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Shrimp Head Protein Hydrolysate as a Potential Feed Attractant for the Asian Redtail Catfish (*Hemibagrus nemurus*) Larvae

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ABSTRACT

Protein hydrolysates are known for their feed attractant properties in various fish species; however, their efficacy in the Asian redtail catfish (*Hemibagrus nemurus*) remains underexplored. This study evaluated the effects of shrimp head protein hydrolysate (SHPH) on feed attractiveness and palatability in the Asian redtail catfish larvae. A completely randomized design was employed, incorporating four dietary treatments: a control (0% SHPH) and three experimental diets with 2.5, 5, and 7.5% SHPH supplementation, respectively. Larval responses were analyzed, including the attractiveness and palatability of the larval feed, the level of feed consumption, feed efficiency, feed rejection, and the time to first feed capture. Results demonstrated significant effects on feed attractiveness and palatability when SHPH was included in the diets. The treatment with 5% SHPH supplementation produced the most favorable outcomes across all measured parameters. These findings suggest that SHPH can significantly enhance the feeding responses of the Asian redtail catfish larvae, with a 5% inclusion level in the diets being the most optimal dose. Incorporating SHPH into the larval diets of the Asian redtail catfish has the potential to improve feed utilization and minimize waste, contributing to more sustainable aquaculture practices for this commercially important species.

INTRODUCTION

The Asian redtail catfish (*Hemibagrus nemurus*) is a highly valued freshwater fish species cultivated extensively throughout Indonesia and Southeast Asia **(Agusnimar *et al.*, 2023; Saputra *et al.*, 2024)**. It is known for its desirable meat quality, characterized by a soft, firm texture, delicate flavor, and rich nutritional profile, particularly its high protein content at a range of 45-53% **(Hasan *et al.*, 2019; Oktavian *et al.*, 2023)**. The economic significance of the Asian redtail catfish is evident in its market value, with prices in traditional Indonesian markets ranging from IDR 70,000−110,000/kg for fresh fish and IDR 325,000−350,000/kg for smoked products **(Heltonika & Karsih, 2017)**.

The high demand for the Asian redtail catfish is accompanied by an increase in fishing activities in the wild. This situation raises concerns that the Asian redtail catfish population will decline and become extinct. One effort to maintain the Asian redtail catfish population is through cultivation. Apart from reducing the intensity of fishing in natural waters, cultivation can also increase the production of good quality fish.

The Asian redtail catfish hatchery requires feed that is suitable for larvae, easy to digest, and highly nutritious. Silk worms (*Tubifex* sp.) are often used due to their excellent protein, fat, and amino acid content, and they are preferred by fish larvae. However, *Tubifex* sp. can be expensive and difficult to obtain during certain seasons, and they also carry the risk of transmitting diseases and parasites.

To reduce dependence on *Tubifex* sp., alternative feeds, such as artificial feed in the form of pasta, have potential. According to **Siagian *et al.* (2022)**, this type of feed fits the larvae's mouth openings, is easy to digest, and has a soft texture.

Pasta-based feeds are promising alternative to live feeds such as *Tubifex* sp. in aquaculture due to their consistent availability, ease of production, customizable nutritional content, and cost-effectiveness. Previous studies have demonstrated the efficacy of pasta-based feeds in various fish species, including the Asian redtail catfish, *Hemibagrus nemurus* **(Aryani *et al*., 2013)**, the African catfish, *Clarias gariepinus* **(Nurhayati *et al*., 2014; Tjodi *et al*., 2016; Mullah *et al*., 2019)**, the bonylip barb, *Osteochilus hasselti* **(Yusuf *et al*., 2014)**, the iridescent shark catfish, *Pangasianodon hypophthalmus* **(Jusadi *et al*., 2015)**, and the climbing perch, *Anabas testudineus* **(Rahmi *et al*., 2016)**. While these studies demonstrated a positive growth performance, enhancing feed attractiveness and palatability remains crucial for optimizing larval feed intake and maximizing growth potential.

Feed attractiveness, which is governed by visual and chemosensory cues, has an important role in the initial detection and location of food by fish **(Lokkeborg *et al*., 2014**; **Olsen & Lundh, 2016)**. Palatability, on the other hand, dictates the ultimate acceptance or rejection of food based on gustatory perception within the oral cavity **(Lokkeborg *et al*., 2014; Olsen & Lundh, 2016)**.

Shrimp head waste, often underutilized or discarded, is a potential source of valuable feed ingredients, including oil and protein hydrolysate, which can be extracted at low cost **(Decarli *et al*., 2016; Silva *et al*., 2017; Diana *et al*., 2023)**. Hydrolysis, a chemical process, breaks down complex proteins into smaller peptides and amino acids, enhancing their digestibility and bioavailability for fish **(Wisuthiphaet *et al*., 2015)**. These low molecular weight nitrogenous compounds, generated through hydrolysis, can act as potent feed attractants by stimulating the olfactory system of fish **(Broggi *et al*., 2017)**.

The efficacy of protein hydrolysates in improving feed attractiveness and palatability has been documented in several studies. For example, sardine protein hydrolysate enhanced food searching behavior in the silver catfish, *Rhamdia quelen* larvae **(Broggi *et al*., 2017)**. Similarly, poultry protein hydrolysate supplementation improved feed intake in the Nile tilapia, *Oreochromis niloticus* larvae **(Alves *et al*., 2019)**. Pig liver protein hydrolysate effectively increased feed consumption in the betta fish, *Betta splendens* larvae **(Oliveira *et al.*, 2022)**, while feather protein hydrolysate demonstrated positive effects on feed attractiveness and palatability in the pacu, *Colossoma macropomum* × *Piaractus mesopotamicus* **(Santos *et al*., 2022)**. Therefore, this study aimed to evaluate the potential of shrimp head protein hydrolysate as a feed attractant and palatability enhancer for Asian redtail catfish larvae.

MATERIALS AND METHODS

This study was conducted at the Microalgae and Fish Nutrition Laboratory, Faculty of Agriculture, Universitas Islam Riau, Pekanbaru, Riau, Indonesia, from May to August 2024.

Production of shrimp head protein hydrolysate

The tiger shrimp head protein hydrolysate was prepared following the method described by **Suparmi *et al*. (2019)**, with slight modifications. Briefly, 500g of finely ground shrimp head flour was mixed with 500mL of distilled water (1:1 w/v ratio) and homogenized for 2 minutes. The pH of the mixture was adjusted to 7.0 using 1 M NaOH or 1 M HCl to achieve the optimal pH for papain enzyme activity. Papain enzyme was added at a concentration of 15%, and the mixture was incubated at 60°C for 24 hours. Enzyme deactivation was achieved by boiling the mixture in a water bath at 85°C for 15 minutes. The supernatant (liquid phase) containing the shrimp head protein hydrolysate was separated from the precipitate (residue) by centrifugation at 3000rpm for 15 minutes, followed by filtration.

Experimental diets

Four isonitrogenous (45%) and isoenergetic (5,000 kcal/kg) experimental diets were formulated following the nutritional recommendations proposed by **Eguia *et al.* (2000)**. The diets evaluated were: SHPH 0 (control, diet with 0% shrimp head protein hydrolysate), SHPH 2.5 (diet with 2.5% shrimp head protein hydrolysate), SHPH 5 (diet with 5% shrimp head protein hydrolysate), and SHPH 7.5 (diet with 7.5% shrimp head protein hydrolysate). The composition of the diets is shown in Table (1).

Table 1. Composition of experimental diets used in evaluating the attractiveness and palatability of SHPH for the Asian redtail catfish (*H. nemurus*)

Ingredient	Diet (% dry ingredients)			
	SHPH 0	SHPH 2.5	SHPH 5	SHPH 7.5
Fish meal	54	53.5	53	52.5
Soybean meal	28	26	24	22
Wheat flour	12	12	12	12
Fish oil	2	2	2	2
Vitamin mix[a]	2	2	2	2
Mineral mix[b]	2	2	2	2
SHPH[c]	0	2.5	5	7.5
Total	100	100	100	100

[a] Vitamin mix (mg/100 g diet): thiamine 5.0; riboflavin 5.0; Ca-pantothenate 10.0; niacin 2.0; pyridoxine 4.0; biotin 0.6; folic acid 1.5; cyanocobalamin 0.01; inositol 200; ρ-aminobenzoic acid 5.0; menadion 4.0; vitamin A palmitate 15.0; chole-calciferol 1.9; α-tocopherol 20.0; choline chloride 900.0.
[b] Mineral mix (mg/100 g diet): KH_2PO_4 412; $CaCO_3$ 282; Ca (H_2PO_4) 618; $FeCl_3.4H_2O$ 166; $ZnSO_4$ 9,99; $MnSO_4$ 6,3; $CuSO_4$ 2; $CuSO_4.7H_2O$) 0,05; KJ 0,15; Dekstrin 450; Selulosa 553,51.
[c] SHPH (Shrimp Head Protein Hydrolysate).

Feed preparation and proximate analysis

Pasta-form artificial feeds were prepared by thoroughly mixing the primary ingredients (fish meal, soybean meal, and wheat flour). Fish oil, vitamin and mineral premix, and shrimp head protein hydrolysate were then added according to the designated treatments. Water was gradually incorporated and mixed until a homogenous paste consistency was achieved, as described by **Syamsunarno and Sunarno (2022)**. Proximate analysis (Table 2) and amino acid profiling (Table 3) of the prepared pasta feeds were conducted following the standard procedures outlined by **AOAC (2005)** and shown in Tables (2, 3). Prepared feeds were stored frozen to maintain freshness and thawed 15 minutes prior to feeding, as described by **Siagian *et al*. (2022)**.

Table 2. Chemical composition of the experimental diets used in evaluating the attractiveness and palatability of SHPH for the Asian redtail catfish (*H. nemurus*)

Chemical composition	Diet (% dry matter)			
	SHPH 0	SHPH 2.5	SHPH 5	SHPH 7.5
Protein	45.49	45.78	45.73	45.79
Fat	16.46	12.78	11.47	10.68
Ash	12.81	11.94	12.78	11.98
NFE[a]	23.35	28.01	28.08	29.66
Energy (GE kcal/g)[b]	509.06	494.39	481.89	481.13

[a] NFE is calculated based on the difference (100 – protein – fat – ash) **(Hasan *et al*., 2019)**.
[b] Gross energy was calculated according to **Chandan *et al.* (2021)** as 5.7, 9.5 and 4.0 kcal/g for protein, fat and NFE.

Table 3. Amino acid profile of experimental diets used in evaluating the attractiveness and palatability of SHPH for the Asian redtail catfish (*H. nemurus*)

Type of amino acid	Diet (%)			
	SHPH 0	SHPH 2.5	SHPH 5	SHPH 7.5
Essential amino acids				
Arginine	1.36	1.44	1.42	1.74
Histidine	0.33	0.71	0.83	0.61
Isoleucine	1.18	1.18	1.22	1.18
Leucine	2.37	2.44	2.47	2.49
Lysine	2.16	1.89	2.34	2.41
Methionine	0.66	0.67	0.66	0.68
Phenylalanine	1.03	1.11	1.26	1.22
Threonine	0.75	0.79	0.77	0.75
Valine	1.53	1.27	1.33	1.31
∑EAA	11.37	11.50	12.30	12.39
Non-essential amino acids				
Alanine	1.34	1.36	1.28	1.31
Aspartic acid	2.35	2.88	2.47	2.67
Cystine	0.89	0.74	0.87	0.91
Glutamic acid	3.27	3.55	3.26	3.54
Glycine	1.84	1.56	1.88	1.85
Proline	1.06	1.46	1.84	1.77
Serine	1.42	1.24	1.60	1.42
Tyrosine	1.02	0.95	0.99	0.88
∑NEAA	13.19	13.74	14.19	14.35
EAA/NEAA ratio	0.86	0.84	0.87	0.86

Experimental units and fish acclimation

Twelve aquaria (60×33×35cm), each filled with 30L of freshwater and equipped with aeration, were used. 1800 healthy Asian redtail catfish larvae (average weight 0.02g) were obtained from the Technical Service Unit of Fish Seed Center, Universitas Islam Riau. Upon arrival at the laboratory, the fish were acclimated in a holding tank (2×1×0.5m) for 30 minutes before being randomly distributed into each aquarium at a density of 150 fish per tank (5 fish/L), as described by **Nasution *et al*. (2021)**.

Experimental diets and feeding regime

During a 10-day acclimation period, the fish were fed a combination of *Tubifex* sp. and pasta feed containing SHPH according to the assigned treatments. Following the adaptation period, a 10-day observation period commenced. During that time, fish were fed the experimental diets five times daily (7:00 am, 11:00 am, 3:00 pm, 7:00 pm, and 11:00 pm) to apparent satiation **(Nasution *et al*., 2021)**.

Water quality management

Water quality was maintained through daily filtering. Parameters including temperature, pH, dissolved oxygen, and ammonia were monitored at the beginning, middle, and end of the experiment. Temperature and pH were measured using a pH tester (H198108, Romania), DO using a DO meter (Lutron PDO-519, Taiwan), while ammonia was evaluated using an ammonia MR (HI715, Romania). The ranges of these parameters: temperature of 27.7-29.8°C, pH of 5.32-6.57, DO of 5.5-6.4mg/ L, and ammonia of 0.0607-0.1821mg/ L, and were within the optimal levels for growth and survival of the Asian redtail catfish larvae.

Experimental design and data analysis

A completely randomized design with three replicates per treatment was employed. Fish feeding behavior was recorded for three minutes after feed introduction using a camera. Observation of the fish responses lasted for ten days **(Alves *et al*., 2020)**. The palatability index was calculated based on the method described by **Kasumyan and Sidorov (2012)** and **Alves *et al*. (2020)** using this equation:

$$\text{Palatability Index} = \left(\frac{\text{consumption of test feed (g)} - \text{consumption of control feed (g)}}{\text{consumption of test feed (g)} + \text{consumption of control feed (g)}}\right) \times 100$$

The following feeding behaviors were analyzed: a) refusal of food after being caught (RFBC); b) not responding to food (NRF); c) time of first capture of food; level of feed consumption (LFC); and feed efficiency (FE) following these formulas:

$$\text{RFBC} = \left(\frac{\text{number of fish that refuse food after being caught (heads)}}{\text{number of sample fish (heads)}}\right) \times 100$$

$$\text{NRF} = \left(\frac{\text{number of fish that do not respond to food (heads)}}{\text{number of sample fish (heads)}}\right) \times 100$$

LFC = weight of feed provided at the beginning (g) – weight of feed at the end (g)

$$\text{FE} = \frac{(final\ weight\ (g) + weight\ of\ dead\ fish\ (g)) - initial\ weight\ (g)}{\text{total feed during rearing}} \times 100$$

Data were analyzed using one-way ANOVA, and significant differences between treatments were determined using Duncan's multiple range test at 95% confidence level using SPSS software.

RESULTS

Diets supplemented with SHPH demonstrated greater palatability and feed consumption compared to the control diet (without SHPH) (Table 4). The SHPH 5 diet significantly enhanced both palatability index ($P<0.05$) and feed consumption ($P<0.05$) compared to all other treatments. Fish fed the SHPH 5 diet also exhibited significantly higher feed efficiency ($P<0.05$) than those on other diets, with the exception of the SHPH 7.5 group. Furthermore, SHPH supplementation significantly impacted the time to first feed capture. Fish fed the SHPH 5 diet exhibited the fastest time (5.20 seconds), followed by SHPH 7.5 (6.60 seconds), SHPH 2.5 (10.20 seconds), and lastly, the control diet (13.80 seconds).

Table 4. Average value of attractiveness and palatability of SHPH for the Asian redtail catfish (*H. nemurus*)

Treatments (%)	Palatability index (%)	Feed consumption (g)	Feed efficiency (%)	Feed refusal after being caught (%)	Fish not responding to feed (%)	First feed capture time (second)
SHPH 0 (Control)	0.00 ± 0.36^{a}	40.38 ± 0.29^{a}	53.22 ± 2.56^{a}	1.67 ± 0.14^{c}	3.02 ± 0.16^{c}	13.80 ± 0.60^{d}
SHPH 2.5	14.01 ± 0.93^{b}	53.55 ± 1.01^{b}	56.14 ± 2.34^{a}	1.20 ± 0.27^{ab}	2.53 ± 0.24^{b}	10.20 ± 0.60^{c}
SHPH 5	20.64 ± 0.54^{d}	61.38 ± 0.69^{d}	67.38 ± 0.95^{b}	1.11 ± 0.16^{a}	1.93 ± 0.17^{a}	5.20 ± 0.35^{a}
SHPH 7.5	17.82 ± 0.36^{c}	57.89 ± 0.44^{c}	65.82 ± 1.52^{b}	1.54 ± 0.12^{bc}	2.46 ± 0.12^{b}	6.60 ± 0.00^{b}

Description: SHPH (shrimp head protein hydrolysate). The values shown are the mean ± SD. Different superscript letters in the same column indicate significant differences between treatments ($P<0.05$).

DISCUSSION

Supplementation of SHPH in the feed for the Asian redtail catfish larvae led to better outcomes compared to the control group without SHPH supplementation. This result can be attributed to the presence of low molecular weight peptide chains in SHPH **(Ribeiro *et al*., 2017; Resende *et al*., 2023; Nikoo *et al*., 2024; Saputra *et al*., 2024)**. These peptides are known to provide essential amino acids **(Mach & Nortvedt, 2011; Santos *et al*., 2022)**, improve protein digestibility **(Khosravi *et al*., 2015; Sandbakken *et al*., 2023)**, and enhance feed consumption **(Alves *et al*., 2019; Pham *et al*., 2021)**. These findings are consistent with other studies that have demonstrated the positive effects of protein hydrolysate on palatability in farmed fish, including the Atlantic salmon, *Salmo salar* **(Refstie *et al*., 2004)**, the barramundi, *Lates calcarifer*

(Chotikachinda *et al*., 2013), and the European seabass, *Dicentrarchus labrax* **(Leduc *et al*., 2018)**.

The feeding behavior of fish is strongly influenced by low molecular weight compounds including amino acids, peptides, and nitrogen compounds **(Alves *et al*., 2020)**. These compounds are detected by the fish's senses of smell and taste, and play critical roles in controlling feeding behavior by assessing the attractiveness and palatability of food **(Siikavuopio *et al*., 2017; Oliveira *et al*., 2022)**. In addition, various fish species exhibit different degrees of sensitivities and preferences for specific amino acids, resulting in differences in their acceptance or rejection of food **(Olsen & Lundh, 2016; Alves *et al*., 2019; Oliveira *et al*., 2022)**.

There are certain amino acids such as cysteine, betaine, glutamic acid, serine, glycine, alanine, proline, methionine, phenylalanine, arginine, tyrosine, valine, leucine, and glutamine, which act as feeding stimulants in fish. These amino acids provide chemical signals to the taste system of this fish, enhancing the perceived attractiveness of food **(Santos *et al*., 2022)**. Numerous studies have demonstrated the stimulatory effects of low molecular weight substances, including amino acids, peptides, and nitrogen compounds, on fish feeding behavior, feed consumption, digestive enzymes, and antioxidant enzyme activity **(Sudagar *et al*., 2010; Silva *et al*., 2017; Giri *et al*., 2021; Sandbakken *et al*., 2023; Santos *et al*., 2023; Fan *et al*., 2024; Nikoo *et al*., 2024)**.

Palatability is a critical factor in food selection for fish, as these influences whether the fish accepts or rejects the feed. When food enters the mouth of the fish, the taste receptors detect and recognize the taste-providing substances. Based on this sensory information, the fish decides whether to swallow or reject the food and determines the amount that will be consumed **(Lokkeborg *et al*., 2014**; **Olsen & Lundh, 2016; Olsen & Toppe, 2017)**.

The results obtained in this study (Table 4) indicate that a 5% SHPH supplementation level is the optimum dose for enhancing palatability and feed consumption, which in turn improves the growth of the Asian redtail catfish larvae compared to other treatments. This finding can be attributed to the ability of this dosage of supplementation to fulfill the free amino acid requirements of the test feed (Table 3). This optimal supplementation level enhanced the attractiveness and palatability of the feed, resulting in improved feed efficiency. These observations are consistent with the findings of **Ndobe *et al*. (2022)**, who reported that an optimal attractant dosage in fish feed can enhance the feed conversion ratio by positively influencing feed intake, growth, and palatability. Research on various fish species, such as freshwater eels, gourami, and seabass, have shown that attractants such as squid meal, garlic extract, turmeric extract, and other natural ingredients can significantly increase feed consumption. In addition, **Afifah *et al.* (2021)** reported that attractants in optimal amounts can stimulate fish appetite, make food more attractive, and increase the speed at which fish find and consume food.

Several studies show that the use of hydrolysate as a food attractant for fish yielded positive results. **Broggi *et al.* (2017)** reported that the use of sardine protein hydrolysate in the feeds for the South American catfish, *Rhamdia quelen* increased the feed intake of this species. Furthermore, **Alves *et al.* (2019)** evaluated the palatability of protein hydrolysates from industrial products for tilapia, *Oreochromis niloticus*. The findings in the study were that all diets containing poultry by-products exhibited higher palatability indices, feed consumption rates, and reduced feed rejection behavior. **Oliveira *et al.* (2022)** found that protein hydrolysates from agro-industrial by-products, such as chicken protein hydrolysate, pig liver protein hydrolysate, feather protein hydrolysate, and pig mucosa protein hydrolysate, have good attractiveness and palatability indices to replace fish meal in the feeds for the betta fish, *Betta splendens*. Additionally, **Santos *et al.* (2022)** found that feather protein hydrolysate is proven to have good attractiveness and palatability for the monkfish, *Colossoma macropomum* × *Piaractus mesopotamicus*). Furthermore, recent research by **Santos *et al.* (2023)** elucidated that feather protein hydrolysate can be used to replace fish meal in the diets of the Nile tilapia, *Oreochromis niloticus* without changing the taste and eating behavior of the fish.

The decreased feed palatability index observed at a 7.5% SHPH supplementation level suggests that this concentration exceeded the optimal limit for SHPH as an attractant. This over-supplementation likely reduced the palatability of the feed, leading to decreased consumption. According to **Peng *et al.* (2022)**, excessive attractant supplementation can cause feed to become unpalatable and cause nutritional imbalances, which can reduce appetite. Similarly, **Yue *et al*. (2022)** noted that exceeding the optimal attractant supplementation level can reduce the appeal of the feed to fish, resulting in decreased feed consumption and growth.

Feed rejection in fish is closely linked to their ability to detect and recognize favorable or undesirable substances in their food, ultimately influencing their decision to accept or reject the feed **(Lokkeborg *et al*., 2014; Moraes, 2016; Olsen & Lundh, 2016)**. Increasing the supplementation percentage of SHPH (2.5, 5, and 7.5%) in the feed led to a decrease in both the rejection rate and the number of fishes exhibiting no response to the feed ($P<0.05$). The control feed (SHPH 0%) had the highest values for rejection and non-response, reaching 1.67 and 3.02%, respectively. This outcome highlights the importance of SHPH in enhancing feed palatability and, consequently, acceptance by the Asian redtail catfish larvae. These findings are congruent with those of **Alves *et al*. (2019)**, who observed a similar impact on feed rejection and non-response in tilapia when industrial by-product protein hydrolysate supplementation was absent in the diets.

Rapid food perception is a crucial aspect of fish feeding behavior, and it can be effectively triggered by providing highly attractive food. This attraction stimulates an extra-oral response, so that the fish will try to find food upon sensing that stimulus **(Kasumyan & Doving, 2003; Moraes, 2016)**. To reduce leaching of nutrients into the water, rapid capture of feed is also important to ensure complete and balanced consumption and to reduce the impact of nutrient losses to the environment due to exposure to feed in water **(Cyrino *et al.*, 2010; Oliveira *et al.*, 2022)**. Statistical analysis revealed a significant difference in the time taken to capture the first feed among the treatment groups (*P<0.05*). The optimal result was achieved with 5% SHPH supplementation, where the time to first feed capture was 5.20 seconds. Research by **Alves *et al.* (2019)** showed that the Nile tilapia, *O. niloticus* fed chicken protein hydrolysate, liver and sea urchins had an average time to catch the first feed at 0.82 seconds. **Santos *et al*. (2022)** attributed this rapid response to a well-developed extra-oral gustatory system, which enables fish to efficiently locate or avoid food by exhibiting various movements, including stopping, returning, swimming backward, turning sideways, and zigzagging.

CONCLUSION

This study demonstrates that dietary SHPH supplementation can significantly enhance both the attractiveness and palatability of feed for the Asian redtail catfish larvae. A 5% SHPH inclusion rate proved optimal, yielding significantly better results than other tested doses. These findings suggest that SHPH could potentially be used as a feed attractant for the Asian redtail catfish larvae.

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