A STUDY OF CHEMOSENSORY SYSTEMS IN FRESHWATER AND MARINE FISHES OF VIETNAM

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1. INTRODUCTION

Chemosensory systems are involved in regulation of the most important behavioral patterns in fish. Olfaction provides fish with information about presence of predators and partners in the group, allows fish to search for food, home territory and migration routes, to distinguish conspecifics and their population, hierarchical position, sex and readiness of specimens to spawn [1]. Based on taste system fish evaluate the food organisms and feed with appropriate ones [2]. Most of the information about chemosensory systems concerns fish inhabited water bodies of the temperate zone. Tropical fish, especially coral reef fish, are much less studied despite the richness of the ichthyofauna in low latitudes. Systematic studies of the chemosensory systems of Vietnamese fish have not previously been carried out. The aim of the work that has been carried out in recent years in the Coastal branch of Joint Vietnam-Russia Tropical Science and Technology Research Centre within the framework of the project Ecolan 3.2 - task 2 was to study the morphology of the olfactory organ in fish, the most common in freshwater and marine waters of Vietnam. The aim of the work also included the study of taste reception and its role in the regulation of trophic relationships between fish and invertebrates of coral reefs.

2. MATEARIALS AND METHODS

The study of the morphology of the main structures of the olfactory organ was analyzed in fish caught with a netting gear in the coral reefs of the Nha Trang Bay (the East Sea), afterward fixed in 10% formalin and transferred to 70% ethanol. The examination was conducted using a binocular MBS-1 microscope. An ocular micrometer was applied for the measurements, and Levenhuk M500 Base digital camera was used to take photographs.

The taste perception of fish was studied using a method based on the behavioral response of fish to pellets made from agar-agar gel (2%) containing chemicals or water extract of various marine animals and plants (300 mg/ml). Extracts and solutions were prepared on the basis of seawater. The fish were placed in aquariums combined into a closed system with water circulation (29 ± 1°C) through a biofilter. The consumption of grasped pellets, the total number of grasps in a trial, and the pellet retention time in fish mouth were recorded.

3. RESULTS AND DISCUSSION

The olfactory organ in *Abudefduf sexfasciatus* and *A. vaigiensis*, like in many damselfish (Pomacentridae), has one nostril. In *A. sexfasciatus*, the nostril is slightly elongated and slightly widened in the caudal part but in *A. vaigiensis* in widened in the rostral part. The arrow-shaped olfactory rosette in both species is located on the medial side of the olfactory cavity. In both species, the rosette is oval, elongated in the rostrocaudal direction, it has a similar number of olfactory lamellae, symmetrically attached to the septum, regularly increased in size and changed in shape towards the caudal edge of the rosette. The maximum number of olfactory lamellae in *A. sexfasciatus* is 15 (in specimens, 13-14.5 cm body length), in *A. vaigiensis*, 19 (in specimens, 12-15 cm). There is no secondary folding on the lamellae. The absolute dimensions of the olfactory rosette—the length and width, and the length of the olfactory lamellae slightly differ in both species having similar body length. Both species have two ventilation sacs, with the lacrimal sac much larger than the ethmoidal one. The similarity of the general structure of the olfactory organ in these two closely related fish species confirms the existing data on the structural conservatism of the olfactory system in fish (Fig. 1, 2) [3, 4].

There are also obvious differences in the structure of the olfactory organ. In *A. vaigiensis*, the olfactory rosette occupies a smaller part of the olfactory cavity; in *A. sexfasciatus*, the rosette fills almost the entire cavity. The close arrangement of the rosette in *A. sexfasciatus* is achieved mainly due to its smaller size compared to *A. vaigiensis*. This is typical for fish with a well-developed sense of smell and is considered an adaptation that increases the sensory capabilities of the organ. The process of water exchange in the olfactory organ in *Abudefduf* differs due to the ratio of the size of olfactory cavity and rosette and different role of olfactory lamellae in this process. In *A. vaigiensis*, the shape of the olfactory lamellae is extremely peculiar which not found in other fish. The distal part of the largest lamellae is designed so that under the action of water entering the organ, the lamellae move apart, and when water comes out, lamellae fold to the center of the rosette. This streamlines the flows and favors water exchange in the olfactory cavity, but requires additional space. In *A. sexfasciatus*, the olfactory folds are flat (Fig. 3) [3, 4].

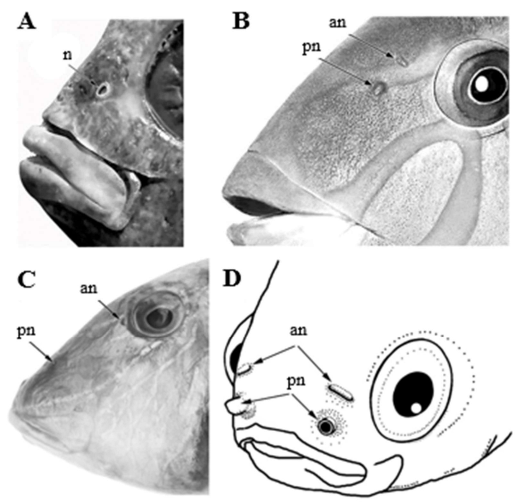

Fig. 1. The location of the olfactory organ on the head of fish
A - *Abudefduf sexfasciatus*; B - *Thalassoma lunare*; C - *Parupeneus indicus*;
D - *Anabas testudineus*; an and pn - anterior and posterior nostrils

The openings of the ventilation sacs in the *Abudefduf* species are located similarly. The lacrimal sac in *A. vaigiensis* is much larger than the ethmoidal one and the olfactory cavity; in *A. sexfasciatus*, the lacrimal sac is smaller in volume and have two compartments. In *A. vaigiensis*, in contrast to *A. sexfasciatus*, the septum (central fold) has an extension that receives the water flow entering the olfactory organ and directs it between the lamellae of the rosette and further into the ventilation sacs [3, 4].

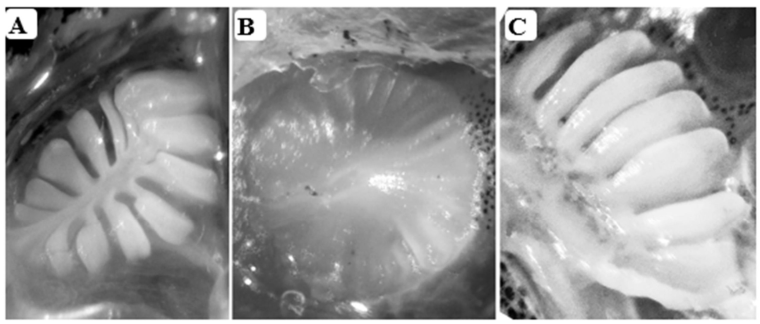

Fig. 2. Olfactory rosette: A - *Abudefduf sexfasciatus*; B -*Thalassoma lunare*; C - *Anabas testudineus*

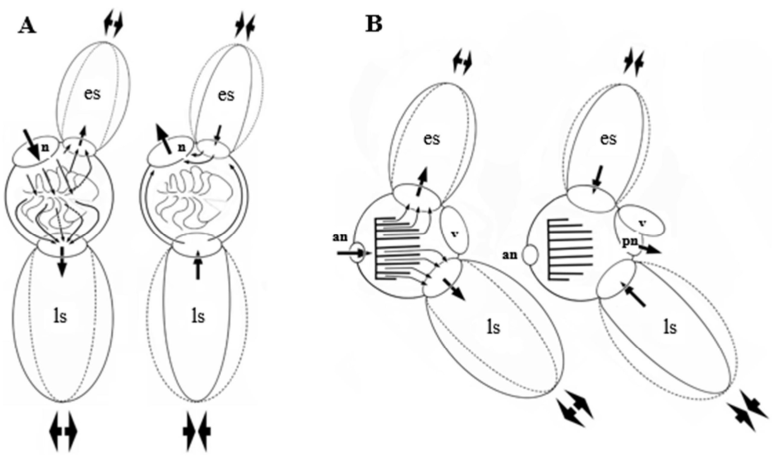

Fig. 3. Scheme of ventilation of the olfactory organ

a - *Abudefduf sexfasciatus*; b -*Anabas testudineus*; ls and es - lacrimal and ethmoidal sacs, v - valve, others - as in Fig. 1

In the moon wrasse *Thalassoma lunare* and the sixbar wrasse *T. hardwicke* (Labridae), the olfactory organ has two nostrils. The posterior nostril is equipped with a valve. The olfactory rosette is absent, but there is the olfactory disc and a vertical membrane, structures previously unknown in fish. The olfactory disk is located at the bottom in the rostral part of the olfactory cavity and is a conglomerate of merged rudiments of the primary olfactory lamellae. In *T. lunare*, the rudiments look like low ridges on the olfactory disc surface; in *T. hardwicke*, the disc surface is smooth. Nerve cords under the rudiments confirm their belonging to the olfactory lamellae. The reduction may be owing to the fact that the olfactory cavity in wrasses is small and flattened. The ridges-lamellae of the moon wrasse and sixbar wrasse differ in thickness. Both species have one large ventilation sac (lacrimal) adjoining the olfactory cavity from below; its entrance is located at the bottom of the cavity caudal to the disc [5].

The nostrils in wrasses (*Thalassoma*) can close due to the sticking of the thin walls in the anterior nostril and the lowering of the valve in the posterior nostril. The closing of nostrils is related to the water exchange in the olfactory cavity and prevent the entering of small particles to it when fish are buried in the ground. Ventilation of the olfactory cavity occurs due to the respiratory movements of the fish, which increase the volume of the ventilation sac during the opening the mouth and decrease the volume when fish close the mouth [5].

The olfactory organ of goatfish (Mullidae) has two nostrils, separated from each other by a great distance. The anterior nostril is small in size and has inner diameter in 0.6 mm and 0.25 mm in *Parupeneus indicus* (body length 28.2 cm) and *Upeneus tragula* (TL 9.8 cm) respectively. The posterior nostril has crescent shape, its size is 1.2-1.5 times that of the anterior one, and is located directly in front of the eye. Olfactory rosette of arrow-shaped type and comprise 29 and 29-33 lamellae in *P. indicus* and in two species of *Uropeneus* in fish with body length 28.2 and 9.8cm. The septum is wide, especially in rostral part. There is no secondary lamellae. Additional nasal sacs are absent, which, together with other structural features of the olfactory organ indicates the principal role of the ciliated epithelium in the ventilation of the olfactory cavity in these fish.

The morphology of the olfactory organ in the climbing perch *Anabas testudineus* (Anabantidae) has been studied in detail for the first time. The anterior nostril is a short forward-facing tube. The posterior nostril in large individuals (TL > 8-10 cm) is almost completely closed by a fold-valve directed backward from the anterior edge of the olfactory opening; the valve develops at a fish with body length of 6.0 cm. The valve can prevent the drying up of the olfactory epithelium when the climbing perch emerge to land, but the main purpose of valve is related with the olfactory organ ventilateion. The olfactory rosette of a parallel type is located in anterior part of the olfactory cavity; the number of lamellae in the rosette increases with the growth of the fish, but does not exceed 11 even in fish having maximal size (22 cm in body length). The olfactory lamellae in the center of the rosette are the biggest, their sizes decrease towards medial and lateral edges of rosette. In fish with body length > 6.5 cm, secondary folds appear on the lateral surface of the lamellae, they are located along the rostrocaudal axis of lamelle. Secondary folding increases the surface of the olfactory epithelium and increases the functional potential of the olfactory system. There are large lacrimal and small ethmoid ventilation sacs. Their openings are located in the caudal part of the olfactory cavity. Ventilation of the olfactory cavity in climbing perch can occur during gill and air-breathing respiration. The obtained data allow us to consider that using olfaction for orientation during the trips on land is quite possible for climbing perch [6].

In the coastal marine ecosystems of Vietnam, animals and plants are widespread, which use taste deterrents to protect themselves from fish - the special substances that accumulate by potential preys and have a repulsive taste for fish [7]. Many properties of such natural deterrents remain poorly studied, including their role in the regulation of complex trophic relationships between coral reefs species. This information is important for the development of programs and activities for the conservation and restoration of the unique coral reef ecosystems, which rapidly degrading now.

To obtain new data on taste deterrents, we compared the palatability of 10 species of comatulid sea lilies (Comatulida) and 6 species of their symbiotic animals for 4 fish species: *A. vaigiensis, A. sexfasciatus, Neoglyphidodon melas* (Pomacentridae), and *Cantigaster valentini* (Tetraodontidae). All objects are common inhabitants of the coral reefs of Vietnam. It was found that sea lilies have a repulsive taste, but their taste aversiveness is different for different fish. In *N. melas*, extracts of all 10 species of sea lilies reduced of flavored pellets intake, with the effect of lilies *Stephanometra indica, Cenometra bella*, and *Lamprometra palmata* being the highest (reduced pellets intake by 15 - 20 times compared with the control). Extracts of *Clarckomanthus alternans, Comaster nobilis* and *Himerometra robustipinna* had the weakest effect (2-fold decrease). For *A. vaigiensis*, extracts of *Cenometra bella* (> 5-fold decrease) and *Comanthus parvicirrus* (complete blocking of consumption) had the highest negative effect. For *A. sexfasciatus*, extracts of 8 species of sea lilies out of 10 tested were taste aversive, and *Comanthus parvicirrus* extract reduced pellet consumption by almost 50 times. Unlike sea lilies, all symbionts had an attractive taste except for the ophiura *Gymnolophus obscura*, which had an indifferent taste, regardless of which host it was collected from [8].

The results of the study indicate that the taste deterrents of sea lilies are not universal and may only be effective for certain fish. Symbionts of sea lilies do not possess deterrence and do not participate in the chemical defense of their hosts as well. Moreover, by presence of highly palatable symbionts provokes attacks of predators and reduces the safety of the hosts. However, the chemical defense of sea lilies is beneficial for symbionts and increases their survival. The results allow us to consider the relationship between crinoids and their symbionts as commensalism.

Although many marine animals accumulate taste deterrents or use other means of defense, it is not clear how the animal's chemical defense is related to their coloration. The study this problem, we used cowries - the gastropod mollusks Ovulidae, in which the colored mantle covers the shell, masking some species of mollusks but making others bright and noticeable. The palatability of the mollusks *Ovula ovum*, the color of which is considered to be aposematic (warning), and *Calpurnus verrucosus*, the color of which cannot be confidently attributed to either aposematic or cryptic, were compared. Testing of extracts of mollusks, as well as 6 species of soft corals that mollusks feed on (Sarcophyton, Sinularia), was performed on *A. vaigiensis* and barramundi *Lates calcarifer*. It was found that extracts of the foot and mantle of *O. ovum* have a strong repulsive taste, which proves the aposematic coloration of this mollusk. On the contrary, *C. verrucosus* has an attractive taste, which makes it possible to consider its coloration as cryptic. Soft corals have a strong deterrent taste to fish. Since both mollusks differ sharply in their taste properties, it can be assumed that *O. ovum* and *C. verrucosus* transform soft coral deterrents supplied with food in different ways. It cannot be ruled out that deterrents are synthesized de novo in *O. ovum* [9].

The taste preferences of barramundi *L. calcarifer*, an important object of cultivation, have been clarified. The palatability of free amino acids and other substances for barramundi was determined, and the feeding behavior was studied in detail [10]. The study of the taste preferences of fish is directly related to the solution of problems related to modern aquaculture. Currently, the using of fishmeal as a source of protein for fish feed is a major constraint to the progress of aquaculture. One way to address this challenge is to look for new, less expensive and more accessible sources of protein. Echinoderms (starfish, sea urchins and holothurians) are common and numerous in marine coastal areas, but only some of the echinoderms are used for food or for other purposes. We have evaluated the palatability of several echinoderm species for barramundi, including the crown-of-thorns star *Acanthaster planci*, whose abundance outbreaks lead to catastrophic consequences for coral reef ecosystems [11]. It was found that extracts 5 out of 6 echinoderm species (starfish *Linckia laevigata*, *A. planci, Culcita novaeguineae, Fromia milleporella*, holothurian *Holothuria atra*) were found to cause a dramatic reduction in pellet intake. But sea urchin *Diadema setosum* extract has an attractive effect. The results show that most of the echinoderms cannot be recommended for use as ingredients for feeds intended for cultivating barramundi. Among starfish, repulsive taste qualities are the least pronounced in *A. planci* [11]. Since some fish are naturally able to feed on *A. planci*, the inclusion of A. planci products in the diet of some farmed fish may be feasible. The the started research has clear prospects, especially considering that during outbreaks the *A. planci* biomass reaches significant values. For example, almost 13 million of *A. planci* were collected from the reefs of the Ryukyu Islands (East China Sea, Japan) from 1970-1983 as part of one of the local programs.

4. CONCLUSION

Chemoreception plays an important role in behavior of fish. Knowledge of the morphological and functional characteristics of the olfactory and gustatory systems is necessary to understand the mechanisms that ensure the existence and relationships between organisms of complex aquatic communities, such as coral reefs. As a result of our studies, the first data on chemoreception of Vietnamese fish were obtained. The new structures previously unknown for the olfactory organ of fish were discovered. The protective adaptations of marine animals based on the function of chemosensory systems in fish were elucidated. The universality and specificity of natural taste deterrents of marine animals and plants were significantly clarified. Further progress in studies of chemoreception and chemocommunication in fish of Vietnam ichthyofauna will make also possible to solve current problems related to artifical feeds used in fish aquaculture.

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SUMMARY

The sensory systems of tropical fish remain poorly studied despite the richness and diversity of the tropical ichthyofauna. Studies of the olfactory and gustatory systems in fishes of Vietnam have not been previously carried out. The article for the first time provides information about the morphology of the olfactory organ in several species of freshwater and marine fish of families Anabantidae, Pomacentridae, Labridae, Mullidae. The morphological peculiarities of olfactory cavity, olfactory rosette and lamellae and ventilation olfactory sacs are discussed. Structures previously unknown for the olfactory organ of fish are described for the first time. The mechanisms of ventilation of the olfactory cavity are considered. Data on the effectiveness of natural taste deterrents and their significance in providing chemical protection for marine animals and plants against fish are presented. The relationships between hosts and symbionts in the example of echinoderms and other invertebrates of coral reefs of Vietnam are discussed. How the animal chemical defense and their coloration are related is analyzed. Data on taste preferences in cultivated fish are obtained.

Keywords: *Olfactory organ, olfactory folds, ventilation sac, taste deterrents, symbionts, cơ quan khứu giác, nếp gấp khứu giác, túi thông khí, chất có vị khó chịu, sinh vật cộng sinh.*

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