



Figure 3. Effect of PS-1 on tumor formation of potato tuber disc (*Solanum tuberosum*). A control; B treated with PS-1.

The findings above suggest that PS-1 assists the attachment of *A. tumefaciens* to plant cultured cells. To confirm this assumption, the effect of PS-1 on attachment of the bacterium to the tobacco cell surface was investigated. To a suspension of cultured tobacco cells in 2 ml of Murashige-Skoog medium were added 1 ml of suspension of *A. tumefaciens* and 1 ml of PS-1 solution at various concentrations. Two hundred  $\mu$ l of this mixture was taken at regular time intervals and filtered through JK wipes (TM) to separate the attached and the free bacterial cells. The numbers of attached and free bacterial cells were measured by a viable cell count method, and the percentage of bacterial inoculum attached to tobacco cells was calculated. Regardless of the addition of PS-1, the percentage of bacterial inoculum attached to tobacco cells reached a plateau within 80 min and the percentage at

the plateau increased from 35% to 55%, depending on the concentration of PS-1 (fig. 2).

The effect of PS-1 on plant cell transformation using *A. tumefaciens* was examined by the potato tuber disc assay first described by Anand and Heberlein<sup>10</sup>. This assay promises a semi-quantitative examination of the frequency of plant transformation by *A. tumefaciens*<sup>11,12</sup>. Tumors with a diameter bigger than 2 mm were counted (fig. 3). On average, 15.6 tumors were formed on a treated potato disc compared to 9.0 in the control. PS-1 apparently enhanced the crown gall formation.

It was concluded that owing to its affinity for both *A. tumefaciens* and plant cells, PS-1 increased the frequency of transformation of plant cells by *A. tumefaciens* by assisting the attachment of the bacterium to the plant cell surface. This compound would be useful for use with *A. tumefaciens* cells as vectors in gene recombination of plant cells.

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## Application of decision theory in understanding food choice behavior of hatchling loggerhead sea turtles and chemosensory imprinting in juvenile loggerhead sea turtles<sup>1</sup>

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**Summary.** Food choice behavior of hatchling loggerhead sea turtles, *Caretta caretta*, and chemosensory choice behavior of juvenile loggerhead sea turtles artificially imprinted prior to emergence from the nest were examined using models derived from choice threshold and set releasing value theories of decision making. Modelling results indicate that food choice behavior of hatchlings is better described by a model based on set releasing value theory and that choice behavior of chemically imprinted juveniles is better described by a model based on choice threshold theory.

**Key words.** Feeding behavior; food choice; sea turtles; decision making; imprinting; chemoreception.

There is little information on the ontogeny of feeding behavior in turtles, generally, and relatively little experimental evidence to support or refute chemosensory imprinting in sea turtles<sup>2</sup>. Understanding the early feeding behavior and food preference development of sea turtles and chemosensory imprinting in sea turtles is important for the conservation of these endangered animals<sup>4</sup>. The purpose of this study was to use theoretical mathematical models based on ethology and

decision theory to examine the underlying mechanism/s of food choice behavior in hatchling loggerhead, *Caretta caretta*, sea turtles and of chemosensory choice behavior in juvenile animals, behaviors which have been previously described ethologically<sup>3,4</sup>.

**Feeding experiments.** It has been proposed that early feeding experience in hatchlings may affect later feeding behavior and may constitute an important component of early

chemosensory learning, including chemical imprinting to the natal beach<sup>4</sup>. Both suggestions raise important questions related to conservation efforts in rearing hatchling turtles to a larger size prior to release since many of these projects use commercial pelleted food.

An experiment was conducted to examine food imprinting in loggerhead sea turtles. Methods used in the original feeding experiments are detailed in Grassman and Owens<sup>3</sup>. Hatchling loggerhead sea turtles were initially fed daily for 14 days according to the following regimen to establish an initial hierarchy of food preference for each group. Group S animals (N = 12) were fed only chopped shrimp, group F animals (N = 11) only chopped flounder, and group P animals (N = 21) only commercially prepared turtle pellets. The 14-day feeding period was followed immediately by two tests (I and II), one on each of two consecutive days, to determine the values of each paired comparison required for modelling the food choice behavior.

In each test hatchlings were placed into a behavioral arena (18 × 28 × 10 cm deep) and presented with equally-sized pieces of shrimp, flounder or a pellet (test I), with each food item suspended from a string to maintain its position in the water (spaced 1 cm apart). The order of the food choices was varied systematically between trials. Typically, the turtles would swim toward a food item, mouth agape, and either grasp it, or attempt to do so. This was the criterion for deciding when a choice had been made. If a choice was not made within 15 min, that turtle was excluded from analysis, therefore, the number of observations varied among tests. Test II was conducted similarly except that the food items were wrapped in white cloth to disguise the food items and to test the ability of the turtles to choose on the basis of olfaction.

This test was followed by a third one examining the plasticity of the initial food preference hierarchy. Prior to conducting test III, each group of turtles was fed a different diet for 14 days, beginning immediately after the completion of test II. Groups S and P were fed flounder and group F was fed pellets. The hatchlings were then retested for their food preferences according to the protocol of test I.

**Chemical imprinting experiments.** The imprinting hypothesis (first discussed in the late 1950's by Carr<sup>5</sup>) states that sea turtles learn an identifying cue or set of cues characteristic of their natal beach. The most appropriate model for sea turtle imprinting is that of the olfactory or chemical imprinting found in salmonid fishes<sup>4</sup>, demonstrated by Hasler and his associates using an 'artificial imprinting' protocol for salmon<sup>6</sup>. In sea turtles, as in salmon, it is unlikely that chemical imprinting explains the entire process of migration. However, the demonstration of such a system in sea turtles would provide a strong argument for continued consideration of chemical imprinting to the natal beach as an explanation of the site fidelity seen in sea turtle nesting<sup>4</sup>, as well as provide a measure of confidence for the success of conservation efforts using artificial imprinting of hatchlings to 'attractant' chemicals and to natural waters in attempts to establish new nesting populations of sea turtles.

To investigate further the chemical imprinting hypothesis in sea turtles, a laboratory experiment modeled after the studies of Hasler and co-workers<sup>6</sup>, including similar 'artificial imprinting' protocol, 'attractant' chemicals, and chemical concentrations, was conducted with loggerhead sea turtles. The complete experimental procedure for the chemosensory imprinting studies with loggerheads is detailed in Owens et al.<sup>4</sup>. *Caretta caretta* eggs were divided into four treatment nests (N = 10, each). The following treatments were used: 1) The nest was kept moist with 5 × 10<sup>-5</sup>M morpholine dissolved in 0.9% artificial seawater (ASW) until after hatching, when the turtles were placed in a tank of 5 × 10<sup>-5</sup>M morpholine in ASW (MPP group); 2) the nest was kept moist with ASW

only and hatchlings were placed in a tank of 5 × 10<sup>-5</sup>M morpholine in ASW (MPO group); 3) the nest was kept moist with 5 × 10<sup>-5</sup>M 2-phenylethanol in ASW and the hatchlings were placed in a tank of 5 × 10<sup>-5</sup>M 2-phenylethanol in ASW (PPP group); 4) nest and hatchlings were exposed only to ASW (UNT, untreated group). Treatments were continued in tank water until testing was completed. Behavioral tests of the ability of these animals (as juveniles) to orient to the chemical conditions experienced began 5 months after the hatchlings were placed in the tanks and continued for another 5 months. Each choice test involved placing a single turtle into an electronic monitoring tank and presenting the animal with simultaneous choices between ASW or ASW containing 5 × 10<sup>-5</sup>M morpholine or 2-phenylethanol in order to determine the values of each paired comparison required for modelling the chemical-choice behavior. Chemical cues were presented to the animals by infusing the chemicals into separate compartments in the arena. A choice was considered to have been made when a turtle entered a compartment sufficiently to break a photoelectric beam inside the entrance. We postulated that hunger would increase movement of the turtles in the behavioral arena by motivating food-searching, thus the animals were unfed for 24 h prior to experimentation.

**Modelling and data analysis.** The experimental protocols described above are those of typical sign stimulus-releaser situations. Dawkins<sup>7</sup> proposed the Choice Threshold Model to explain such decision making in choice behavior when choosing between pairs of alternate stimuli. The model is an application of earlier ethological models attempting to describe decision making between alternate motor patterns. Although formulated some years ago, it is a most appropriate mathematical modelling approach for a simple sign stimulus-releaser situation, and remains relevant and useful to modern ethology, as indicated by its recent review by Guthrie<sup>8</sup>.

The model is based on the hypothesis that an animal may choose either of two stimuli equally, and contains three assumptions: 1) there exists in the animal a motivational variable which fluctuates in time; 2) this motivational variable must exceed a certain threshold for a response to a particular stimulus to occur; and 3) there is a state of 'suprathreshold indecision' which means that if the thresholds of more than one alternate stimulus is exceeded, each stimulus whose threshold is exceeded is equally likely to be chosen. The formulation is given by:

$$P_{AC} = 2(P_{AB} + P_{BC} - P_{AB} \cdot P_{BC}) - 1$$

where  $P_{AC}$  is the proportion of times 'A' is chosen in preference to 'C', when these two stimuli are presented together, and so forth. The three possible stimuli, A, B, and C, are listed in order of preference, and are presented in all possible pair combinations<sup>7</sup>. The model does not predict the timing of choices, which is relegated to a 'go/no go decision maker'<sup>8</sup>.

An alternative model, presented for comparison with the Choice Threshold Model, is the Product Rule<sup>7</sup>, which is derived from the Set Releasing Value Model<sup>7</sup>. This model assumes that each alternate stimulus has a 'fixed' stimulating value for an individual. Thus the number of responses to a stimulus is always proportional to this set releasing value, which may be 'fixed' for only certain periods of time, after which a new 'fixed' releasing value may be established. Its formulation is:

$$P_{AC} = P_{AB} \cdot P_{BC} / (P_{AB} \cdot P_{BC} + Q_{AB} \cdot Q_{BC})$$

where, again,  $P_{AC}$  is the proportion of times 'A' is chosen in preference to 'C', when these two stimuli are presented to-

gether, etc.;  $Q_{AB} = 1 - P_{AB}$  (e.g. the proportion of times 'A' is *not* chosen in preference to 'B', when these two stimuli are encountered together), and so forth.

To use the prediction equation of each model<sup>7</sup>, the percentage preferences ( $P_{ij}$ 's) of animals for each alternate stimulus must be determined experimentally by presenting the alternate choices in all three possible pair combinations. The choices are then listed alphabetically, with A being the most preferred, B the next preferred, and C the least preferred. Predictions of percentage preference are then made for  $P_{AC}$ . To examine the success of the two models offered for comparison, predicted values of  $P_{AC}$  were determined for each model<sup>7</sup> from the data on food preferences of the hatchling loggerheads and on chemical cue choices of the juvenile loggerheads. Each predicted value was first compared with the experimentally determined  $P_{AC}$  and a percent error calculated by subtracting the predicted value from that observed, then dividing the result by the observed value. For the food preference studies, two-tailed Wilcoxon sign-rank-tests were used to compare the percent errors of  $P_{AC}$  between the models for each test for each data set. The sample size (number of paired predictions) was too small for statistical inference in the artificial imprinting studies.

**Results.** Observed percentage preferences and predicted values for the hatchling loggerhead food preferences are in table 1. Results of the two-tailed matched-pairs Wilcoxon tests indicate a significant difference ( $p < 0.01$ ) in the distributions of the predictions between the Choice Threshold (CT) model and the Product Rule (PR) model. The CT model had consistent, significantly greater percent error ( $p < 0.01$ ) and predicted values ( $p < 0.01$ ) than the PR model. Observed percentage preferences and predicted values for the juvenile chemical cue preferences are in table 2. The PR model had consistently greater percent error and predicted values than the CT model.

**Discussion and conclusion.** When using theoretical models, it can be inferred that the similarity between the observed behavior of the biological system and that of the mathematical system is due to a similarity of internal mechanism<sup>7</sup>.

Table 1. Observed and predicted percentage preferences of food items for hatchling loggerhead sea turtles

Tests Foods	Observed preferences*			Predicted values for $P_{AC}$ **			
	$P_{AB}$	$P_{BC}$	$P_{AC}$	CT	% Err	PR	% Err
Group S (N = 12)							
I. (n = 11)	63.6	54.6	68.2	66.9	1.9	67.8	0.5
A: shrimp, B: flounder, C: pellets							
II. (n = 12)	58.4	58.3	66.7	65.3	2.1	66.2	0.7
A: flounder, B: pellets, C: shrimp							
III. (n = 11)	54.6	59.2	63.7	62.9	1.2	63.6	0.2
A: flounder, B: shrimp, C: pellets							
Group F (N = 11)							
I. (n = 8)	75.0	62.5	87.5	81.2	7.2	83.3	4.8
A: flounder, B: shrimp, C: pellets							
II. (n = 11)	77.2	54.6	81.8	79.3	3.1	80.3	1.8
A: flounder, B: pellets, C: shrimp							
III. (n = 11)	55.0	65.0	70.0	68.5	2.1	69.4	0.9
A: flounder, B: shrimp, C: pellets							
Group P (N = 21)							
I. (n = 19)	94.7	52.6	97.4	94.9	2.6	95.2	2.2
A: pellets, B: shrimp, C: flounder							
II. (n = 18)	72.2	55.5	77.8	75.4	3.1	76.4	1.8
A: pellets, B: flounder, C: shrimp							
III. (n = 18)	58.4	66.7	75.1	72.3	3.7	73.8	1.7
A: shrimp, B: fish, C: pellets							

\*  $P_{ij}$ , Percentage preference of choice i when presented with choice j;

\*\* CT, choice threshold model; PR, product rule model, % Err = percent error in prediction. N = number of animals in each group; n = number of animals making a choice in each test. See text for description of tests and models.

Table 2. Observed and predicted percentage preferences of chemical cues for juvenile loggerhead sea turtles

Group Chemical cue*	Observed preferences**			Predicted values for $P_{AC}$ ***			
	$P_{AB}$	$P_{BC}$	$P_{AC}$	CT	% Err	PR	% Err
MPP	69.2	55.5	72.8	72.6	0.4	73.7	1.1
A: Mor, B: Cont, C: Phenyl							
MPO	56.6	67.9	66.0	67.2	1.7	68.0	2.9
A: Cont, B: Phenyl, C: Mor							
PPP	69.6	53.3	73.3	75.0	2.3	76.2	3.9
A: Cont, B: Mor, C: Phenyl							
UNT	52.9	72.7	75.0	74.98	0.03	74.94	0.08
A: Mor, B: Cont, C: Phenyl							

\* Mor, morpholine; Cont, control (untreated); Phenyl, phenylethanol;

\*\*  $P_{ij}$ , percentage preference of choice i when presented with choice j;

\*\*\* CT, choice threshold model; PR, product rule model;

% Err = percent error in prediction. For each group, N = 10. See text for description of groups and experiments.

Visual examination of the percent errors in prediction between the Choice Threshold (CT) model and the Product Rule (PR) model suggests that both models have approximately equal predicting power for the results of food choice behavior of the hatchling loggerhead sea turtles (table 1). However, statistical analyses indicate a significant difference ( $p < 0.01$ ) between the models in percent prediction error, with the CT model having significantly greater errors in prediction. Thus the PR model appears to be the better predictor of the observed food-choice behavior of the hatchling turtles. Apparently the early exposure to a certain diet 'set' a 'fixed' stimulating, or releasing, value for that food item. The 'fixed' releasing value is apparently reset by feeding with an alternate diet. Such an underlying mechanism insures behavioral plasticity in food preference and has obvious adaptive advantages in the turtles' natural environment due to the variable availability of food sources. It also suggests that no long-term adverse effects on feeding behavior of these sea turtles is likely to be sustained in 'headstart' rearing programs where hatchlings are fed commercially produced foods.

Examination of the percent errors in prediction between the CT model and the PR model (table 2) suggests that the chemosensory choice behavior of juvenile loggerhead sea turtles in groups MPO, MPP and PPP are better described by a model based on choice threshold theory. Although the sample size is too small for statistical inference, a definite trend is evident. Neither model was better at describing the choice behavior of UNT animals (table 2), which is not unexpected since 1) they had no prior experience with the chemical cues, and 2) morpholine and 2-phenylethanol are not naturally-occurring chemicals, thus there is no evolutionary reason for the animals to orient to either one spontaneously. Why would juvenile sea turtles be expected to orient to a chemical cue even if they were imprinted to it? Normally the response would be expected only in reproductively active adults. Because the motivation to orient to a chemical cue in this study cannot be reproductive, a response could still be shown to a substituted motivation. Since the turtles were unfed, food searching in the behavioral arena could possibly be elicited in place of reproductive responses normally associated with the stimulus in adults. If a group of animals had been imprinted during the critical period, a prolonged orientation to the stimulus could be demonstrated. A demonstrated recognition to a chemical cue might then be achieved without attempting the logistically difficult experiment on migratory adults. Although there is evidence of chemical preferences exhibited by these animals, the results are equivocal<sup>4</sup>. The indication that choice threshold theory underlies chemosensory choice behavior of these animals offers a partial explanation of these equivocal results, as the lack of

sufficient reproductive motivation to orient to a chemical stimulus, not itself a food odor, may not have been adequately replaced by the motivation to search for food in 'home' waters.

Finally, comment must be made about the apparently different mechanisms underlying food choice behavior of the hatchlings and chemosensory choice behavior of the juveniles. These results may be due to an underlying biological difference in selecting food versus responding to non-food chemical cues, or to the difference in experimental protocol, i.e., a 'reward' versus a 'non-reward' situation. Because juvenile loggerheads are subject to the same patchy distribution of food in the marine environment as are hatchlings and thus would equally benefit from food choice behavior following the PR model, we speculate that the former is true. This is an easily-testable hypothesis, and is the focus of research planned by the third author.

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### Locomotory response of *Phreatichthys andruzzii* Vinciguerra (Pisces, Cyprinidae) to chemical signals of conspecifics and of closely related species

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**Summary.** The phreatic cyprinid *Phreatichthys andruzzii* Vinciguerra, tested singly in a choice-apparatus, is strongly attracted to the chemical signals of unfamiliar conspecifics, while it is completely indifferent to the chemical cues of *Barbus semifasciatus* var. *schuberti*, a species that is close systematically. The stimulus and response are evidently species-specific.

**Key words.** Cave fish; chemoreception; locomotory response.

The attraction of isolated specimens to conspecific odor has long been known in several species of epigean fish<sup>1-4</sup>. Recent experiments verified the existence of an analogous phenomenon in the blind cave forms of *Astyanax mexicanus* Filippi<sup>5,6</sup> and in the hypogean species *Caecobarbus geertsi* Boulenger<sup>7</sup> and *Phreatichthys andruzzii* Vinciguerra<sup>8</sup>. Specimens of the cave forms of *A. mexicanus* can discriminate not only between the odor of conspecific groups of different size (4, 8, 16, 32 individuals) tested in pairs, systematically preferring the larger ones<sup>9,10</sup>, but also between familiar and unfamiliar groups of equal size, where they show a preference for the familiar group<sup>6</sup>. Similarly, in *P. andruzzii* the attraction to chemical information of conspecifics is more immediate and intense when they are familiar to the test fish<sup>8</sup>. In the light of these findings we decided to investigate whether the reaction of this species – which shows very marked morphological regression and functional adaptations related to the subterranean habitat<sup>11,12</sup> – has remained species-specific or has become merely a response to generic fish-odor. The loss of the species-specificity of the chemical stimulus and/or of the response to this, brought about in the process of their adaptation to a subterranean habitat, where there are no known sympatric species, would not have compromised either the biological function of the stimulus or the significance of the biological response. The locomotory response of *P. andruzzii* to the chemical traces of unfamiliar conspecifics and of *Barbus schuberti*, the golden variety<sup>13,14</sup> of *B. semifasciatus* Günther, which is an epigean species close to it systematically, was tested using the method employed in earlier experiments<sup>7,8</sup>.

**Materials and methods.** Three groups of native *P. andruzzii* and one group of *B. schuberti* were used. Two of the three groups of *P. andruzzii* (A1: 32 specimens; A2: 33 specimens) came from wells in the locality of Bud-Bud (Central Soma-

lia) and one (B: 31 specimens) from a well in Gheriale, 7 km south of Bud-Bud. The group of *B. schuberti* (C: 35 specimens) was purchased from a pet store. Both the groups of *P. andruzzii* and *B. schuberti* were kept in similar glass aquariums (100 × 40 × 50 cm, water height 40 cm) with an adsorbing charcoal filter and aerator, taking all the necessary precautions so that there was no passage of chemical information between the various aquariums either during normal maintenance or testing. Sixty days before testing, the aquariums were emptied and washed, and both the filter material and water renewed. Six days prior to testing the operation was repeated without renewing the water.

The test aquarium was a narrow glass corridor (105 × 8 × 20 cm, water height 8 cm) divided by removable partitions into three compartments 35-cm long. The test fish was selected from either group A1 or A2 and placed in the central section of the test aquarium where it was allowed to acclimatize for 9 to 13 h, considered sufficient time for the animal to overcome the stress of transfer. Then two 300 cc samples of water – one odorless and one taken from either the home aquarium of group B (A × B tests) or group C (A × C tests) – were simultaneously poured into the two end compartments. For each experiment, the introduction of the 'odorous' sample in the right or left end was determined using a table of random numbers. For both types of tests (A × B; A × C) the odorous sample was poured the same number of times in either end. After 5 min, sufficient for the two samples to spread in the end compartments, the partitions were removed and the fish was then free to swim the length of the test aquarium. At this point the presence of the fish in each of the three compartments, whose borders were indicated by the position of the partition slots, was recorded every 30 s for 30 min for a total of 60 successive registrations. During each experimental session an equal number of both types of tests