***Original Research Article***

**Effect of Mixed Zooplankton (Daphnia/Moina) on The Growth Performance and Survival of *Hemigrammus Caudovitatus* (Albino Buenos Aires Tetra)**

**Abstract**

A sixty (60) day feeding trial was conducted to evaluate effect of mixed Zooplankton (Daphnia/Moina) on the growth performance and survival of *Hemigrammus caudovitatus* (Albino buenos aires tetra). The culture was carried using four different locally available nutrient sources viz, yeast, Mustard Oil Cake, cow dung and poultry manure. Proximate analysis was carried out for the mixed zooplankton cultured on different media. The highest zooplankton density was observed in tanks with chicken manure (3266.50±6.85 individuals/litre on day 21), followed by cow dung (2203.5±1.29 individuals/litre), Mustard Oil Cake (1481.5±4.20 individuals/litre), and yeast (1110±3.55 individuals/litre). Protein analysis showed the highest crude protein content in zooplankton fed with poultry manure (55.76±0.19%) and lowest protein content was observed in zooplankton fed with yeast (47.41±0.23%). Highest lipid content was observed in T4 (7.84±0.) group, while as the lowest lipid content was observed in the T1 (6.36±0.15) group. Highest ash content was observed in T2 (15.57±0.29) group, while as the lowest ash content was observed in the T1 (7.44±0.28) group. Fish fed on these zooplankton exhibited the highest weight gain (86.6±0.8 mg) and survival rate (92.5±1.29%). Nutrient source significantly influenced zooplankton growth and composition, with poultry manure proving to be the most effective, leading to better growth and survival of *Hemigrammus caudovitatus.*

**Keywords**

*Hemigrammus caudovitatus*, growth, Daphnia, Moina, zooplankton

**Introduction**

The ornamental fish trade is a multibillion-dollar industry with participants in more than 125 countries. More than 2 billion live ornamental fish are handled in the estimated $20 billion worldwide ornamental fish trade (King, 2019) . In 2021, Freshwater Ornamental Fish ranked 3575th among the world's traded products, amounting to a total trade value of $307 million. From 2020 to 2021, the exports of these fish increased by 21.2%, rising from $253 million to $307 million. The trade volume in Freshwater Ornamental Fish accounts for 0.0014% of the overall global trade (OEC, 2023). Developing countries are the major producers and suppliers in the world supplying more than 60% of the ornamental fish. India's domestic trade in ornamental fish is thought to be worth over 25 crores, whereas its exports are only worth 6.0 crores, or 0.3% of the world market. The major part of the export trade is based on wild collection. There is very good domestic market too, which is mainly based on domestically bred exotic species (Satam *et al.,* 2018). It’s important to highlight that Ornamental fish have shown significant growth, with a 312.42% increase in quantity and a 153.54% increase in US dollar earnings, respectively. The export performance of ornamental fish trade increased from 54 Metric Tonnes in 2020-21 to 222 Metric Tonnes in 2021-22 (MPEDA, 2022).

Live food organisms are sometimes referred to as "living capsules of nutrition" since they are full of nutrients like vital proteins, lipids, carbohydrates, vitamins, minerals, amino acids, and fatty acids (New, 1998). Zooplanktons are the main sources of natural food for fish and shellfish which is directly related to the survival and growth and these form the base of food chains and food webs in all aquatic ecosystems. They also have a significant impact on the recycling of nutrients and energy in their particular ecosystems. Fish that are omnivorous and planktivorous depend on zooplankton for food (Alam *et al.,* 1987) and the most essential for larvae culture (Bardach *et al.,* 1972). When managing successful ornamental operations, both the qualitative and quantitative abundance of zooplankton in a fish pond are crucial since they vary from location to location and pond to pond within the same area even under identical ecological conditions (Boyd, 1982). Zooplankton exhibit distinct advantages over artificial feeds, their natural and diverse nutritional profile aligns closely with the needs of ornamental fish juveniles, offering essential fatty acids, proteins, vitamins, and minerals crucial for healthy growth. Their easy digestibility benefits many ornamental fish species, contributing to improved immune systems, better growth rates, and lowered disease vulnerability. Additionally, their use as feed reduces the environmental impact associated with producing and disposing of artificial feeds, fostering a more sustainable aquatic ecosystem. In certain instances, zooplankton can also prove more cost-effective, especially when locally harvested or cultured, mitigating the expenses linked to feed production. Even though there are many zooplankton species in freshwater and they are all vital to aquaculture, rotifers, cladocerans (Daphnia, Moina) are found in a variety of natural environments, particularly in freshwater (FAO 1996). Zooplanktons are vital for the growth and development of fish larvae due to the fact that they contain higher levels of protein as well as a variety of digestive enzymes that can act as exo-enzymes in the fish larvae's gut, including cellulase, proteinases, peptidases, amylases, and lipases. They are the finest and most universally accepted live foods for most fish (Miah *et al.,* 2013).

Albino Buenos Aires tetra, *H. caudovittatus*, belongs to the Characidae family and similar to neon tetra, *Paracheirodon innesi*, or yellow tetra, *Hyphessobrycon bifasciatus*, is among the most popular species of that family in aquarium breeding. In the natural environment they are found mainly in tropical waters of South America although they are also highly numerous outside their natural geographic location being objects of interest in high demand among aquarium keepers worldwide. Their high popularity results from both attractive coloring and ease of breading. Buenos Aires tetra is a small fish reaching 7–9 cm in length. Young fish hatch within 24 hours from fertilization of the eggs and after several days they start independent feeding. Body is yellowish-orange and the odd fins are intensely red. A white horizontal belt runs along the body. The eye pupil is red and the iris is white (Kucharczyk *et al.,* 2008). Live feed is nutritionally complete food supplying all essential amino acid for growth and survival of larvae. It is the most palatable feed for larvae owing to their small mouth size. Furthermore, live feed is easily accepted by fish because of their mobility. Therefore, the current study aims to evaluate the impact of local nutrient sources for growth of zooplankton and the proximate analysis will suggest the best substrata for growth and culture of zooplankton. On the other hand, albino tetra can thrive in cold water, the successful rearing of stock can help to advance research in ornamental field in temperate conditions

**MATERIALS AND METHODS**

**Experimental design, Zooplankton culture , and sampling**

The current experimental trial was carried out at the Fisheries Instructional Farm of the Faculty of Fisheries, Rangil, Ganderbal (340.21', 740.80'). The experiment was divided into four different treatments (T1, T2, T3, T4) each with four replicates following a Completely Randomized Design (CRD) as shown in Table 1. The culture was carried in glass aquaria of 40 L capacity using four different locally available nutrient sources viz, yeast, Mustard oil cake (MOC), cow dung and poultry manure purchased from local farms for the current study. Using an electric grinder, the ingredients excluding yeast were first crushed into a fine powder before being sieved through a sieve with a mesh size of 200 mm. The tanks were filled with water and were then fertilized using MOC in one set up, Cow dung in second, Poultry Manure in third and Yeast in fourth. The dosage is given in Table 1. The water in tanks was then inoculated with mixed zooplankton (daphnia/moina) procured from already existing stock culture @ 50 no. of individuals per tank (Paray and Al-Sadoon, 2016). The aquariums were fitted with the aerators to maintain adequate dissolved oxygen.

**Table 1: Nutrient sources and their concentration in experimental treatments.**

|  |  |  |
| --- | --- | --- |
| **Treatment** | **Nutrient source** | **Dosage** |
| **T1** | MOC | 75mg/L(Das *et al.,* 2012) |
| **T2** | Cow Dung | 20mg/L (Nair *et al.,* 2014) |
| **T3** | Poultry Manure | 50gm/L(Damle and Chari, 2011) |
| **T4** | Yeast | 200mg/L(Das *et al.,* 2012) |

**Quantitative enumeration of zooplankton**

For the quantitative studies ten litres of water was sieved through the plankton net. The plankton sample collected in the polyethylene tube attached to the net was preserved in 4% formalin. The sample was carried to the laboratory where it was concentrated to a known volume and shake gently. 1 ml of sample was placed on Sedgewick rafter counting cell of 1 ml capacity (50mm long, 20mm wide and 1mm deep) and studied under compound microscope LABOMED CXR2 for quantitative enumeration. For accuracy the counting was done in triplicate and average of three counts was used to calculated the population dynamics of various species and hence of total zooplankton in individuals per litre of water by the formula given by Welch (1948).

 $n= \frac{a ×c}{l}$

Where, n = number of individuals per litre.

 a = average no of individuals in 1 ml of concentrated sample.

 c = volume of concentrated sample.

 l = volume of the original water sample sieved.

**Proximate analysis of cultured zooplankton:**

The cultured zooplankton were subjected to proximate analysis according to the standard methods of AOAC (AOAC., 2005) for moisture, protein, fat and ash at the laboratory of Fish Nutrition and Biochemistry, Faculty of Fisheries, Rangil, Ganderbal.

**Rearing Experiment**

One month old juveniles were procured from Fish Farm Faculty of Fisheries, Shuhama, District Ganderbal, Jammu & Kashmir. The mean weight of fish was 25 mg. The fishes were acclimatized for 7 days before the start of experiment under lab conditions. Dead and weak fishes were discarded. During the period, fish were fed with commercial ornamental diet twice a day. Each tank was filled with 40 litres of water and were left for stabilization for one day prior to stocking of fish. The experimental setup was divided into four groups each with four replicates following a completely randomized design**.** Ten albino tetra juveniles having a mean weight of 25 mg were stocked in each tank. Each tank had a continuous supply of oxygen through aerators. 20% of water was exchanged daily to remove left over feed and excreta. Feeding was not done for twenty-four hours before the experiment. With the commencement of experiment, the juveniles were fed with their respective diets i.e., control diet @ 5% of body weight twice a day and mixed zooplankton were given @ 100 individuals/tank. Sampling was done every two weeks (every 15 days), on the 15th, 30th, 45th, and 60th days of the trial.

**Survival and growth performance**

Initially the weight of fishes was recorded. To study the growth parameters, the fishes were sampled after every fifteen (15) days which contributed to a total of five (5) samplings during the sixty (60) days of study period. The weight was recorded using an electronic weighing balance. The following growth parameters were recorded:

**Weight gain:**

Weight gain is defined as the amount of weight gained over a period of time in relation to the total weight. The weight gain was calculated using the following formula:

**Weight gain** = Wf – Wi

**Percentage weight gain:**

Percentage weight gain is defined as the amount of weight gained over a period of time in relation to the total weight. The percentage weight gain was calculated using the following formula:

**Weight gain (%)** = $\frac{  \left(W\_{f}-W\_{i}\right)}{W\_{i}}×100$

**Specific growth rate (SGR):**

Specific growth rate is a term used in aquaculture to estimate the production of fish after a certain period. The Specific Growth rate was calculated by the following formula:

**Specific growth rate (SGR)** = $ \frac{  (lnW\_{f}-lnW\_{i})}{T}×100 $

**Survival Rate (SR):**

It is defined as the ratio of final number of fishes to the initial population.

**Survival rate (%) =** $\frac{final number of fish}{initial number of fish}×100 $

**Statistical analysis:**

The data was statistically analysed by R software. Experimental data was subjected to the statistical analysis following the (Completely Randomized Design) CRD. Data was subjected to one way analysis of variance (ANOVA) followed by Duncan’s Multiple Range Test. Test p< 0.05 was considered as statistically significant. The results were expressed as the mean ± standard deviation.

**Results**

**Zooplankton Density**

The density of mixed zooplankton (Daphnia/Moina) under the influence of different nutrient sources was determined by counting zooplankton samples every 7 days during the experiment. It was determined in terms of number of individuals per litre as shown in Table 2.

At the start of experiment, all the treatments were inoculated with same number of individuals. The maximum density was recorded to be 3266.50±6.85 individual/l on 21st day from tanks fed with poultry waste (T3). The zooplankton number recorded in tanks fed with cow dung (T2) was recorded to be 2203.50±1.29 individual/l while as zooplankton number in tanks fed on MOC (T1) was recorded to be 1481.5±4.20. However, the population fed on yeast yielded the minimum density among all the experimental tanks (1110.00±3.55 individual/l) as shown in Table 2 and Fig. 1.

**Table 2: Weekly changes in Mixed Zooplankton densities (individual/l) at different Nutrient types (Data were presented as: Mean ± SD)**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Treatments** | **7th Day**  | **14th Day** | **21st Day** | **28th Day** |
| **T1** | 284.25±3.77 | 841.5±2.08 | 1481.5±4.20 | 1062±29.22 |
| **T2** | 585±2.94 | 1163.75±2.75 | 2203.5±1.29 | 1348±27.15 |
| **T3** | 793.75±3.50 | 1683.75±3.50 | 3266.5±6.85 | 1704±204.86 |
| **T4** | 238.5±3.87 | 678.5±5.19 | 1110±3.55 | 1012±455.67 |

Data were presented as mean ±SE (n=4). Values within the same column having different superscripts are significantly different (P<0.05)

**Fig. 1. Weekly changes in Mixed Zooplankton densities (individual/l) at different Nutrient types**

T 1 = Mixed Zooplankton cultured on MOC

T2 = Mixed Zooplankton cultured on cow dung

T3 = Mixed Zooplankton cultured on Poultry Waste

T4 = Mixed Zooplankton cultured on yeast

**Proximate analysis of cultured zooplankton**

Crude protein content was found higher in mixed zooplankton fed poultry waste (55.76±0.19) as shown in Table 3. Mixed zooplankton fed Cow Dung and MOC diets showed second level of protein among groups. Lipid content of Mixed Zooplankton was supported by Yeast diet (7.84±0.21) following by both Poultry Manure and Cow Dung diets. The lowest crude lipid content was found in MOC Treatment (6.36±0.15). The highest ash content was recorded in Cow dung Treatments (15.57±0.29) and the lowest in Yeast Treatments (7.44±0.28).

**Moisture:**

Moisture content of the mixed zooplankton was recorded at the end of the experimental trial and the result is shown in the Table 3. Significant difference (p<0.05) was observed in the moisture content between the treatments. Highest moisture content was observed in T2 (7.61±0.31) while the lowest moisture content was observed in the T4 (4.48±0.15).

**Protein:**

The protein content estimated for the mixed zooplankton is given in the Table 3. Significant difference (p<0.05) was observed in the protein content between the treatments. However, no significant difference (>0.05) was observed in T2 and T3 group. Highest protein content was observed in the T3 (55.76±0.19) while the lowest protein content was observed in T4 (47.41±0.23).

**Lipid**

The total lipid content of Mixed Zooplankton samples recorded at the end of the experiment is shown in the Table 3. Significant difference (p<0.05) was observed in the total lipid content between the treatments. Highest lipid content was observed in T4 (7.84±0.) group, while as the lowest lipid content was observed in the T1 (6.36±0.15) group.

**Total Ash**

Total ash content of the mixed zooplankton is shown in the Table 3. Significant difference (p<0.05) was observed in the total ash content between the treatments. Highest ash content was observed in T2 (15.57±0.29) group, while as the lowest ash content was observed in the T1 (7.44±0.28) group.

**Table 3: Mean±SD of Proximate Composition of Mixed zooplankton**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Treatments** | **Moisture** | **Protein** | **Ash** | **Lipid** |
| **T1** | 6.53±0.19b | 55.33±0.11b | 13.45±0.22b | 6.36±0.15a |
| **T2** | 7.61±0.31 d | 55.58±0.13bc | 15.57±0.29d | 6.78±0.12b |
| **T3** | 6.89±0.17c | 55.76±0.19c | 14.51±0.08c | 7.47±0.31c |
| **T4** | 4.48±0.15a | 47.41±0.23a | 7.44±0.28a | 7.84±0.21d |

Data were presented as mean ±SE (n=4). Values within the same column having different superscripts are significantly different (P<0.05)

**Growth parameters**

Growth parameters were evaluated throughout the trial period. The growth performance of *H. caudovitatus* juveniles in terms of body weight gain, percentage weight gain and specific growth rate (SGR) are shown in Table 4.

**Body weight gain**

The body weight of the experimental groups was recorded at 15 days interval. The body weight gain was expressed in milligram. The weight gain was found to be significantly different among the various treatment groups. Highest weight gain was recorded in treatment group T1 (86.6±0.8) and the lowest in the control group T0 (73.38±0.46). However no significant difference was observed in treatments groups T1.T2, T3 as shown in Table 4.

**Percentage weight gain:**

The Percentage weight gain among different treatments is shown in Table 4. Statistically significant difference was observed between percentage weight gain in control group with other experimental groups. Highest % weight gain was observed in T1 (33.89±0.33)and lowest was recorded in T0 (28.73±0.18). However, there was no significant difference observed among the experimental groups.

**Specific growth rate:**

Statistically significant difference was found between treatment groups with control group. The highest SGR was recorded in T1 (0.49±0.01)and the lowest was recorded in T0 (0.42±0.01) as shown in Table 4.

**Survival Rate (SR %):**

The SR (%) of different experimental groups is shown in the Table 4. No significant difference (p>0.05) was observed in the SR% between all treatment groups. Highest survival rate was recorded for T0 (92.5±1.29) group and the lowest was recorded for T2 (91.25±0.96) and T3 (91.25±0.96) group.

**Table 4 : Mean±SD of Growth performance in terms of WG, SGR, and SR of *H. caudovitatus* juveniles fed with different experimental diets**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Treatment** | **IW (mg)****(Mean ± SD)**  | **FW (mg)****(Mean ± SD)** | **WG (mg)****(Mean ± SD)** | **WG (%)****(Mean ± SD)** | **SGR****(Mean ± SD)** | **SR (%)****(Mean ± SD)** |
| **T1** | 255.38±0.18a | 328.76±0.48a | 73.38±0.46a | 28.73±0.18a | 0.42±0.01a | 92.5±1.29a |
| **T2** | 255.56±0.39a | 342.16±0.83b | 86.6±0.8b | 33.89±0.33b | 0.49±0.01b | 92±1.41a |
| **T3** | 255.53±0.36 a | 341.44±0.9b | 85.91±1.19b | 33.62±0.51b | 0.48±0.01b | 91.25±0.96a |
| **T4** | 255.61±0.38 a | 341.44±0.92b | 85.83±1.04b | 33.58±0.43b | 0.48±0.01b | 91.25±0.96a |

IW = Initial weight, FW = Final weight, WG = Weight gain, SGR = Specific Growth Rate, SR= Survival Rate.

Data were presented as mean±SD (n=4). Values within the same column having different superscripts are significantly different (P<0.05)

**Discussion**

**Zooplankton density**

In the present study, the density of mixed zooplankton was observed using different nutrient sources. It was found that on day 21 of the culture, poultry waste showed the highest population density (3266.50±6.85 individual/l) while yeast diet showed comparatively lower production (1110.00±3.55 individual/l). This could be attributed to the fact that poultry manure has fine particle size which allows rapid decomposition (Geiger and Turner, 1990) and releases soluble salts continuously resulting in high production of zooplankton (Sulochana Gaur and Gaur, 2007). The results are in accordance with Kangombe *et al.* (2006) who reported that, the production of zooplankton such as copepods, cladocerans (daphnia and moina) and rotifers was higher in ponds manured with poultry manure. *Nandeesha et al.* (1984) observed that, cladocerans were especially abundant in poultry manure treated waters. Previous researchers have also noted that waters fertilized with chicken manure produced copious amounts of copepods. Almost similar result were also observed for *D. pulex* by Damle (2007), where maximum density was observed using chicken dropping and the minimum density was observed in control (without feed supplement). Suresh Kumar (2000) and *Sivakumar* (2005) reported 5817/l and 6247/l respectively, in chicken manure and mixed algae mixture while as in our present study a zooplankton number of 3266.5/l was reported in case of poultry manure.

**Proximate composition analysis:**

In the present study, proximate composition analysis was also carried. The nutritional content of mixed zooplankton can fluctuate significantly based on their age and the specific dietary sources available in their environment. The study revealed significant difference in proximate composition of mixed zooplankton cultured on different nutrient sources. Higher protein content was found in mixed zooplankton cultured on poultry manure (47.33±0.11). The present study is in agreement with Creswell(1993) who reported 59.12% crude protein of Moina sp. fed with poultry manure. According to the research by Gogoi *et al.* 2016, the nutritional value of Moina sp. demonstrates significant variability depending on food availability. Gogoi *et al.* 2016 observed that the protein content remains relatively stable at an average of 50% on a dry weight basis. While as lowest protein (47.41±0.23) was recorded in mixed zooplankton cultured on yeast. The study is in agreement with Turcihan *et al* (2021). He reported that daphnia magna cultured on yeast has protein content (47.39 ± 0.05).

Many living organisms rely on specific dietary fatty acids to facilitate their somatic growth and overall well-being, as indicated in the study by *Masclaux et al.,* 2012. According to Hiltunen *et al.,* (2016) besides serving as energy storage compounds, certain fatty acids play crucial roles in various physiological processes. Monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) hold particular significance for aquatic organisms, often accumulating through their dietary intake. In the present study highest lipid content was recorded in Mixed Zooplankton fed on Yeast diet (7.84±0.21). The results are in agreement with Turcihan *et al.,* (2021). Daphnia fed on baker's yeast diet showed moderate level (7.33%) of total lipid accumulation in body. similar results were obtained by Cheban *et al.,* (2017) reported that Daphnia magna fed baker's yeast resulted higher total lipid accumulation in their body. While as the lowest crude lipid content was found in MOC Treatment(6.36±0.15). The highest ash content was recorded in Cow dung Treatments(15.57±0.29) while as the lowest was recorded in Yeast Treatments(7.44±0.28). similar results were obtained by Turcihan *et al*., (2021) who reported that lowest ash content of mixed zooplankton fed on baker’s yeast (6.83 ± 0.55).

**Growth parameters**

The present study was conducted to evaluate the effect of mixed zooplankton on the growth parameters of albino tetra juveniles. The juveniles fed with mixed zooplankton showed significant effect in growth performance, measured in terms of weight gain and specific growth rate than the juveniles fed on mixed zooplankton diet.

 Albino Tetra fed with mixed zooplankton exhibited highest weight gain (86.6±0.8) as compared to the control group. Similar results were reported by Degani (1991) also who found that juvenile *Trichogaster trichopterus* when fed with live feed grew faster than those fed formulated feed because of better palatability, high consumption rate and chemical composition. James & Sampath (2004) also reported that feeding live feed to *Xiphophorus helleri* resulted in higher growth rate as compared to artificial diets. Kanazawa (1991) also reported that live feed is preferable for larval nutrition than non-live feeds. The decrease in weight gain in control group can be attributed to the fact that feeding dry diets in the early larval stage leads to insufficient feed intake, digestion, and absorption due to the absence of a functional digestive system. Commercial dry feeds have satisfied the nutritional needs of fish larvae and have been manufactured in acceptable sizes using modern technology, the attractiveness and digestibility of these feeds for the larvae have not been totally enhanced (Koven *et al.* 2001). Furthermore, the present study is also in accordance with the earlier results reported by Ergün *et al.* (2010), who reported that higher dietary protein level resulted in higher mean weight gain and specific growth rate in freshwater ornamental fish, blue streak hap (*Labidochromis caeruleus*), than fish fed with lower dietary protein.

 In the present study, the specific growth rate (SGR) of albino tetra was found highest in all the treatments that were fed with mixed zooplankton as compared to control diet (T0). The results of the present study are in agreement with Janakiraman & Altaff (2014), who reported the higher SGR value of Koi carp *(Cyprinus carpio)* larvae fed live feed than those fed pelletized food. Similar results were observed by Janakiraman & Altaff (2015) who reported a higher specific growth rate in Gold fish (*Carassius auratus*) larvae fed on live feeds. Similar results were reported by Karga and Mandal (2017) in zebra fish when fed with mixed zooplankton.

**Conclusion**

In conclusion, the present study revealed good mixed zooplanktons grow in abundance when cultured on poultry waste in comparison to other nutrient diets. The body composition also varied with the nutrient source wherein zooplankton cultured on poultry waste has higher protein content. The study also revealed that zooplankton is an ideal source of food for juveniles improving growth parameters.

**Data availability statement**

Data generated or analysed during this study are provided in full within the published article.

**Disclaimer (Artificial intelligence)**

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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**COMPETING INTERESTS**

Authors have declared that no competing interests exist.

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