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Empirical feed formulations for the marine ornamental fish, striped damsel, *Dascyllus aruanus* (Linné 1758) and their physical, chemical and nutritional evaluation

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Abstract

Formulated feeds containing a common ingredient mixture (CIM) consisting of fishmeal (anchovies), shrimp meal (*Acetes*), squid meal (*Loligo*) and soybean meal incorporated in ascending levels to obtain protein levels ranging from 180 to 560 g kg^{-1}(18.34%, 25.35%, 36.27%, 46.61% and 56.28%) and an energy level of 19 MJ kg^{-1} were fed to the marine ornamental fish, striped damsel, *Dascyllus aruanus* <200 mg and 200–300 mg in size for periods of 35 and 63 days. The <200 mg fish accepted particles <0.5 mm in size and showed maximum growth in terms of absolute growth rate (AGR), relative growth rate (RGR) and specific growth rate (SGR) with the feed containing 380 g kg^{-1} CIM having a protein content of 362 g kg^{-1}. Second-degree polynomial regression equations fitted confirmed these observations with a predicted requirement of 360 g kg^{-1} protein. In fish weighing 200–300 mg, growth was not significantly different ($P>0.05$) in fish fed with feeds containing 380 and 580 g kg^{-1} CIM with 360 and 470 g kg^{-1} protein. With these data, the second-degree polynomial regressions showed that a protein level of 464 g kg^{-1} would result in an RGR of 107%. The feeds were well accepted by the damselfish, showing good colour retention and health.The cost of the feeds excluding processing costs ranges from US $ 1.35 to 3.36 kg^{-1}. This is the first report on the development of formulated feeds for damselfish that would help in rearing and aquarium keeping of damselfish worldwide.

Keywords: three-striped damsel, humbug damsel, hatchery-reared *Dascyllus aruanus*, formulated feed, nutrition, colour retention

Introduction

The uniqueness of the marine ornamental fish trade is that fish are traded by price per fish than by weight. The estimated global trade is US $3 billion in retail and US $900 million in wholesale (FAO 2006). The three-striped damsel, humbug damsel or white-tailed damselfish *Dascyllus aruanus* (Family *Pomacentridae*) is one of the marine ornamental fish distributed in coral reef areas and is often found to be associated with branching corals. They are small, attractive fish sold at US $1–5 fish^{-1} in the international trade of marine ornamental fish (http://www.animal-world.com, www.liveaquaria.com). Hatchery technology for seed production of *D. aruanus* was developed by the Central Marine Fisheries Research Institute (CMFRI 2006) and several batches were hatchery produced. Fry are fed a paste of clam meat in batches of 500–1000 fish obtained in one spawning in 5 tonne fibre-reinforced plastic (FRP) tanks. There is a need to develop formulated dry feeds for holding these fish in hatchery rearing systems and also for use in aquarium keeping by hobbyists and traders of marine ornamental fish. Feeds available in local pet shops in India are imported coloured granules (1 mm) meant for feeding freshwater ornamental fish, which are not suitable for feeding marine ornamentals such as damsels.

Information available in the public domain regarding the nutrient requirements of marine ornamentals is scanty (Sales & Janssens 2003). Available reports are on the clownfish (*Amphiprion percula*), which is not only the most popular marine fish species in the aquarium trade but is also considered to be a reference fish for scientific studies on nutrition, egg and larval quality (Delbare, Lavens & Sorgeloos 1995).

It was shown in the marine clownfish (*A. percula*) that fry can be weaned onto a formulated dry feed from 7 days after hatch with no significant reduction in survival as compared with controls receiving enriched *Artemia*, although the optimum time of weaning on a dry formulated feed was found to be between 15 and 20 days after hatch. From 32 days after hatch, supplementation of diets with live or natural feeds is unnecessary (Gordon, Kaiser, Britz & Hecht 1998). Higher survival and growth of larvae was observed on feeding of a combination of rotifer and copepod, which indicates the suitability of copepods to *artemia* (Ignatius, Gaurav, Jagadis, Kandasami & Victor 2001). Woods (2003) demonstrated that juvenile seahorses (*Hippocampus abdominalis*) 1–2 months of age could be successfully weaned onto both frozen and artificial foods, but newborn juveniles were not successfully weaned onto the artificial food. This study reports the formulation and nutritional evaluation of feed for aquariculture of hatchery-reared striped damsel, *D. aruanus*.

Materials and methods

Feed preparation and analysis

Major feed ingredients, e.g., squid meal (*Loligo* spp.), shrimp meal (*Acetes* spp.), fish meal (anchovies), soy flour (defatted) and micro-ingredients, were procured from the local market (Cochin, India). All ingredients were pulverized through a 0.5 mm sieve and stored in airtight polypropylene containers. After analysis of the ingredients for their proximate composition (Tecator Instruments, Sweden), a common ingredient mixture (CIM) was blended first and subsequently this CIM was incorporated in ascending levels into five formulations fortified with vitamins, minerals and additives. After blending of all feed ingredients by addition of water at 180 g kg^{-1}, the feeds were extruded through a laboratory model extruder (Basic Technologies, Calcutta, India) with a uniform time–temperature (10 s and 80 $\pm$ 5 °C) combination for all the five experimental feeds. These formulated feeds were then mechanically crushed and passed through American Society for Testing and Materials (ASTM) sieves to obtain three particle sizes: $\leq$0.5, 1.00 and 1.5 mm. Feed ingredient proximate composition, ingredient composition and proximate composition of CIM, ingredient composition of the experimental diets inclusive of cost, their proximate composition, all details and sources of micronutrients and additives are presented in Table 1, with the experimental diets identified as 18, 26, 36, 47 and 56 based on assayed crude protein content.

Two feeds that performed the best among the five tested (nos. 36 and 47) were subjected to amino acid analysis as follows: powdered feed samples (0.1 g) with 10 mL 6 N HCl were digested at 110 °C in sealed tubes for 24 h. The solution was filtered flash evaporated thrice, using distilled water to remove the acid. The acid-free sample was then made up to 5 mL with 0.05 N HCl, and filtered in a 0.2 μm nylon filter syringe. Pre-column derivatization of amino acids was performed with phenylisothiocyanate (PITC) to form phenylthiocabamyl (PTC) amino acids. Twenty microlitres of derivatized sample was injected into HPLC (Waters reversed-phase PICO.TAG amino acid analysis system), fitted with a packed column (dimethylocatadecylsilyl-bonded amorphous silica). The elution buffer used was sodium acetate trihydrate (pH 6.4) and acetonitrile. The detector (Waters 2487 dual λ absorbance detector) was set at 0.1 absorbance units full scale (AUFS) at 254 nm and the column temperature was set at 38 °C. Standard (PIERS amino acid standard H) was run before each sample injection. Samples were injected in triplicate and the output was analysed using BREEZE software. The amino acid profiles of these two feeds are presented in Table 2.

Physical qualities of the feed

Bulk density

Weight by volume of all the five experimental feeds was assessed by determining the weight of a fixed volume of the feed, before crushing and sieving and after size reduction to $\leq$0.5, 1.0 or 1.5 mm.

Hydrostability

Approximately 5 g of each experimental feed sieved through $\geq$0.5 mm weighed to the nearest milligram was introduced into pre-weighed net pouches in triplicate and immersed with sinkers in a seawater tank where the experimental conditions were simulated. The pouches were removed at intervals of 15, 30, 45 and 60 min, rinsed carefully in double distilled-water and weighed after drying in a hot-air oven at 65 $\pm$ 4 °C. The dry matter loss was calculated in percentage.

Nutritional evaluation

Two sets of nutritional trials were conducted with the feed formulations, e.g., experimental feeds identified as

Table 1 Feed ingredient proximate composition* (% DM matter), ingredient composition (g kg^{-1}) of experimental diets and their proximate composition (% DM matter)

	CP	EE	CF	NFE	Ash
Ingredients					
Squid meal	61.42	6.81	0.98	11.91	18.88
Shrimp meal	65.46	3.78	5.29	0.04	18.35
Fishmeal	69.54	7.22	0.23	0.07	17.69
Soya flour	51.95	0.59	2.91	26.98	6.96
Wheat flour	13.45	1.39	2.95	75.79	0.73
Feed nos.	18	25	36	47	56
Ingredients					
CIM†	10	180	380	580	780
Wheat flour‡	865	685	505	315	120
Fish oil§	40	50	30	20	15
Vitamin mixture¶	20	20	20	20	20
Mineral mixture‖	10	10	10	10	10
Other additives**	55	55	55	55	55
Cost (US $ kg^{-1})	1.64	1.96	2.56	3	3.36
Proximate composition of experimental feeds					
CP	18.34	25.35	36.27	46.61	56.28
EE	5.30	5.84	5.47	5.11	5.25
CF	0.91	0.92	1.11	1.41	1.60
NFE	72.78	64.12	51.50	38.09	25.14
Ash	3.43	4.46	6.13	9.21	12.63
AIA	0.00	0.01	0.45	0.57	0.84
DE (MJ 100 g^{-1})	14.170	14.512	14.744	14.781	14.924
GE (MJ 100 g^{-1})	19.180	19.460	19.586	19.446	19.424

*CP, crude protein; EE, ether extract; CF, crude fibre; NFE, nitrogen-free extract.

†Common ingredient mixture consisted of squid meal, shrimp meal, fishmeal and soy flour in equal quantities (0.25:0.25:0.25:0.25) with a proximate composition of CP 65.56, EE 5.40, CF 2.96, NFE 10.99 and Ash 15.03.

‡From the local grocery shop.

§Cod liver oil – sea cod.

¶Supplevite-M from Jubiliant Organosys Limited, Samlaya Unit, Block 138, Village Samalaya, Taluka Savil, Vadodara 391520, India. Marketed by Sarabhai Zydus Animal Health, Administration Building, Gorwa Road, Vadodara 390029, India, containing kg^{-1} vitamin A, 2 000 000 IU; vitamin D_3 400 000 IU, vitamin B_2 0.4 g; vitamin E 250 IU.

‖Salt mixture for biological test diets from Sisco Research Laboratories, Mumbai 400093, India U.S.P. XIV (1950). Percentage composition: calcium carbonate 6.86000, calcium citrate 30.83000, calcium phosphate monobasic 11.28000, magnesium sulphate · $7H_2O$ 3.83000, manganese carbonate 3.52000, potassium chloride 12.47000, dipotassium phosphate 21.88000, sodium chloride 7.71000, copper sulphate · $5H_2O$ 0.00777, ferric citrate (16–17% Fe) 1.52815, manganese sulphate · H_2O 0.02008, potassium aluminium sulphate 0.00923, potassium iodide 0.00405, sodium flouride 0.05070.

**Other additives include Wockcee^{TM} ascorbylpolyphosphate from Wockhardt Life Sciences, (1 g kg^{-1}) Mumbai 400061, India, β-carotene (0.5 g kg^{-1}) and spirulina from Parrys Nutraceuticals, Chennai, India, and a probiont *PS-102* from Vijayan *et al.* (2006) @ 2 g kg^{-1}).

DE, digestible energy; GE, gross energy; MJ, mega joules GE calculated multiplying the analysed protein lipid and carbohydrates by 5.5, 9.1 and 4.1, respectively, and DE values using the conversion factors 4.25, 8 and 3 for protein, lipid and carbohydrate and converted to MJ kg^{-1}.

Table 2 Amino acid composition of the feeds indicating optimum performance (g kg^{-1})

	Feed nos.	
Amino acids	36	47
Asp	26.23	43.34
Glu	47.94	68.40
Ser	17.95	28.91
Gly	34.73	33.50
His	6.93	10.55
Arg	14.23	22.30
Thr	13.36	19.06
Ala	25.81	27.92
Pro	27.39	31.68
Tyr	9.09	10.51
Val	18.34	21.26
Met	6.64	4.93
Cys	0.87	2.66
Ile	15.53	18.63
Leu	27.85	36.29
Phe	22.60	19.35
Lys	25.46	41.83

18, 25, 36, 47 and 56 based on their protein content: one with fish weighing < 200 mg for 35 days, and another with fish weighing 200–300 mg for 63 days.

Hatchery-reared *D. aruanus* (225 fish) from a single brood of uniform size were hand sorted and quickly weighed individually in a top-loading electronic balance (Shimdazu, Japan) without any anaesthetization for use in both the experiments. These fish were divided into 15 groups of 15 fish each and introduced into rectangular FRP tanks of 250 L in triplicate containing 200 L seawater, fixed with two *in situ* biological filters, each fabricated with food-grade PET bottles (1000 mL). Aeration was continuous using an airlift pump mechanism operating through the biological filter. Water in the tanks remained clear in the majority of the tanks throughout the experimental duration and water exchange was carried out only in tanks where the water became cloudy mainly due to malfunctioning of the biological filter, which was immediately removed and replaced with a freshly fabricated one. Water quality was monitored for salinity (refractometer, Atago, Tokyo, Japan), dissolved oxygen (DO), pH and temperature (Eutech, Singapore) to ensure that they did not drop below the standards specified for marine aquatic life (salinity 35 ± 2‰, DO > 4.8 mg L^{-1}, pH 6.8 ± 0.9 and temperature 28 ± 1 °C). The whole experimental set-up was housed in a wet laboratory facility exposing the system to a 12:12-h photoperiod with sunlight directly falling on the tanks during the morning

hours at Mandapam Regional Centre of CMFRI, Tamil Nadu, India.

Each experimental feed had three replications and the initial wet weight of each fish was recorded to the nearest milligram. Mortality record was maintained daily and feed weight was adjusted accordingly. Feeding was carried out at the rate of 3% of the body weight divided into equal doses at 09:00 and 18:00 hours. On termination of the experiment, the fish were again weighed individually and weights were recorded.

The final wet weight (mg), absolute growth rate (AGR) (mg day^{-1}), relative growth rate (RGR) (per cent weight gain over initial weight) and specific growth rate (SGR) (% weight gain day^{-1}) were calculated and subjected to analysis of variance (ANOVA) in EXCEL data analysis menu. Data in per cent were arcsine-transformed before ANOVA. Using critical difference values, 'Student's *t*-test' for equality of means was used to compare the differences between means ($P < 0.05$). With the RGR as the dependent variable and levels of protein in the feeds as independent variables, a second-degree polynomial regression of the form $y = a + bx + cx^2$ was fitted and plotted to arrive at the theoretical optima. The significance of the second-order regression was also tested here using the '*t*-test'.

Table 3 Bulk density of the experimental feeds (g mL^{-1})

		Crushed and sieved through		
Feed nos.	**Uncrushed**	**≤ 0.5 mm**	**1.0 mm**	**1.5 mm**
18	0.201	0.399	0.257	0.242
25	0.287	0.542	0.412	0.359
36	0.297	0.558	0.445	0.405
47	0.321	0.607	0.484	0.427
56	0.372	0.656	0.579	0.514

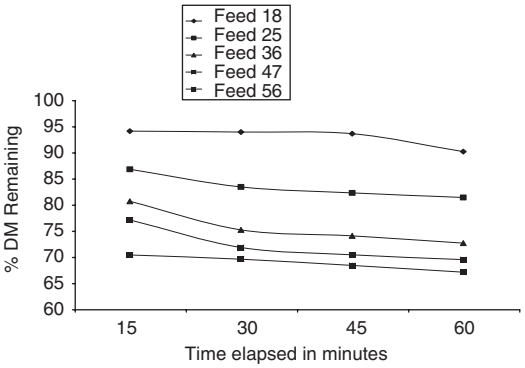

Figure 1 Hydrostability of the crushed and sieved experimental feeds of ≤0.5 mm from 0 to 60 min with intervals of 15 min.

Results

All the five feeds offered were attractive to the fish, which consumed them with very little feed waste. Throughout the experimental duration, the fish were healthy and retained their natural colour.

Bulk density and hydrostability

The feed containing the maximum amount of wheat flour had the minimum bulk density (weight/volume) due to puffing of starch (wheat) as shown in Table 3. In the extruded form, feeds 1–4 floated and feed 5 sank. The variation in bulk densities after crushing and sieving is also depicted in Table 3. Dry matter loss varied from 6% in feed 17, 13–14% in feeds 25, 36 and 47 and 30% in feed 56 in the first 15 min of immersion. Lixiviation rates are depicted in Fig. 1.

Nutritional evaluation

Among the five experimental feed formulations when tested with < 200 mg damsels, fish fed feed 35 exhibited significantly higher growth ($P < 0.05$). In terms of AGR, RGR and SGR also, the same trend was depicted with statistically significant differences ($P < 0.05$) as shown in Table 4. After a gradually significant increase in growth with feeds 18, 25 and 36, later,a decline in growth could be observed in fish fed with feeds 47 and 56. No discoloration or disease was observed with all the five feeds throughout the experimental duration of 35 days and survival percentages were 93% in fish reared on first two feeds and 100%, 90% and 83% for feeds 36, 47 and 56 respectively. Observed maximum growth with the feed containing 380 g kg^{-1} CIM was 96.8% over the initial weight. Optimum protein level deduced was 360 g kg^{-1} with an RGR of 97.3% (Fig. 2).

In the second experiment with damsels of 200–300 mg, feeds 36 and 47 performed in concert, yielding significantly higher growth ($P < 0.05$). These feeds contained 380 and 580 g kg^{-1} CIM. All other indices also recorded the same trend as shown in Table 5. In this experiment, similar growth was recorded with feeds 36 and 47 and, with feed no. 56, there was a decline in growth. Fish showed good colour expression and good health during the 63-day experimental period. The survival rates in this

Table 4 Growth and survival of *Dascyllus aruanus* (<200 mg) reared on formulated feeds for 35 days (mean ± SE)

Feed nos.	18	25	36	47	56
Wet initial weights	0.179 ± 0.021	0.179 ± 0.058	0.179 ± 0.032	0.179 ± 0.079	0.179 ± 0.048
Wet final weights	0.286 ± 0.012^{a}	0.320 ± 0.039^{ab}	0.353 ± 0.038^{bc}	0.335 ± 0.018^{bc}	0.256 ± 0.015^{a}
AGR	0.003 ± 0.01^{a}	0.004 ± 0.04^{ab}	0.005 ± 0.02^{b}	0.004 ± 0.02^{ab}	0.002 ± 0.01^{ac}
RGR	59.64 ± 6.86^{a}	78.37 ± 21.84^{ab}	96.77 ± 20.89^{bc}	86.73 ± 9.96^{abc}	42.70 ± 8.31^{a}
SGR	1.34 ± 0.12^{a}	1.65 ± 0.34^{ab}	1.93 ± 0.29^{b}	1.78 ± 0.15^{c}	1.02 ± 0.17^{a}
Survival per cent	93	93	100	90	83

Values are means of three replicates ± SE. Values in the rows sharing the same superscript do not differ significantly ($P<0.05$).
AGR, absolute growth rate (g day^{-1}); RGR, relative growth rate (%); SGR, specific growth rate (% day^{-1}).

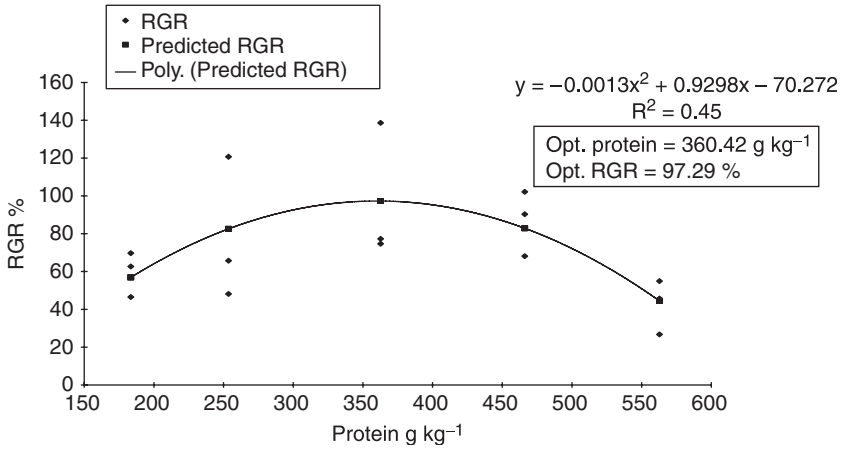

Figure 2 Polynomial regression of relative growth rate (RGR) on the varying levels of protein in feeds of marine ornamental fish *Dascyllus aruanus* (<200 mg initial weight).

Table 5 Growth and survival of *Dascyllus aruanus* (200–300 mg) reared on formulated feeds for 63 days (mean ± SE)

Feed nos.	18	25	36	47	56
Wet initial weights (g)	0.210 ± 0.005	0.249 ± 0.038	0.225 ± 0.013	0.228 ± 0.020	0.230 ± 0.031
Wet final weights (g)	0.253 ± 0.002^{a}	0.327 ± 0.008^{b}	0.450 ± 0.004^{c}	0.462 ± 0.004^{c}	0.434 ± 0.006^{c}
AGR	0.0007 ± 0.0001^{a}	0.0012 ± 0.0005^{b}	0.0036 ± 0.0003^{c}	0.0037 ± 0.0003^{c}	0.0032 ± 0.0006^{c}
RGR	20.43 ± 2.23^{a}	36.13 ± 16.60^{b}	101.78 ± 14.13^{c}	105.77 ± 17.57^{c}	95.94 ± 25.86^{bc}
SGR	0.29 ± 0.03^{a}	0.46 ± 0.20^{b}	1.11 ± 0.11^{c}	1.13 ± 0.13^{c}	1.04 ± 0.23^{d}
Survival (%)	90	81	95	95	95

Values are means of three replicates ± SE. Values in the rows sharing the same superscript do not differ significantly ($P<0.05$).
AGR, absolute growth rate (g day^{-1}); RGR, relative growth rate (%); SGR, specific growth rate (% day^{-1}).

experiment were observed to be 90% and 81% with the first two feeds and 95% with feeds 36, 47 and 56.

Maximum growth recorded in terms of RGR for the aforementioned feeds were 101.8% and 105.8%, respectively, which were statistically similar (Table 5). Theoretical optima derived were 464 g kg^{-1} protein and 107.4% RGR (Fig. 3).

Discussion

In the present study, formulated feeds for the rearing and maintenance of striped damsel were developed and tested for the first time. Growth, colour retention and health were the major nutritional aspects investigated and above all the acceptance of a formulated feed reduced to <0.5 mm in size. As feeding progressed, all the five experimental feeds used were well accepted by the damsels. Marine ornamental fish used for aquarium keeping are normally collected from natural coral ecosystems, through destructive methods. Wild-caught *D. aruanus* has been shown to be one such fish by Durville, Bosc, Galzin and Conand (2003) amenable to artificial feeding. In this context, the first step to promote aquarium

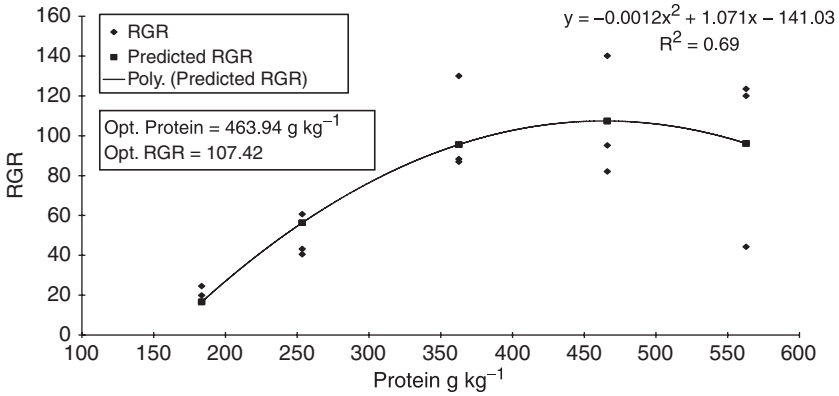

Figure 3 Polynomial regression of relative growth rate (RGR) on the varying levels of protein in feeds of marine ornamental fish *Dascyllus aruanus* (200–300 mg initial weight).

keeping of marine ornamentals has been to breed them in captivity and close the life cycle of the fish, followed by the use of artificial feeding for rearing in closed systems. When CMFRI was able to breed this fish (CMFRI 2006), hatchery-produced striped damsels became available for scientific investigations.

While evaluating the physical qualities of aquatic feeds, bulk density is a measure describing the weight of any material or feed in this situation per unit volume. In general, bulk density can be increased by reducing the particle size to increase the weight of a feed ingredient or complete feed per unit of volume (Knott & Shurson 2006). Feeds with less than 480 g L^{-1} bulk density are reported (Rokey & Plattner 2006) to be floating in seawater (Table 6). Feed 56 in this study, containing 780 g kg^{-1} CIM with 120 g kg^{-1} wheat flour, was found to be sinking in its original (uncrushed) form and had a bulk density of 372 g L^{-1} probably due to its high level of protein-rich CIM with an ingredient particle size <0.5 mm. Particles of all the experimental feeds floated initially and then sank slowly on absorption of water. Bulk densities worked out for uncrushed and crushed feed would aid in selection of the volume of containers for packing these feeds either for bulk sale or retailing. A lixiviation rate of 13–14% of the dry matter in feeds 25, 36 and 47 in the first 15 min of immersion is indicative of the potential nutrient loss from the feed particles. Lixiviation rates in actual fish culture would be much lesser because the feed dispensed is consumed before lapse of the first 15 min throughout the experiment.

At the beginning of the experiment, the fish fed only on floating and suspended particles. Within a week, fish could be seen feeding on the particles settling on the tank bottom, also resulting in better utilization of the feed available in the water column also. Switching off aeration itself became a cue to the fish to expect feed. Feeding activity could be observed while the feed particles float, suspend in the water column and sink to the bottom, leading to utilization of exogenous nutrition at all levels in the water column. Thus, residual feed being the source of water spoilage is minimal, which is highly desirable in aquariculture.

Table 6 Final product bulk density (g L^{-1}) correlation with float-sink properties for aquatic feeds*

Feed characteristics	Seawater at 20 °C (3% salinity)	Fresh water at 20 °C
Fast sinking	640	>600
Slow sinking	580–600	540–560
Neutral buoyancy	520–540	480–500
Floating	<480	<440

*From Rokey and Plattner (2006).

The optimum protein range that elicited the best growth in this investigation is between 360 and 470 g kg^{-1}. Durville *et al.* (2003) reported growth rates recorded in terms of SGR as 1.1 with a formulated feed containing 520 g kg^{-1} protein and 150 g kg^{-1} lipid in *D. aruanus*. In the present study, growth was found to be higher, i.e., 1.93 with a feed containing only 360 g kg^{-1} protein and 55 g kg^{-1} lipid in the experiment with fish weighing <200 mg for a period of 35 days. This could have been due to the higher level of adaptation of the hatchery-reared fish to confined conditions in this study as they were bred in captivity compared with wild-caught fish used by Durville *et al.* (2003). Higher mortality rates reported by Durville *et al.* (2003) also point towards the same. When fish weighing >200 mg were fed for a period of 9 weeks in the second experiment, SGR reported by Durville *et al.* (2003) was similar to feeds of lower nutrient densities used in this study (Table 4).

However, mortalities in this experiment were lower compared with Durville *et al.* (2003). The observed protein level that promoted maximum growth (RGR 97%) in the first experiment was 362 g kg^{-1}. The derived optimum was 360 g kg^{-1} protein and 97% RGR. In the second experiment, the observed maximum is between 380 and 580 g kg^{-1} for CIM providing 360–470 g kg^{-1} protein for an RGR of 102–107%. The derived values with these data were 464 g kg^{-1} protein, resulting in a growth of 107.42%. Even though similar data on *D. aruanus* are not available for comparison, in freshwater ornamentals, 450 g kg^{-1} is the optimum protein level reported for the discus (Chong, Hashim & Ali 2000) and the sword tail (Kruger, Britz & Sales 2001). In sebae clown (*Amphiprion sebae*), a marine ornamental fish, Varghese (2004) reported an optimum protein requirement of 460 g kg^{-1}. Freshwater ornamental fish with significantly lower protein requirements than the aforementioned reports are dwarf gourami (Shim, Landesman & Lam 1989), gold fish (Loachman & Phillips 1994) and tinfoil barb (Elangovan & Shim 1997). Growth depression above the optimum level seen in this study is reported in many other fish. (Dabrowski 1977; Teng, Chua & Lim 1978; Jauncey 1982; Siddiqui, Howaldar & Adam 1988; Vergara, Fernadez-Palacios, Robiana, Jauncy, De La Higuera & Izquierdo 1996 and Lee, Park & Band 2002). Unproductive energy expenditure involved in deamination and excretion of excess amino acids absorbed is one of the reasons cited for this phenomenon (Jauncey 1982). Reduction in energy available for growth due to use of non-protein energy for deamination and excretion of excess amino acids is also reported by Lim, Sukhjawongs, and Pascula (1979). The toxic effect of high-protein diets with low non-protein energy leading to decline in growth could be another reason as reported by Prather and Lovell (1973) in catfish. A linear increase in growth, followed by a plateau reported in fish by El-Sayed and Teshima (1992), Lee, Kanf, Lee and Kim (1993) and Kang, Lee, Hwang and Bai (1998) was also observed in this study, especially in the second experiment of 9 weeks' duration, with slow and steady growth maintaining the healthy status of the fish. All the five feeds used in this investigation support aquariculture of *D. aruanus* without any adverse effects on their colour and health. Differences in growth, however, reflected macronutrient variations in diet design that followed generally reported patterns in fish.

The cost of these feeds is another aspect looked into without including the processing or packaging costs. The cost kg^{-1} worked out to 1.35–3.36 US $ approximately. In India, freshwater ornamental fish feeds retail at US $ 4.44 kg^{-1}. Most of these feeds are imported in bulk and repacked in India. Development of dedicated indigenous formulated feeds for marine ornamentals such as damselfish would contribute to the development of marine ornamental aquariculture in India and elsewhere.

This investigation reveals that the striped damselfish *D. aruanus* is amenable to rearing with artificial feeds, provided the right particle size is provided as a slow sinking granule or crumble. However, refinements of the formulated feed with reference to absolute macronutrient requirements and micronutrient and ingredient requirements have to be investigated further for improvements in feed design and development because *a priori* additives such as carotenoids, spirulina and a probiotic (Vijayan, Bright Singh, Jayaprakash, Alavandi, Somnath Pai, Preetha, Rajan & Santiago 2006) could have also contributed to the health, growth and colour retention of these fish. Seventy-five per cent of the CIM used in this investigation contained proteins of marine origin. Reduction of the levels of costly marine proteins to minor ingredients or attractants should be attempted for further cost reduction and value addition.

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