Ventilation Rate of Cuttlefish, *Sepia officinalis*, in Response to Visual Stimuli

by

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Abstract. Ventilation rate can provide information about the detectability and salience of stimuli. Octopus ventilation rate responds to chemical stimuli; here we tested whether cuttlefish ventilation rate is sensitive to visual stimuli. We measured the changes in ventilation rate of juvenile *Sepia officinalis* in response to a general disturbance (being moved to a new tank), to the sight of prey items (live fish and live crabs), and to the sight of conspecifics (familiar and unfamiliar). Ventilation rate increased relative to controls in all cases, but most to general disturbance. Responses to prey items were stronger than reponses to conspecifics, a finding consistent with the semi-solitary lives of free-living juvenile cuttlefish. Although cuttlefish prefer crabs to fish and hunt them differently, no differences in responses were found between types of prey. Responses to familiar and unfamiliar conspecifics also did not differ. We conclude that analysis of ventilation rates is a good method for measuring the perception and relative significance of broad classes of visual stimuli in cuttlefish.

INTRODUCTION

It is not always easy to discern what animals perceive. The appearance of stimuli important to an animal can cause increased alertness, with a possible concomitant increase in ventilation rate (hereafter, referred to as "arousal"). A directly observable behavior, ventilation rate has the potential for providing a convenient measure of the relative salience of different stimuli. To investigate this possibility, we measured changes in ventilation rates of cuttlefish in response to visual stimuli.

Cephalopods change the rate or volume of water respired in response to changes in activity level. The common octopus, *Octopus vulgaris* Cuvier, 1797, increases its ventilation rate with even small increases in activity level (Boyle, 1983). The common cuttlefish, *Sepia officinalis* Linnaeus, 1758, increases ventilation volume, with a reduction in ventilation rate, when it jets (Trueman & Packard, 1968).

Cephalopods also change the rate or volume of water respired in response to chemical stimuli. In *Octopus vulgaris*, ventilation rate increased in response to crab extract and to water from a crab holding tank (Boyle, 1983). Ventilation rate in the octopus *Eledone cirrhosa* Lamarck, 1798, changed in response to several common chemical constituents of arthropod flesh (betaine, glycine, proline, constituents of arthropod flesh (betaine, glycine, proline, alanine, lactic acid, and serine) (Boyle, 1986). Chase & Wells (1986) observed respiratory changes in *O. vulgaris* in response to weak solutions of glutamic acid, glycine, and adenosine 5′-monophosphate.

The feasibility of using ventilation rate to measure responses to chemical stimuli was demonstrated by Boyle (1983), using two different methods: (1) direct observation by an experimenter, and (2) automated measurement of the electrical impedance of the water surface, which fluctuates with the disturbances caused by ventilation movements. Automated measurements were sometimes disrupted by movements of the subject within the enclosed space or by spontaneous bursts of hyperventilation. In the experiments reported here, we measured ventilation rates by the simpler method of direct observation.

We measured the ventilation rates of *Sepia officinalis* in response to visual stimuli: live fish, crabs, shrimp, and familiar and strange conspecifics. We addressed four questions: is ventilation rate affected by (1) general disturbance (moving the animal into the test tank); (2) the presence of a food item (live shrimp); (3) the sight of a conspecific (familiar or unfamiliar); and (4) the type of live stimuli (fish, crab and other cuttlefish)? We discuss the possible relationship between relative magnitudes of ventilation changes and significance of stimuli to the cuttlefish.
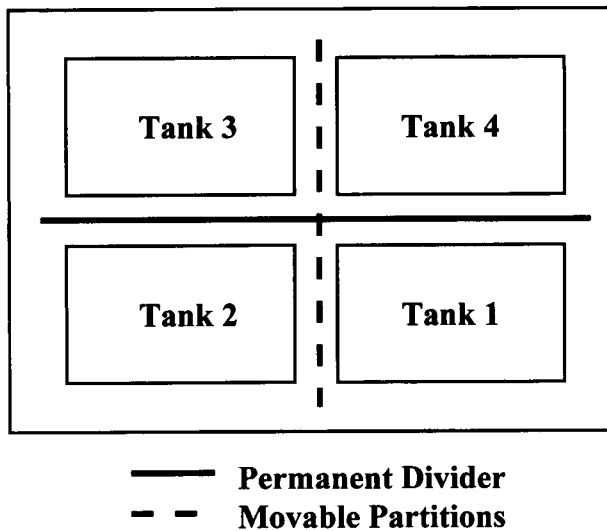
MATERIALS AND METHODS

Subjects (7.5–10.5 cm mantle length) were taken from a cohort of juvenile laboratory-cultured *Sepia officinalis* (Forsythe et al., 1991). This cohort of approximately 120 animals was maintained in groups of about 10 cuttlefish per 1.52 m diameter round tank. All 12 holding tanks, as well as the test tank, were interconnected to the same recirculating water system (see Forsythe et al., 1991). Water was obtained from the Gulf of Mexico, and ranged from 19–21°C with a salinity of 35 ppt. Light was provided with a combination of natural light and artificial light on a 12 hr light/12 hr dark cycle. The animals were fed frozen shrimp twice a day *ad libitum*.

The test tank was a glass-bottomed aquarium (109 × 90 × 90 cm). Stout timbers set on building blocks supported the tank along the outside perimeter and allowed an experimenter to view the tanks from beneath. The outer side walls of the tank were covered with dark green camouflage cloth. Four identical smaller aquariums (51 × 25 × 40 cm) were placed within the test tank in a 2 × 2 array (Figure 1). A lid made from plastic mesh fit over the four smaller tanks. A permanent opaque divider separated the tanks into two pairs; movable opaque partitions could be placed between the two tanks of each pair. A

——— **Permanent Divider**

− − **Movable Partitions**

Figure 1

Schematic of test tanks. Tanks had glass bottoms, and cuttlefish were viewed from below in order to observe the ventilation movements of their funnels.
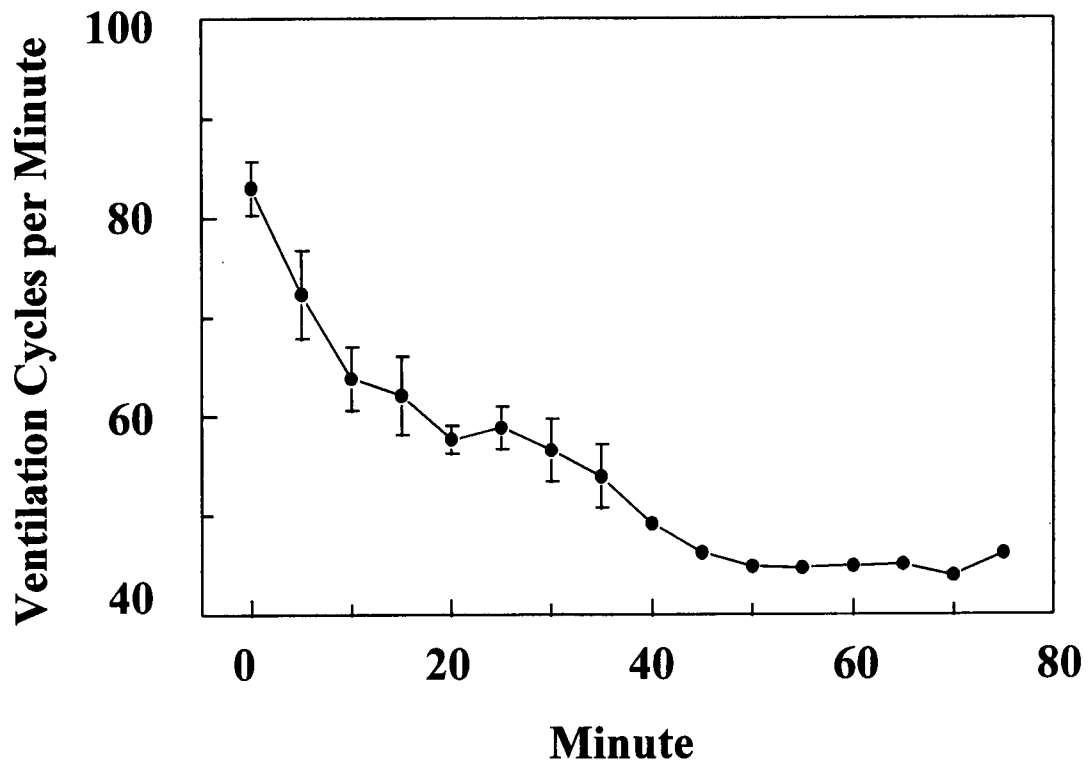

Figure 2

Experiment 1: Ventilation rate of cuttlefish ($n = 20$) after being transferred from holding tanks to test tanks (mean ± SE).
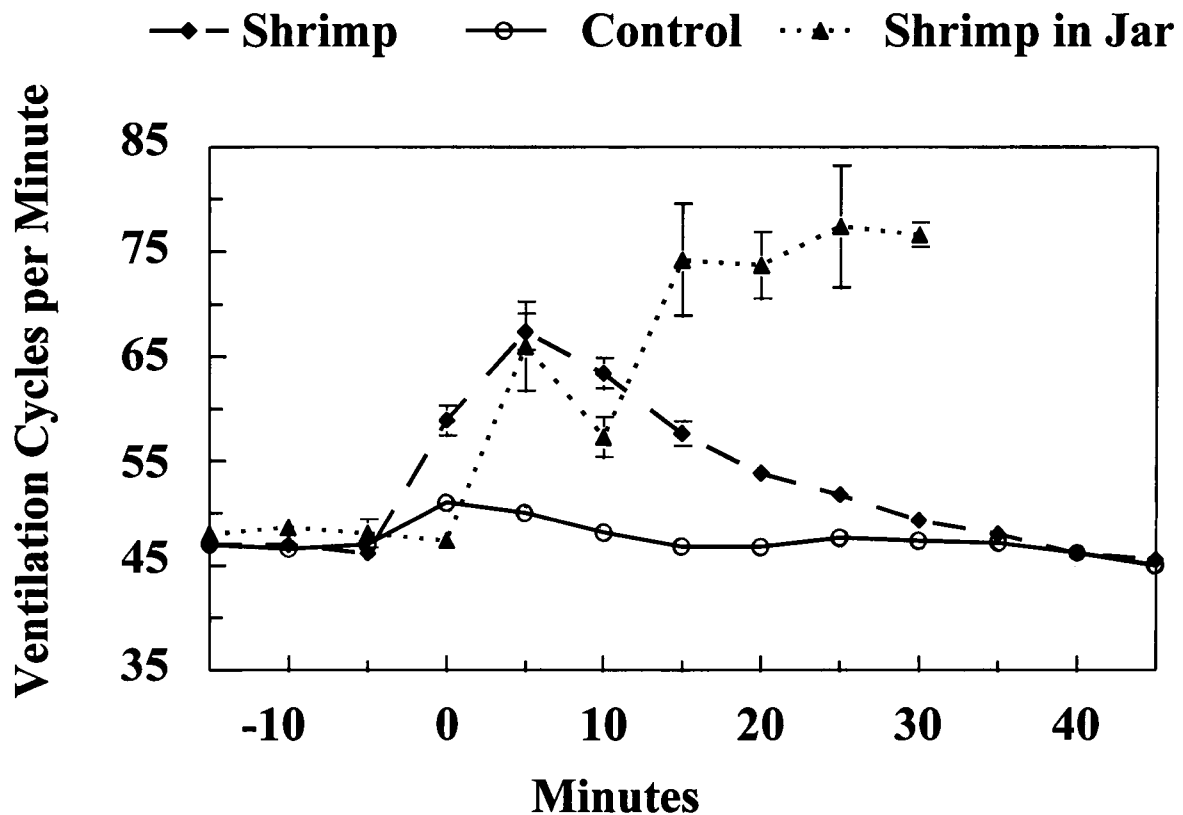
Figure 3

Experiment 2: Ventilation rate in response to the appearance of a prey item at minute 0 (mean ± SE).

small desk lamp (60 watts) was placed below the test tank, shining upward. The experimenter lay quietly beneath the tank, observing the ventral sides of the subjects.

Subjects were selected at random from the 12 holding tanks and placed in the test tank 1 hour before trials. When trials began, the experimenter recorded at 5 minute intervals the total time for 30 inhale/exhale cycles for each subject. Cycles could be observed directly from the ventilation movement of the funnel with the pressure changes of inhalation and exhalation. We compared ventilation rates before and after each event for each subject. Pre-event rates were determined by averaging the three measurements immediately prior to the event. Post-event rates were determined by averaging highest recorded rate after the event with the two recorded rates that immediately followed the peak rate.

1. General Disturbance

We recorded the responses of cuttlefish to the general disturbance of being moved to the test tank. One cuttlefish was placed into each of the four small tanks. Subjects were in visual isolation with all barriers in place. Ventilation rate was measured starting immediately after the subjects

were placed into the test tanks. Sixteen subjects were observed for 1 hour; an additional four subjects were observed for 1 hour and 20 minutes.

2. Presence of Food

We recorded the response of cuttlefish to the presence of familiar food items. An inverted PVC cap, about 5 cm in diameter, was placed over a live shrimp in two of the small aquariums; in the other two aquariums the cap was empty (control). Subjects were again placed into each of the tanks, in visual isolation. After 1 hour had elapsed, caps were inverted by means of an attached string. Ventilation rate was measured at 5 minute intervals starting 15 minutes before the cap was inverted, for a total of 1 hour and 15 minutes. Twelve subjects were used, six with the shrimp and six with the control condition. For an additional two subjects, the cap and shrimp were confined within a glass jar in order to determine the effect of solely visual stimulation.

3. Sight of Conspecifics

In this experiment, we recorded the response of cuttlefish to the sight of familiar and unfamiliar conspecifics. One

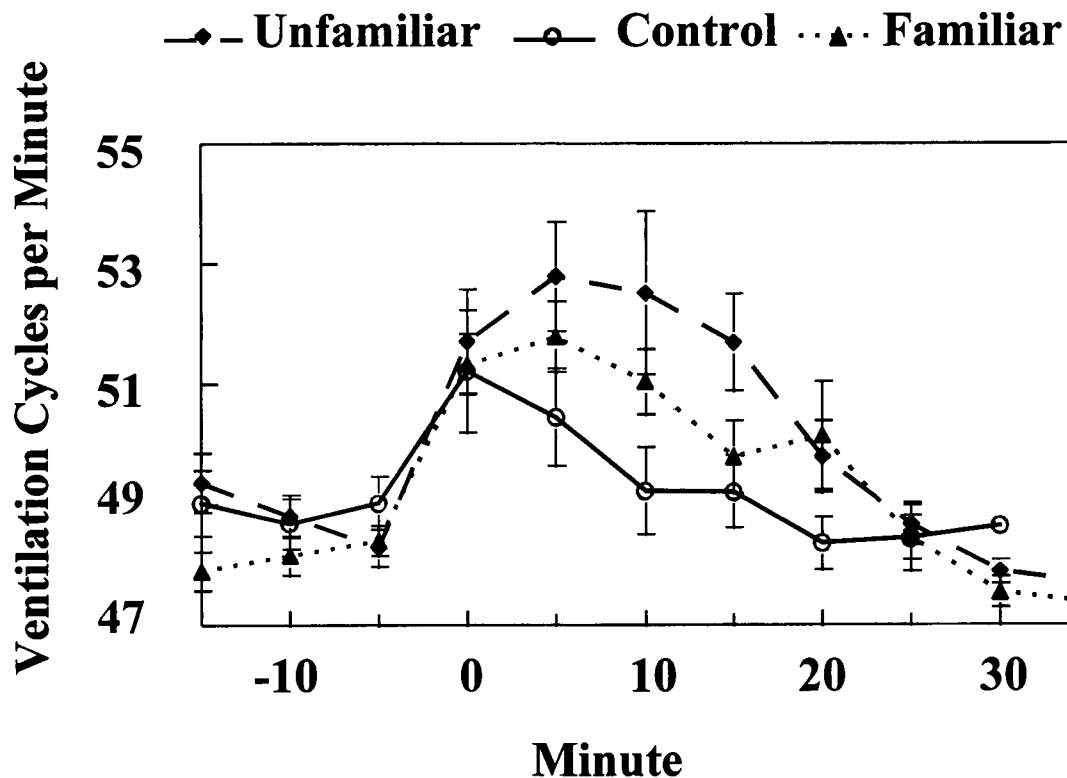## — Unfamiliar —o— Control ··▲·· Familiar

Figure 4

Experiment 3: Ventilation rate in response to the appearance of a conspecific (familiar or unfamiliar) or an empty tank (control) at minute 0 (mean ± SE).

cuttlefish was placed into each of the four small tanks. Subjects were in visual isolation. After 1 hour, the movable partitions were raised by means of an attached string. Each animal could thus see one other subject. For five pairs of subjects, both animals came from the same holding tank (familiar conspecific); for five other pairs, each individual came from a different holding tank (unfamiliar conspecific). For a further eight subjects, the adjacent tank was empty (control). Ventilation rate was measured starting 15 minutes before the barriers were raised, for a total of 1 hour and 15 minutes.

4. Differences between Stimuli

We tested whether cuttlefish arousal, as measured by ventilation rate, differed between similar-sized stimuli. We compared responses to a crab and a fish, each roughly the same size as the subject when viewed from the cuttlefish's perspective (fish total length, crab carapace width, and cuttlefish mantle length), with the responses we found in Experiment 3 to familiar and unfamiliar conspecifics.

For each pair of tanks, one cuttlefish was placed in one of the small tanks; in the other, a large glass jar was placed containing either a blue crab (*Callinectes sapidus* Rathbun,

1896) or a gulf killifish (*Fundulus grandis* Baird & Girard, 1853). The movable partition separated the tanks. After 1 hour, the movable partition was raised. Ventilation rate was measured statrting 15 minutes before the barriers were raised, for a total of 1 hour and 15 minutes. The fish was used for four subjects; the crab was used for four other subjects. Results were compared with those of Experiment 3 with familiar and unfamiliar conspecifics.

RESULTS

1. General Disturbance

Immediately after the subject was moved to the test tank (the disturbance, time 0), ventilation rates averaged 83 ventilation cycles per minute. After 50 minutes, average rates had slowed to 45 cycles per minute, a decrease of about 45%. Rates then stabilized (Figure 2).

2. Presence of Food

Treatment (control, live shrimp, live shrimp in glass jar) had a significant effect (F = 157.51; df = 2,13; $P < 0.001$) (Figure 3); ventilation rates increased about 40% in re-
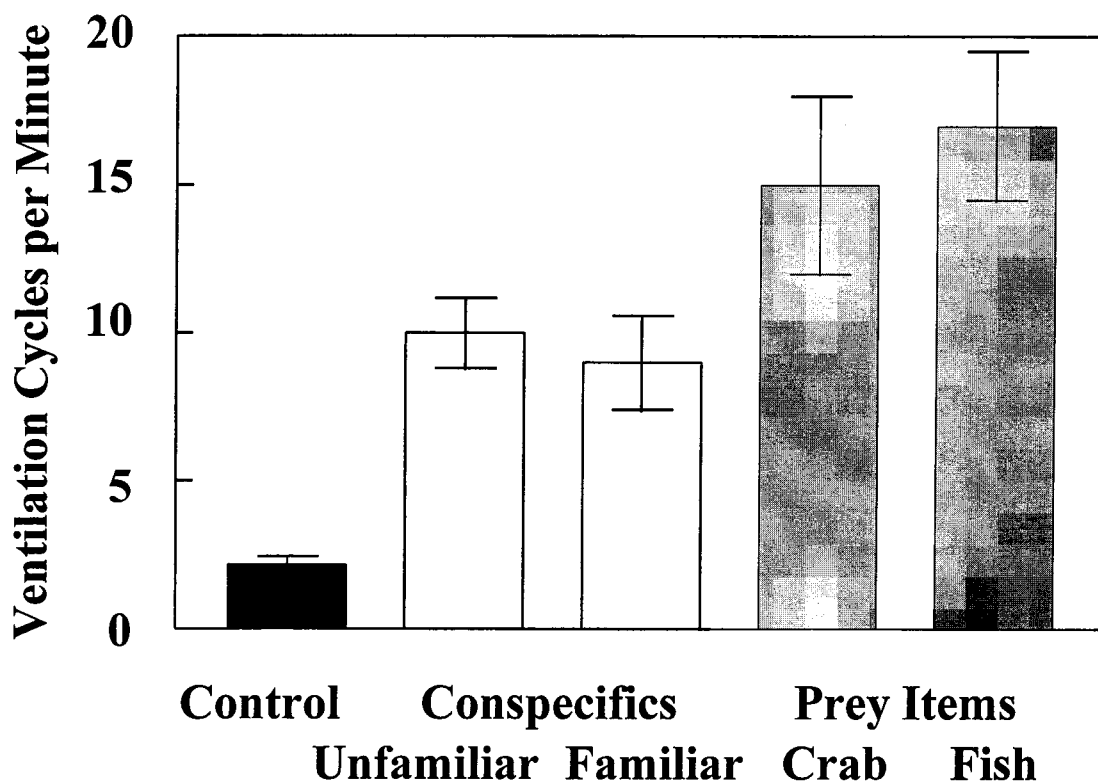
Figure 5

Experiment 4: Ventilation rate increase in response to stimuli, each of the same overall size as the subjects (mean ± SE).

sponse to the presence of the shrimp, as compared to controls. *A priori* tests between means showed that responses with the glass jar were significantly different from those without the glass jar (F = 21.36; df = 1,13; $P < 0.001$).

3. Sight of Conspecifics

In the test for response to conspecifics, treatment (control, familiar conspecific, unfamiliar conspecific) had a very small, yet statistically significant effect (F = 3.53; df = 2,25; $P < 0.05$) (Figure 4). *A priori* tests between the means showed that there was no difference in response to a familiar and an unfamiliar conspecific (F = 0.80; df = 1,25; $P \sim 0.45$).

4. Differences between Stimuli

In the experiment on the reaction of cuttlefish to other living animals of the same size as themselves, we found a small but significant difference in ventilation rate between presentation of other cuttlefish (familiar or unfamiliar) and other animals (crab or killifish) (F = 14.39; df = 2.25; $P < 0.001$) (Figure 5). There was no difference between their response to a crab and a killifish (t = 2.10, df = 3, $P > 0.05$).

DISCUSSION

Disturbance had an effect on ventilation rates of cuttlefish, as did the sight of conspecifics and other animals. These results are consistent with and extend those of Boyle (1983) and Chase & Wells (1986) on the effect of chemical stimuli on octopus ventilation rate. Ventilation rate thus appears to be a good overall indication of cephalopod arousal.

The largest influence on ventilation rate was that of the general disturbance. After the transfer to the test tank (Experiment 1), ventilation rate gradually decreased to the resting rate, a pattern consistent with an interpretation of habituating to their new surroundings.

When presented with prey items (Experiment 2), cuttlefish became quite active and focused intently on the shrimp at the same time that ventilation rates increased. Response to the shrimp in the glass jar differed significantly from that without the jar. This difference was probably a result of sustained responses by the subjects unable to reach the shrimp rather than any effect of the absence of chemosensory information. Initial response to the sight of the shrimp did not differ between the two groups (Figure 3).

Response to the sight of another cuttlefish (Experiment

3) was quite different from that to prey items. Subjects were less active and their activity was less focused in any particular direction. Ventilation rate rose only slightly more than that of controls. We found no significant difference between the response to familiar and to unfamiliar conspecifics. Although results appear suggestive that a difference might be found had we used a larger sample size or more sensitive method (Figure 4), Boal (in press) has found similar lack of evidence for social recognition in adults of the same species.

Response did not differ between the crab and fish presentations (Experiment 4). Both are prey items when they are smaller than the cuttlefish. Cuttlefish will attempt to capture same- or even larger-sized crabs and fish if they are hungry enough. In the laboratory, the cuttlefish appear to prefer crabs over fish. They also approach them differently when preying upon them (Nixon, 1987). It may be that our results did not reflect these differences because our sample size was quite small ($n = 8$). Another possible explanation is that the two live prey items were equally arousing when presented alone to hungry cuttlefish accustomed to receiving only dead food. Satiated animals might respond differently.

The cuttlefish did not respond equally to all live stimuli of approximately the same size; they were clearly more interested in food than in each other (Experiment 4). Our subjects were juveniles; results with sexually mature adults might be different.

We find it noteworthy that with this simple experimental design we were able to support results obtained with more sophisticated equipment (Boyle, 1983, 1986). Cephalopod ventilation rate is indeed responsive to visual stimuli, as well as to chemical stimuli. The magnitudes of changes in ventilation rates were consistent with interpretations of differing salience. Ventilation rate provides a robust, objective, and quantifiable behavioral measure for testing animal perception, and is sensitive enough to demonstrate discrimination between classes of stimuli.

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