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First attempt to fill gaps in the feeding of the axolotl

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ABSTRACT

Objective: To determinate the attractability of four oils, fish, chicken, krill, and red crab (*Pleuroncodes planipes*) for *Ambystoma mexicanum* juveniles, evaluating their feeding behavior using a Y aquarium.

Design/Methodology/Approach: Ten axolotls were used per test, fasted for 48 h. Gels with oils were prepared using gelatin and poured into petri dishes and refrigerated until gelation. A recording of the test was made using two video cameras. In the feed chamber the gelled oil was placed and allowed to stand for 15 min. On the other chamber a gelled disk with no other ingredient than gelatin and water was placed. The video recording began once the 15 min of gel permanence had finished, removing the barrier so that the axolotls could move through the rest of the aquarium. All tests were carried out with a recording time of 30 min.

Results: Fish oil demonstrated a lower attraction effect compared to krill, red crab and chicken oils ($P<0.05$), while chicken oil (30.00 ± 1.73) doubled the attractive effect of krill oil (16.00 ± 1.00).

Limitations of the study/implications: It was necessary to condition a room with controlled environmental temperature for *A. mexicanum* (18 ± 1 °C).

Findings/Conclusions: Krill and chicken oil are good feeding effectors for *A. mexicanum* causing positive feeding behavior. The use of chicken oil is desirable because of its low cost compared to krill.

Key words: axolotl; chicken; krill; chemostimulants.

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INTRODUCTION

Ambystoma mexicanum (Shaw & Nodder, 1798) is an endemic species from Lake Xochimilco, Mexico, that is endangered in its natural habitat. Its population has decreased considerably, it is estimated that today there are less than 35 animals per square kilometer

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(Vance, 2017). Due to its biological characteristics in which it stands out the ability to regenerate almost any part of its body, it has been used as a biological model for years on labs, and it is well appreciated as an exotic pet being distributed in great part of the world. Because of that, *A. mexicanum* has been reproduced largely in captivity which has helped to conserve the species (Gresens, 2004; Vance, 2017).

Despite the high degree of scientific and ornamental interest of the Mexican axolotl (*Ambystoma mexicanum*), studies on its feeding and nutrition in captivity, are practically nil. Keeping them in captivity can be expensive because their diet is mainly made up of live food. In the early larval stages, because their gape size is really small, the live feed has an important role in the development of *A. mexicanum* (Chaparro-Herrera *et al.*, 2011) and other species of the same genre like *A. maculatum* (Freda, 1983), *A. altamiranoi* (Lemos-Espinal *et al.*, 2015), *A. granulosum* (Sarma *et al.*, 2017) being zooplankton their main source of nutrients. Gresens (2004) mentions that once axolotls reach 5 cm, they can begin to receive food in pellets in their diet. However, there is the possibility of them not being accepted because they seem unattractive.

Aquatic organisms can detect compounds (amino acids, sugars, fatty acids) dissolved in the environment and identifying them as possible food using different detection methods (Nolasco-Soria, 2014; Villarreal-Cavazos *et al.*, 2017). Axolotls have a vomeronasal system and an olfactory system. It is believed that the former is used to detect large molecules, especially pheromones for reproduction compared to the olfactory system, which detects smaller molecules (Eisthen & Park, 2005).

It is easier to achieve the change in diet from live feeding to pellets using soft pellets, which sink to the bottom and have a strong smell, as this would help the attractability of the feeding increasing the chances of success (Gresens, 2004).

The production of balanced feed aimed at aquaculture species must consider the attractability. Adding attractant compounds is supposed to increase food detectability and intake, optimize feed conversion rates, reduce waste, and help the transition from live food to pelletes (Dempsey, 1978; Mendoza, 1999; Gresens, 2004; Montemayor-Leal *et al.*, 2005). Among aquafeed producers, the ingredients most used as attractants are fish meals, soluble extracts from marine fish, shrimp head and squid meals, or squid liver oil (Mendoza *et al.*, 1999). In order to measure the attractant power of a molecule, ingredient or food for a determined species, attractability tests are carried out *in vivo* at the laboratory level or in experimental or commercial feeding bioassays.

To the best of our knowledge, the attractability of feeds or their ingredients for *A. mexicanum* has not been formally subjected to behavioral bioassays, nor has feeding behavior been described. Contrarily, some extensive studies have been carried out on the subject for fish (Oikawa & March, 1997; Kolkovski *et al.*, 2000; Barroso *et al.*, 2013; Sanches-Alves *et al.*, 2019) and crustaceans (Mendoza *et al.*, 1997; Cruz-Suárez *et al.*, 2000; Montemayor-Leal *et al.*, 2005; Montoya-Martínez *et al.*, 2018).

Therefore, the objective of this study was to determine the feeding behavior of these animals by including some oils of animal origin as additives to increase the attractability of feeds, helping to contribute on the design of specific diets for *A. mexicanum*.

MATERIALS AND METHODS

Attractability bioassays were carried out using oils from alternative sources: krill oil (SimiKrill®), red crab (*Pleuroncodes planipes*) (obtained from the Centro de Investigaciones Biológicas del Noroeste, SC), chicken oil (Proteínas Marinas y Agropecuarias, S.A. de C.V.®) and fish oil (Proteínas Marinas y Agropecuarias, S.A. de C.V.®) of conventional use in the formulation of commercial feed for aquatic organisms. The juveniles of *A. mexicanum* were donated by the AXOS-PIMVS production center located in Tepic, Mexico (Bahía de Banderas 62, Lomas de la Cruz, 63037). The smallest axolotls (12.2±1.0 cm) that the producer had, accustomed to feeding with pellets, were selected. The animals were transported in individual bags to the Laboratory for Water Quality and Experimental Aquaculture (LACUIC), belonging to the University of Guadalajara, located in Puerto Vallarta, Jalisco. After an acclimatization period of 30 minutes, they were placed in the laboratory with a controlled environment to guarantee a stable temperature of 18±1 °C. They were placed randomnly in two 300-liter ponds and fed high-protein pellets for cold water fish (Silvercup®, 42% protein, 15% fat) ad libitum during the maintenance period prior to the bioassays. For this study, gels with oils were prepared using the method proposed by Nolasco-Soria (2014). Gelatin was weighed and pre-hydrated with cold water. Afterwards, 5 g of the oil to be evaluated was weighed and 20 mL of water was added and stirred using a heating plate. The gelatin (pre-hydrated and heated in the microwave for 8 seconds) was then added. The homogenized mixture (15 mL) was poured into petri dishes and refrigerated until gelation. For the evaluation, a Y-shaped aquarium (Figure 1. Nolasco-Soria, 2014; Montoya-Martínez *et al*., 2018) made with acrylic material was used. For the tests, the aquarium was filled with water (6.5 cm deep) at the same temperature as the maintenance tanks. After each test the aquarium was washed with detergent and neatly rinsed with tap water to discard remains of the used oil. Ten axolotls were used per test, fasted for 48 hours, placed in the R region

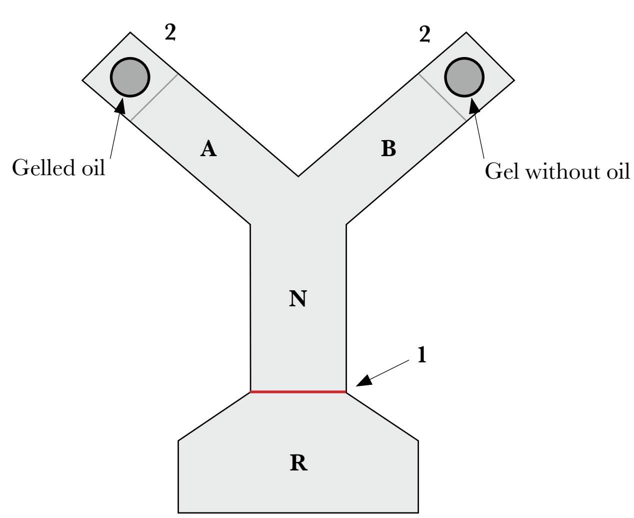

Figure 1. Y system for attractability tests (Nolasco-Soria, 2014; Montoya-Martínez *et al*., 2018). R: Acclimation chamber, N: Transit zone, A and B: Arms, 1: Barrier, 2: Feed chambers.

of the aquarium, and allowed to acclimate for one hour. Between the R and N regions, a barrier was placed to prevent the axolotls from entering the rest of the system. This barrier is a transparent acrylic square, which snaps into the body of the aquarium so that it does not allow the axolotls to perceive the odor during acclimatization. In the feed chamber of regions A or B (arms), alternating between the tests, the gelled oil was placed and allowed to stand for 15 minutes. On the other chamber a gelled disk with no other ingredient than gelatin and water was placed.

A recording of the test was made using two video cameras (GoPro®). One of these was placed framing both arms of the aquarium and a fraction of the "N" zone, and the second one was installed framing the side of the feeding chamber with the gelled oil. The video recording began once the 15 minutes of gel permanence had finished, removing the barrier so that the axolotls could move through the rest of the aquarium (the room was abandoned to avoid human interference). All tests were carried out with a recording time of 30 min, within the same closed room with controlled temperature. The axolotls used in the bioassays were left in isolation for at least 48 hours before being used again for another test. They were fed *ad libitum* during isolation time.

After having performed all the tests in triplicate, the videos were reviewed and the following indices were analyzed: i) time (s) of the first admission (it is the time required for the first axolotl to enter the feeding chamber of the gel arm once the gate was removed); ii) number of axolotls that entered the gel arm; iii) time of permanence of the axolotl in the feeding chamber (s); iv) number of axolotls that entered the feeding chamber remaining close to the gel; v) number of axolotls that fed on the gel.

All bioassays were carried out in compliance with animal welfare protocols. The animals were not subjected to any kind of suffering in a programmed or innocent way. Once the study was completed, the axolotls were relocated to their original reservoirs under optimal maintenance conditions.

A Kolmogorov-Smirnov test was performed to assure the normality and homoscedasticity of the obtained data. Subsequently an ANOVA analysis was performed. In cases where significant differences were found between treatments ($P<0.05$), the Tukey test was performed for comparison. These tests were conducted using SigmaPlot software.

RESULTS AND DISCUSSION

The statistical tests indicate that there were significant differences ($P<0.05$) in the number of organisms that entered the arm containing the gel (far orientation) and number of organisms that entered the feeding chamber remaining close to the gel (close orientation). The response to the fish oil stimulus was lower compared to that of krill, red crab and chicken oils, despite fish oil being the most commonly used for aquatic animal feed. Between chicken oil and krill oil, there were no differences in the number of individuals that accessed the arm with the gel, although the latter attracted twice as many individuals that entered the feeding chamber while remaining close to the gel (Table 1).

Table 1. Average and standard deviation of the effect on feeding behavior of *A. mexicanum* induced by red crab, krill and chicken oils, in 30 min tests.

	Red crab oil	**Krill oil**	**Chicken oil**	**Fish oil**
Time to first admission (sec)	196±129 [a]	284±23[a]	241±72[a]	269±62[a]
Number of organisms that entered the arm with gel	16.67±3.79[a]	15.00±2.00[a]	17.67±1.53[a]	7.67±0.58[b]
Time of the stay in the feeding chamber (sec)	1719±274[a]	2942±316[a]	1994±176[a]	1941±847[a]
Number of organisms that entered the feeding chamber	18.33±2.08[a]	16.00±1.00[a]	30.00±1.73[b]	13.67±4.16[a]
Number of organisms that fed of the gel	4.67±4.619[a]	6.33±0.58[a]	6.67±0.57[a]	3.33±4.16[a]

* Means with different letters in the same line are significantly different according to Tukey's test ($P<0.05$).

This may mean that chicken oil and krill oil are good attractants, but when axolotls are near the stimulus, krill oil is the most striking. In addition, despite the fact that no statistical differences were found between treatments in the number of axolotls that fed on the gels, it was observed that chicken and krill oils are good stimulants for the ingestion of the gel and encourage the axolotls to keep feeding. This was evidenced by the higher consumption rate of these gels observed at the end of each test. It was observed that the axolotls explored the arm with the gelled disc without additives, but did not remain in it, so data recording was not considered. This exploration was rapid, which suggested the search for the origin of the attractant molecules, which in fact was in the other arm and in which they entered and remained at the different times that were recorded.

Since no study was found on the attractability of compounds, ingredients, or foods in the family Ambystomatidae (and probably not in the entire order Caudata), the interpretation of the eating behavior responses was based on the generalized behavior pattern for all type of species described by Lindstedt (1971). This author indicates that there are different chemical effectors that can positively or negatively affect animals and are classified according to their effect on their behavior patterns (Table 2). By observing the videos, it is possible to identify the four behavioral responses, starting with the orientation (far away) towards the food, movement for orientation close to the food, start of feeding and continued intake until its completion. For this reason, the "Y" -shaped aquarium can be considered suitable for obtaining favorable results in the attractability tests with *A. mexicanum*. Unfortunately, studies on nutrition in amphibians are scarce as for many species there are no specific commercial feeds, so pelleted feeds designed for other species need be used (Domínguez, 2005). Most of the research carried out with

Table 2. Types of chemical stimuli that affect the response pattern of feeding behavior on all type of species. Beck (1965) modified by Lindstedt (1971).

Response	**Evoking stimulus**	
	Positive	**Negative**
Orientation (distant)	Attractant	Repellent
Orientation (close)	Arrestant	Repellent
Initiation of feeding	Incitant	Suppressant
Continuation of feeding	Stimulant	Deterrent

balanced feeds for tadpoles is focused on finding the optimal protein requirements and their effects on development (Carmona-Osalde *et al.*, 1996; Martins *et al.*, 2013; Pinto *et al.*, 2015; Godome *et al.*, 2019), without considering the attractiveness and palatability of the ingredients used.

However, there are several studies evaluating the attractability or responses to food effectors (term called for attractants, initiators, and stimulants), ingredients, foods, commercial attractants, animal and plant extracts, pheromones, etc. in other aquatic animals. In studies carried out with fish (Oikawa & March, 1997; Kolkovski *et al.*, 2000; Barroso *et al.*, 2013; Sanches-Alves *et al.*, 2019) and crustaceans (Costero and Meyers, 1993; Jaime-Ceballos, 2007; Suresh *et al.*, 2011; Sacristán *et al.*, 2014; Montoya-Martínez *et al.*, 2018) the authors seek to increase the attractability of the food supplied to improve intake and therefore obtain better results in food consumption, growth, development and survival of organisms.

In general, the chemoreceptors of aquatic organisms are sensitive to low molecular weight water soluble chemicals (Lee & Meyers, 1996; Nunes *et al.*, 2006). Ingredients originating from aquatic animals (such as flour, soluble fish, mollusks, or crustaceans) have a high content of these compounds, which is why they are considered as excellent attractants (Smith *et al.*, 2005; Ali *et al.*, 2007), while by-products of terrestrial animal origin (such as oils and flours from poultry by-products) contain lower levels of them. Therefore, they are considered to have lower attractability and palatability, but few studies have been carried out to confirm this fact (Suresh *et al.*, 2011). In this context some studies have shown in fish that hydrolyzed krill is a good attractant, since when used as a food additive it increases intake and growth in *Oncorhynchus mykiss* (Oikawa & March, 1997) and in *Perca flavescens*, *Vitreous Stizostedion* and *Coregonus clupeaformis* (Kolkovski *et al.*, 2000). However, similar results have been found with the use of hydrolyzed poultry protein (Sanches-Alves *et al.*, 2019) when including it as a food additive for *Oreochromis niloticus*. In crustaceans (*Litopenaeus stylirostris*), Suresh *et al.* (2011) found that poultry by-product meals showed the highest attractability, but the highest palatability was obtained by krill meal. Smith *et al.* (2005) reported that the inclusion of krill increased feed consumption in *Penaeus monodon* and improved growth (Williams *et al.*, 2005), with similar results obtained in *Penaeus vannamei* (Córdova-Murueta & García-Carreño, 2002).

CONCLUSIONS

In conclusion, krill and chicken oil are good feeding effectors for *A. mexicanum*, causing positive feeding behavior. Based on these results, it is suggested to carry out nutritional bioassays using oils as additives in experimental diets to evaluate the increase in the attractability of foods directed to the species, and to contribute to the design of specific diets that allow better development and health status of axolotls. These results provide essential knowledge to improve the quality of nutrition of this endangered endemic species from Mexico, in captivity conditions, which would allow the design of more effective reproduction and assisted repopulation plans.

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