence on the disease outbreak of clown fishes



2.1.2. Salinity
Salinity is also considered to be an important factor. Salinity varied from 36 to 38 ppt (Fig.5

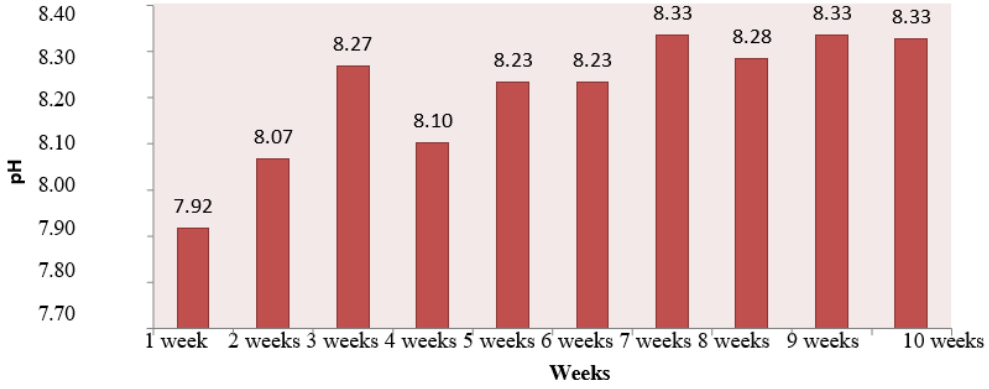
Fig.5 Assessment of Salinity in the quarantine tanks



2.1.3. pH

pH was fluctuated from 7.92 to 8.33 (Fig.6). Entire assessment period was noticed to be alkaline state.

Fig.6 pH assessment in the quarantine tanks



2.2. Disease assessment in clown fishes

Fig. 7. Marine velvet white spots

Fig. 9. Red pest (Body hemorrhage)

Fig. 10. Mouth fungus

Table 2. Various clown fish diseases and its numbers observed in the study period.

Category	Disease	week 1	week 2	week 3	week 4	week 5	week 6	week 7	week 8	Week 9	week 10
Bacterial Disease	Tail rot	2	1	2	3	2	1	1	0	0	0
	Fin rot	2	2	1	2	3	2	0	0	0	2
	Body hemorrhage	6	3	2	6	6	7	2	4	2	0
Fungal Disease	Fungal disease	3	4	6	4	6	3	4	3	4	5
Protozoan Disease	Clown fish (Brooklynella)	0	0	6	5	6	7	5	4	2	0
	White spot disease	0	0	6	8	8	9	8	6	5	0
	Velvet disease	0	0	5	4	4	6	3	4	0	0

Table.3. Correlation coefficient for environmental factors and diseases.

	Salinity	pH	Temp	Bacterial disease	Fungal Disease	Protozoan disease
Salinity	1.000					
pH	-0.482	1.000				
Temp	-0.978	0.601	1.000			
Bacterial disease	0.602	-0.652	-0.676	1.000		
Fungal Disease	0.118	0.336	-0.056	-0.073	1.000	
Protozoan disease	0.461	0.322	-0.360	0.372	0.170	1.000

Preventive measure:

Table 4. Diseases, possible cause and medication

Symptom	Possible Cause	Medication
Small white spots on fins / skin, clamped fins	Ich	For fresh water species (Sea water Bath) and for Sea water species (Fresh water Bath), Formalin and Malachite green is preferably used for this type of cause.
Peppery coating, yellowish, clamped fins	Velvet	Copper sulphate bath for 1-2 minutes
Gray or white fluffy	Fungus	Methyl Blue, Antibiotics for

patches		secondary infections.
Gray or white fluffy patches around mouth	Mouth Fungus	Erythromycin, Kanacyn, Fish Pen (penicillin), Maracyn antibiotics for secondary infections. (Use Maracyn simultaneously with Maracyn II)
Pale appearance	Neon Tetra disease	No Known Cure
Unusual racing around tank. Black to red nodules beneath skin.	Flukes	Paragon, Clout, Proxipro, Fluke-Tabs
Milky cloudiness on skin	Costia, Chilodone lla	Copper sulphate, Acriflavine
Destruction of fins or tail	Tail or fin rot	Maracyn, Methylblue, Antibiotics, Tetracycline, Chloromycetin
Red streaks on body	Red pest, Fin rot	Tetracycline, Penicillin. Acriflavine, Chloromycetin, Fish Pen (penicillin)
Yellow to black nodules on skin	Ichthyosporidium	
Ulcerated patches on skin	Red pest, Ichthyosporidium	
Emaciation, hollow belly, possibly sores	Tuberculosis	No Known Cure
Protrusion of scales with bloated body	Dropsy	Feed Anti-Bacteria medicated food
Protrusion of scales, body normal	Scale protrusion	
Eyes protrude	Pop eye	Penicillin or amoxicillin
Cloudiness of eyes	Eye problems, Ich, velvet	Maracyn, Maracyn Plus, Antibiotics for bacterial infection, Increase vitamin A.
Hole in head, ulceration of lateral line, loss of appetite	Head and Lateral Line Disease (Hole-in-theHead)	Copper sulphate
White slimy feces, loss of appetite, swim backwards	Hexamita	Metronidazole
Crustaceans on skin	Argulus, Ergasilus	Trifon, Anti-Fluke treatment
Flukes on skin or gills	Flukes	
Worms hanging from anus	Nematoda	Trifon, Worm Parasitic treatment
Heart shaped worms	Leeches	
Nodular white swellings on fins or body	Lymphocystis,Gl ugea, Henneguya	Since lymphocystis is not harmful and will drop off after some time, no cure is necessary.
Glancing off rocks or plants	Velvet, Ich, flukes, anchorworm, Chilodonella, Costia	Ich Medication (Ich) freshwater bath

Severe loss of balance	Swim bladder disease	Check aquarium parameters; look for signs of other disease.
Gasping at surface	Oxygen/ O2deficiency, CO2 excess, tank too hot, toxins, shock	Oxygenex, Oxygen stones - (short-term). Provide better water circulation, lower temperature
Jumping out of water	pH wrong, toxins	Check for phextremes, do water changes with dechlorinated water.
Appetite dwindles,	Constipation or Internal Parasites	Medicinal parrafin oil, change in diet, Anti-Parasitic Medicated Food
Fins frayed or split, scales missing	Injuries	Wound Treat, Bio Bandage, Stress relievers. Look for and remove bully fish.

Discussion:

The present study demonstrated that disease occurrence in Amphiprion sebae maintained under quarantine conditions was associated with variations in water-quality parameters. During the three-month observation period, marine velvet/white-spot-like symptoms, body haemorrhage and mouth fungal lesions were observed. The temperature ranged from 26.83 to 31.60°C, salinity from 36 to 38.50 ppt and pH from 7.92 to 8.33.

Temperature showed negative correlations with bacterial disease ($r = -0.676$) and protozoan disease ($r = -0.360$), whereas the correlation with fungal disease was weak ($r = -0.056$). Salinity showed positive correlations with bacterial ($r = 0.602$) and protozoan diseases ($r = 0.461$), while pH showed a negative correlation with bacterial disease ($r = -0.652$) and positive correlations with fungal ($r = 0.336$) and protozoan diseases ($r = 0.322$). These findings suggest that environmental conditions may influence disease occurrence through their effects on fish physiology and host–pathogen interactions. However, the correlations should be interpreted as associations rather than direct causal relationships.

The comparatively stronger relationship between bacterial disease and the physicochemical parameters is important for A. sebae culture. Ramesh et al. (2017) reported Vibrio parahaemolyticus as a causative agent of tail-rot disease in A. sebae, highlighting the susceptibility of this species to bacterial infections. Similarly, Binesh et al. (2013) reported viral nervous necrosis-associated mortality in hatchery-reared A. sebae, demonstrating the importance of disease surveillance in clownfish culture. Pirarat et al. (2011) also documented lymphocystis disease in cultured Amphiprion ocellaris, indicating that clownfishes may be affected by several infectious diseases.

The present findings emphasize the importance of quarantine, stable water quality, sanitation and continuous observation of fish behaviour and clinical signs. Fioravanti and Florio (2017) highlighted water quality, nutrition, stocking density and biosecurity as important factors in managing diseases of marine ornamental fishes. Therefore, regular monitoring of temperature, salinity and pH, together with early detection and laboratory confirmation of pathogens, can help reduce disease-related losses in captive A. sebae.

Overall, the study indicates that physicochemical conditions were associated with bacterial, fungal and protozoan disease occurrence in A. sebae. Further studies incorporating pathogen isolation, microscopy, histopathology and molecular identification are recommended to confirm the causative agents and establish stronger relationships between environmental factors and disease development.

Conclusion:

The present study provides useful information on the occurrence of diseases in the Sebae clownfish, Amphiprion sebae, maintained under quarantine conditions and highlights the importance of water-quality management in ornamental fish culture. During the three-month observation period, several clinical manifestations, including marine velvet/white-spot-like symptoms, body haemorrhage and mouth fungal lesions, were observed. The physicochemical parameters of the quarantine water showed variations, with temperature ranging from 26.83 to 31.60°C, salinity from 36.00 to 38.50 ppt and pH from 7.92 to 8.33. These variations indicate that maintaining suitable and stable environmental conditions is essential for minimizing stress and disease occurrence in captive clownfish.

Correlation analysis demonstrated that the occurrence of bacterial, fungal and protozoan diseases was associated differently with temperature, salinity and pH. Bacterial disease showed comparatively stronger associations with the measured environmental factors, particularly negative correlations with temperature and pH and a positive correlation with salinity. Protozoan disease also showed a positive association with salinity, whereas fungal disease exhibited relatively weak relationships with the examined parameters. These findings suggest that changes in environmental conditions may influence disease occurrence by affecting fish physiology and host–pathogen interactions. However, the observed correlations represent associations and should not be considered evidence of direct causation.

Overall, the study emphasizes the importance of effective quarantine practices, regular monitoring of temperature, salinity and pH, proper tank sanitation, adequate husbandry and early recognition of clinical symptoms for maintaining the health of A. sebae. Such preventive measures can reduce disease-related losses and improve the survival and

productivity of clownfish in hatchery and ornamental fish culture systems. Further investigations involving pathogen isolation, microscopic examination, histopathology and molecular identification are necessary to confirm the specific causative agents and establish stronger relationships between environmental conditions and disease development. The findings of the present study can therefore serve as a baseline for developing improved disease-management and sustainable hatchery practices for A. sebae.

References

1. Binesh, C. P., Renuka, K., Malaichami, N., & Greeshma, C. (2013). First report of viral nervous necrosis-induced mass mortality in hatchery-reared larvae of clownfish, Amphiprion sebae Bleeker. Journal of Fish Diseases, 36(12), 1017–1020.
2. Fioravanti, M. L., & Florio, D. (2017). Common diseases in marine ornamental fishes. In R. Calado, I. Olivotto, M. P. Oliver, & G. J. Holt (Eds.), Marine Ornamental Species Aquaculture. Wiley-Blackwell.
3. Lam, C., Khairunissa, I., Damayanti, L., Kurobe, T., Teh, S. J., Pfahl, H., Rapi, S., Janetski, N., & Baxa, D. V. (2020). Detection of a new strain of lymphocystis disease virus (LCDV) in captive-bred clownfish Amphiprion percula in South Sulawesi, Indonesia. Aquaculture International, 28, 2121–2137.
4. Pirarat, N., Pratakpiriya, W., Jongnimitpaiboon, K., Sajjawiriyakul, K., Rodkhum, C., & Chansue, N. (2011). Lymphocystis disease in cultured false clown anemonefish (Amphiprion ocellaris). Aquaculture, 315, 414–416.
5. Pouil, S., Tlusty, M. F., Rhyne, A. L., et al. (2020). Aquaculture of marine ornamental fish: Overview of the production trends and the role of academia in research progress. Reviews in Aquaculture, 12(2), 1217–1230.
6. Ramesh, R., et al. (2017). Vibrio parahaemolyticus, a causative bacterium for tail rot disease in ornamental fish, Amphiprion sebae. Aquaculture Reports, 8, 39–44.
7. Sivakumar, S., et al. (2017). Vibrio parahaemolyticus, a causative bacterium for tail rot disease in ornamental fish, Amphiprion sebae. Aquaculture Reports, 8, 39–44.