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Does Type of Food Affect The Prey Preferences of Three-Spined Sticklebacks?

Awad Lamin Hosan[1,*], Abdalbast H. Fadel[1], and Aisha Saleh M. Amar[2]

[1)] Department of Marine Resources, Faculty of Natural Resources and Environmental Sciences, Omar Al-Mukhtar University, Albayda, Libya.
[2)] Biology Department, Faculty of Education, University of Tripoli, Qaser Ben Gasher, Libya.

[*]**Corresponding author:** awad565@yahoo.com

هل يؤثر نوع الغذاء في تفضيلات الفريسة لأسماك ثلاثية الأشواك؟

عوض لامين حسين[1، *]، عبدالباسط فضيل[1]، عائشة صالح عامر[2]

[1)] قسم الموارد البحرية، كلية الموارد الطبيعية وعلوم البيئة، جامعة عمر المختار، البيضاء، ليبيا.
[2)] قسم الأحياء، كلية التربية، جامعة طرابلس، قصر بن غشير، ليبيا.

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Abstract

Three-spined sticklebacks (*Gasterosteus aculeatus*) are carnivorous feeders that can eat a wide variety of foods, although this animal's relatively small size limits its prey choice. An assessment of the diet and feeding patterns of fish can provide helpful insights. In this study, a three-spined sticklebacks model system was used to hypothesize that, whether a number of factors including the prey's visual appearance in terms of coloration, texture, size, shape, and movement capability of food can affect the preference for food, it might be expected that patterns for food consumption will alter. Therefore, changes in feeding behavior may be caused by the size, color, smell, visibility, and palatability.

The current study examined the effects of host nutrition on the preferences of diet. Here present the results of a diet type study in which fed sticklebacks on a diet of either *Artemia* spp., *Daphnia*, or bloodworms (*Chironomus* larvae). The results showed significant effects of preferences of diet. Further, fish showed preferred bloodworm and *Artemia* rather than *Daphnia.* In the total number of different bites consumed, the time (sec) spent by sticklebacks in various diet areas, and the number of visits by sticklebacks to the three diet areas. The results have demonstrated that the influence of factors such as variation in nutritional composition, color, size, coloration, texture, size, and shape as well as the movement of nutrients in the diet has a significant effect on the preferences of diet the fish in the study.

Keywords: *Prey preferences, Three-spined sticklebacks, Host nutrition, Behavior.*

الملخص

تعتبر أسماك ثلاثي الأشواك أنموذج ممتاز من خلاله يتم دراسة أسئلة حول النظم الأيكولوجية للأسماك. فالأسماك ثلاثية الأشواك هي أسماك حيوانية التغذية يمكنها تناول مجموعة واسعة من الأغذية، على الرغم من أن حجمها الصغير نسبيا يحد من قدرتها على اختيار نوعية الغذاء، فتقييم النظام الغذائي وأنماط الغذاء يعطى رؤية مفيدة حول السلوك الغذائي للأسماك. في هذه الدراسة تم استخدام ثلاثة أنواع من مكونات الأغذية الطبيعية لأسماك أبوشوكة لفرضية أن ما إذا كان عدد العوامل بما في ذلك المظهر البصري واللون والملمس والحجم والشكل والقدرة على الحركة يمكن أن يؤثر في سلوك تفضيل الغذاء للأسماك. في هذه الدراسة تم تغذية أسماك أبوشوكة على ثلاثة انواع من الغذاء الطبيعي وهي الأرتيميا والدافنيا ودودة الدم (يرقه حشرات ثنائية الاجنحة) لدراسة سلوك تفضيل الغذاء لأسماك أبوشوكة.

أظهرت النتائج ان هناك فروق معنوية في التفضيل بين أنواع الغذاء، حيث أظهرت الأسماك تفضيل دودة الدم (يرقات الحشرات ثنائية الأجنحة) الأرتيميا بدلاً من الدافنيا وكان هذا الاختلاف في عدد لقيمات الطعام والوقت الذي تقضيه الأسماك

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بالثواني في منطقة الغذاء وكذلك عدد الزيارات إلى منطقة الغذاء. كما أظهرت نتائج الدراسة أن هنالك بعض العوامل مثل التباين في تركيب الغذاء والحجم واللون والملمس والشكل والرائحة في النظام الغذائي والتي لها تأثير على تفضيلات النظام الغذائي للأسماك.

الكلمات الدالة: تفضيلات الفريسة، أسماك ثلاثية الأشواك، تغذية المضيف، السلوك.

1. Introduction

Three-spined sticklebacks are carnivorous feeders that can eat a wide variety of foods (Wootton, 1976a) although the relatively small size of this animal limits its choice of prey. An assessment of the diet and feeding patterns of fish can provide useful insights. This process generally comprises undertaking an analysis of fish stomach contents as an indicator of food availability, as well as monitoring habitual feeding behavior (Andrian, 1996).

The three-spined stickleback *Gasterosteus aculeatus* is a small teleost fish belonging to the Family Gasterosteidae (Class: Actinopterygii, Super-Order: Teleostei, Order: Mesichthyes) (Heuts, 1947). It is a small fish length between 3 and 8 cm (Falter, 1987) and has the special characteristic that it can be identified by its three sharp spines on the back and in front of the dorsal fin.

The dietary choice of sticklebacks is influenced by the way, in which, they select their prey. In this regard, Wootton (1984b) identifies features such as size, type of movement, and perceived color variations as being strongly correlated with the capacity to locate their prey. The diet of three-spined sticklebacks has been the focus of many studies. (Wootton, 1976b; and Allen and Wootton, 1984) number of factors including the prey's visual appearance in terms of coloration, texture, size, and shape, as well as its movement capability Ibrahim (1988), and this constitutes a wide variety of zooplankton, especially copepods larvae and the pupae of chironomids (Hynes, 1950). Juveniles at one week post-fertilization begin to feed (*Artemia*, nauplii), whilst adults can eat a variety of foods including live feed crustaceans such as Cyclops and *Daphnia* sp., chironomid larvae, tubifex, Asellus, and Duphniu. Wootton (1984b), and even other stickleback eggs (Allen and Wootton, 1984).

Eating habits change according to the season in three-spined stickleback (Allen and Wootton, 1984). Sticklebacks have the capacity to feed on surface-floating food, such as commercial dried fish, or on food bottom habitats such as tubificid and enchytraeid (Wootton, 1976a). The dietary nutrition of three-spined sticklebacks has been investigated in many studies (Maitland, 1965). Three-spined sticklebacks have a varied diet (Barber, 2013). and are characterized by their small size, making it abide in the food size Sticklebacks have large eyes so that they can detect prey from approximately 44 cm away in clear water and 10 cm in muddy water (Moore and Moore, 1976); they are carnivorous and eat a range-wide of small animals including crustaceans, the larvae of chironomids, and fish eggs; they even consume stickleback eggs (Wootton, 1976b). Prey features could be one of the key factors influencing the food consumption and growth (Sun *et al*., 2010) of the fish; in this regard, movement, color contrast, and size are particular physical characteristics that have been demonstrated as closely linked to the detection of prey (Wootton, 1984a). The variation in prey availabilities

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can highly influence the growth of three-spined sticklebacks (Hoxmeier *et al*., 2004); Bloodworm and *Daphnia* were selected because these species are found in the natural environments of sticklebacks (Webster *et al*., 2007) The purpose of this study is to elucidate the preferences of three-spined sticklebacks for certain prey types.

1.1. Aims of The Study

This study describes an experimental study investigating the effect of qualitative differences in prey choice on food preference type. The experiment investigates the proximate factors affecting selection among different prey types by three-spined sticklebacks and the description of three-spined sticklebacks' diet, as well as the preferences of three-spined sticklebacks for certain prey types, the type of food and color could affect the dietary preferences or selectiveness of fish; therefore, the study aims to understand whether this food type changes the dietary preferences of fish in terms of the quality of food they select. This was investigated by undertaking replicated experimental fish diet preference tests.

2. Materials and Methods

2.1. Fish Collection and Husbandry

The experimental fish were offspring of wild, naturally-spawning parents. Juvenile, young-of-the-year yearling (0+) sticklebacks were collected using hand nets and minnow traps from the River Soar at Abbey Park, Leicester (N52:38:42, W1:07:49) in early. These fish were transferred to the aquarium facility of the Department of Biology at the University of Leicester.

2.2. Tank Preference Trials

The experimental diet preference trials were undertaken in small aquaria, measuring 20 cm×35 cm×20 cm (length×width×height), which were filled to a depth of 30 cm, and divided into two equal compartments (a resting compartment and a test compartment) measuring 20×17.5×20 cm (length×width×height) which divided by plastic divider. To minimize disturbance, the aquaria were concealed in housings made of black plastic that covered the four sides. Each aquarium was supplied with compressed air which was delivered through an air stone and a plastic plant for shelter as well as gravel. Three watch glasses were placed in the aquaria on one side (the test compartment) thereafter, the fish were placed individually in the aquaria.

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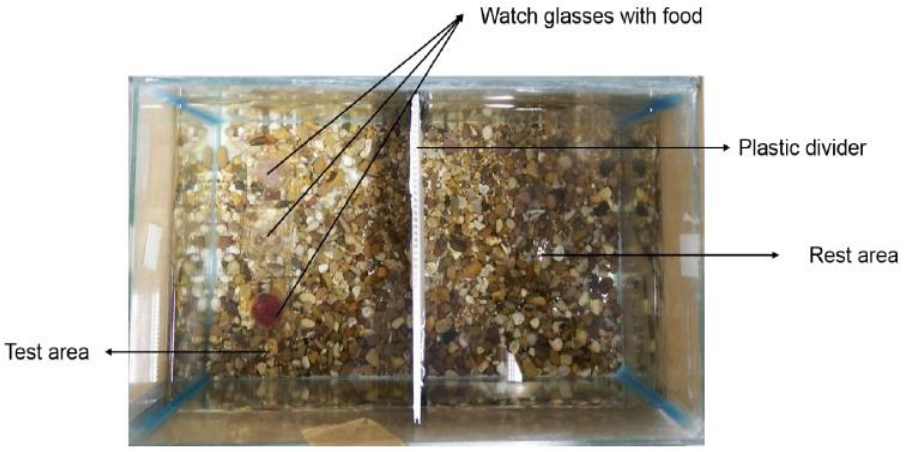

Figure 1. Experimental test tank (Rest area, Test area with three glasses dishes with experimental food, and the white plastic divider in the middle).

2.3. Experimental Diet

Three commercially available and pre-frozen food types were used to feed the fish in this project: adult water fleas *Daphnia* sp., adult brine shrimps *Artemia* sp., and bloodworms, which are larvae of *Chironomus* sp. Midges. All frozen foods were purchased from commercial suppliers and manufactured by 3F Fish Food (The Netherlands, www.frozenfish-food.nl).

2.4. Fish Collection and Husbandry

The experimental fish were offspring of wild, naturally-spawning parents. Juvenile, young-of-the-year yearling (0+) sticklebacks were collected using hand nets and minnow traps from the River Soar at Abbey Park, Leicester (N52:38:42, W1:07:49). These fish were transferred to the aquarium facility of the Department of Biology at the University of Leicester. The fish were inspected for characteristic signs of *S. solidus* infection (Barber, 1997), and their fins were inspected for any visible ectoparasites.

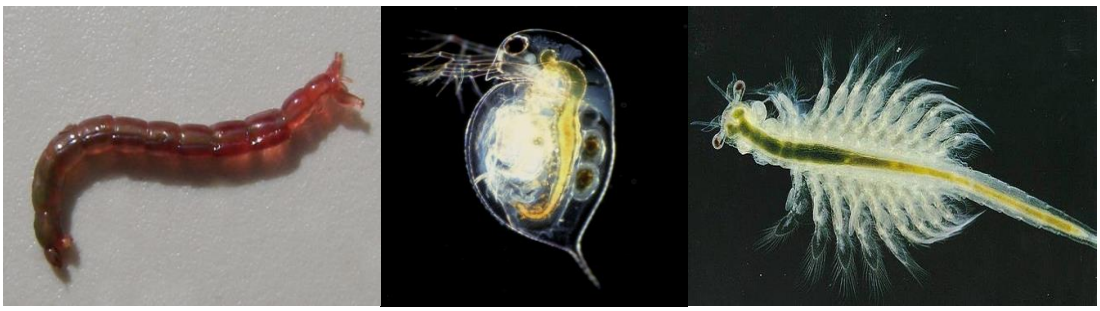

Figure 2. Basic nutritional of three different food type used in experiment data provided by the food manufacture (*Artemia*, *Daphnia*, and Bloodworm).

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Table 1. Basic nutritional composition of the three different types of commercially available frozen food used in the experiments.

Constituent	*Artemia*	*Chironomus* sp.	*Daphnia* sp.
Crude Protein	5%	5%	2.4%
Crude fat	1%	1%	0.7%
Crude fiber	0.9%	0.9%	0.3%
Ash	0.8%	0.8%	0.7%
Moisture	92.2%	92.0%	96.3%

2.5. Experimental Procedure

Each test fish was deprived of food for 24 hrs., to increase appetite and reduce food residue in the gut (standardization of hunger). Immediately prior to the preferences trials, individual fish were moved from their holding tank to the test tank where they left to acclimatize for 60 minutes. Following the settling period prior to the experiment, watch glasses were introduced into the experimental tanks via pipette. Each food type covered a similar within each watch glass, to avoid food partially or complete food depletion, and fish were fed libitum. After 10 min. of acclimation, the divider was removed giving the fish access to the feeding compartment. A megapixel USB webcam was fixed above the tanks and connected to a computer, enabling the movements and behavior of the fish to be recorded without disturbing the fish. The observations of each fish were continued for 10 minutes. Individual prey items were clearly visible on the screen; all experimental trials were video-taped, and each trial replicated three times per fish, with the experiment being conducted for 18 fish in total.

2.6. Video Analysis

The diet preferences of each fish were recorded during each round on three separate occasions, with the physical position of the food type being switched each time.

During each diet preference trial, the following variables were recorded: the total number of seconds spent by the fish in the rest area; the proportion of time spent in each food type "zone"; the total number of prey items taken by the fish; the proportion of bites directed to each food type.

3. Results

The preference shown by sticklebacks for various prey is presented in Figures (3-5). Sticklebacks preferred Bloodworm, *Artemia*, and *Daphnia*; in this case, their preference led them to the more nutritious prey items. an overall preference for *Artemia* and Bloodworm rather than *Daphnia*. In the total number of different bites consumed, the time (sec) spent by sticklebacks in various diet areas, and the number of visits by sticklebacks to the three diet areas.

To test for the effect of a number of bites of prey eaten by sticklebacks in three replicates Subsequent statistical tests showed that the total number of bites versus diet was significant in the case of chironomid larvae and *Daphnia*, but not in other cases, (one-way ANOVA, $F_{2,53}=6.04$, $P=0.004$) post hoc test Tukey was then used to identify significant

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differences between treatment, and revealed that a number of the bite of prey were significantly higher in bloodworm-fed fish than fish fed in *Daphnia* (P<0.05).

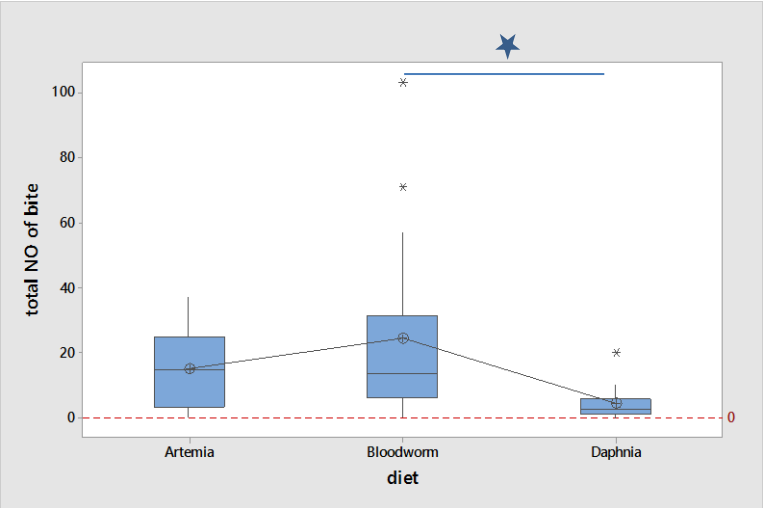

Figure 3. The total number of different bites (*Artemia*, Bloodworm, and *Daphnia*) consumed by sticklebacks

The time spent by the fish in each diet zone and handling each prey type are presented in Figure (2), the time spent was longer for bloodworm than for either *Artemia* or *Daphnia*. The median time was lower in the case of *Daphnia* than *Artemia* and Chironomid larvae (one-way ANOVA, F2, 49=4.44, P=0.017).

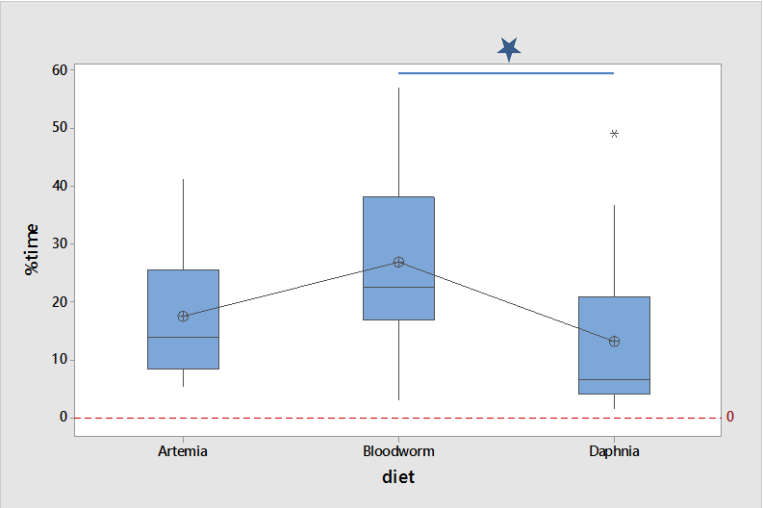

Figure 4. The time (sec) spent by sticklebacks in various diet areas (*Artemia*, Bloodworm, and *Daphnia*)

There was a significant difference in the number of prey visits (*Artemia*, Bloodworm, and *Daphnia*) by the sticklebacks. A high number of visits by the fish was observed in the diet

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areas for Artemia and Bloodworm (these differences were not significant), while the number of visits was lower for *Daphnia* than for either *Artemia* or Chironomid larvae (one-way ANOVA, F2,35=5.43, P=0.017).

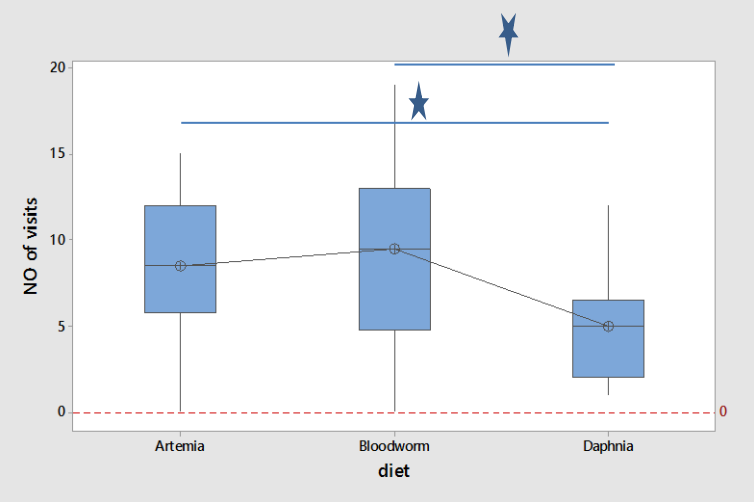

Figure 5. The number of visits by sticklebacks to the three diet areas.

4. Discussion

The type of nutrient used in the test of dietary preference of three-spined sticklebacks used in this study consisted of aquatic crustaceans (*Artemia*), the larvae of *chironomids* (bloodworms) *Daphnia*. Bloodworms were selected because they have previously been used in preference tests on sticklebacks (Barber and Huntingford, 1995). Also, they were used because they are routinely fed to fish in aquaria, and are natural components of stickleback diets, and were found to facilitate fish growth and health in other experiments.

Meanwhile, *Artemia* was chosen because they have previously been used in feeding the fry of three-spined sticklebacks Hahlbeck *et al.* (2004), they represent an alternative prey source replicating other natural components of the fish's diet.

Daphnia was chosen because this is known to be the major sticklebacks. Daphnia is also considered to be a key source of protein for fish (Ibrahim, 1988; and Bogatova *et al.*, 1971).

The present investigation was designed to discover the food preferences of three-spined sticklebacks in relation to prey profitability, the total number of different bites, the median time (sec) spent by fish in the various diet areas, and the number of visits thereto. The experiments have shown that there are clear differences in feeding behavior between the three types of diet (*Artemia*, bloodworm, and *Daphnia*) with an overall preference for *Artemia* and Bloodworm rather than *Daphnia*. However, the observed differences between *Artemia* and Bloodworm in this study were not significant; the higher consumption of bloodworm may be due to the fact that three-spined sticklebacks prefer prey whose color is discernible (Popham, 1966). The color of the Bloodworm is red, which derives from the red pigment of blood

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hemoglobin (Armitage *et al*., 2012). These results further support the idea that three-spined sticklebacks prefer prey whose color is conspicuous. Another possible explanation for this is that prey size also affects food choice; (Werner and Hall, 1974) suggested that a large size could be eminently useful in selecting prey. The average size of *Artemia* was 10-15 mm (Lavens and Sorgeloos, 1993) while the *Daphnia* were on average more than five times smaller than Artemia; ranging between 2.06 to 1.75 mm (Infante and Abella, 1985). This finding confirms the suggestion of large size selection by fish.

The findings of this research also demonstrate that the preferences towards *Artemia* decline with time through the study, the reason behind this observation is not obvious. A possible explanation for this might be that *Artemia* is a genus of Crustacea (Nunes *et al*., 2006; and Triantaphyllidis *et al*., 1998). Therefore, the exoskeleton of this species is approximately 50-80% chitin (Shiau and Yu, 1999). The main structure of the exoskeleton is chitin (Talens-Perales *et al*., 2017). The carnivorous fish lack the carbohydrate-degrading enzymes such as amylase and glucanase, therefore, they probably consume few amounts of chitin (German *et al*., 2010).

In this study, tests of prey preference were carried out on individual fish in isolation. This contrasts with the behavior of three-spined sticklebacks in the wild which live in large shoals (Poulin and FitzGerald, 1989). Compared to individual fish, shoaling fish were observed to be quicker in detecting limited sources of food (Pitcher, 1986). Therefore, the reduced foraging I observed is likely to be due to keeping fish in isolation during experiments.

One additional justification for the same can be given by the fact that fish have flexible eating habits that are subject to food availability and they turn to consume food that is available in abundance in the environment around them (Azevedo, 1972). Three-spined sticklebacks showed a tendency to select bloodworm as their preferred diet. The present investigation was designed to discover the food preferences of three-spined sticklebacks in relation to prey profitability. Experiments have shown that there are clear differences in feeding behavior between the three types of diet (*Artemia*, Bloodworm, and *Daphnia*) with an overall preference for Bloodworm.

The higher selection of Bloodworm may be due to the fact that three-spined sticklebacks prefer prey whose color is discernible Popham (1966); the color of Bloodworm is red as a result of the red pigment of blood hemoglobin (Armitage *et al*., 2012). In support of the present study, similar results have been reported for Thinlip mullet (*Liza ramada*) larvae fed a diet with different colored food. The best performance and survival were achieved in fish fed on dark-colored diets (red, dark blue, and dark brown) (El-Sayed and El-Ghobashy, 2011).

However, the *Artemia* and offered to three-spined sticklebacks was of the same color as *Daphnia*, as it was colored using commercial food colorants. Therefore, this suggests that three-spined sticklebacks may not be visual feeders and the food color was not a factor in food preference. Nevertheless, the influence of the color of diet in preference of fish might be controversial. Jegede and Olusola (2010) demonstrated that tilapia zillii fed feeds with different colors showed better growth and feed efficiency with yellow and light-green food than those fed on dark-colored diets. Conversely, Nile tilapia larvae, for example, are visual

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feeders that favor food that is dark in color, although their fingerlings will consume food of any color (El-Sayed *et al*., 2013). In a previous study by (Johannesen *et al*., 2012), three-spined sticklebacks use their sense of smell to find food, especially when visibility is poor as in turbid water. Conversely, in the indigenous habitat, augmented algal turbidity results in a higher dependence on olfactory signals in the mating process in contrast with clear waters (Heuschele *et al*., 2009). In addition, other non-visual characteristics such as size, form, and palatability are considered to be some of the important factors when foraging for food (Villamizar *et al*., 2009; Wootton, 1984b; and Gibson & Hirst, 1955). This is supported by earlier studies; for example, (Gibson and Hirst, 1955), found that the foraging behavior of three-spined sticklebacks showed a preferred relation to size, where the fish select larger, rather than smaller, prey.

Multiple investigations concur with the contention and designated that supplementary physiological procedures, excluding the vision-feeding relationship, assume a greater role in encouraging the behavior of juvenile and adult-developed fish. Kallayil *et al*. (2003) it was discovered that even in non-sensory surroundings, the foraging behavior of cod could be artificially stimulated by utilizing bait odor.

However, in this experiments, sticklebacks were selecting prey according to physical appearance, over the study period this experiment was conducted in acceptable circumstances, and to aquarium maintenance, water was changed regularly. In results of this study, the three-spined sticklebacks showed a tendency to select Bloodworm. Results suggest that three-spined stickleback relied more on olfactory cues than visual in clear water, although this may not always compensate for the reduction in visual cue availability caused by turbidity. Presented results suggest that sticklebacks, use their olfactory sense to indicate their prey location and their eyes for discovering sites.

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