Fish as a Model to Study Non-verbal Numerical Abilities

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Numerical abilities are widespread among vertebrates

Abilities such as recording the number of events, enumerating items in a set, or comparing two different sets of objects can be adaptive in a number of ecological contexts. Lyon [1], for instance, reported a spontaneous use of numerical information (egg recognition and counting) in a natural context as a strategy to reduce the costs of conspecific brood parasitism in American coots. McComb and co-workers [2] using playback experiments found that wild lions based the decision whether or not to attack a group of intruders on a comparison of the number of roaring intruders they had heard and the number and composition of their own group. Extensive laboratory research carried out on apes and monkeys has revealed the existence of non-verbal systems of numerical representation that non-human primates apparently share both with infants and with human adults tested in comparable conditions [3]. In the last five years, an increasing number of studies has focused their attention also on numerical abilities of species more distantly related to humans, such as fish, in order to broaden our knowledge on the evolutionary origin of number processing.

Spontaneous quantity discrimination in poeciliid fish

There is substantial evidence that, in social situations, individual fish in unfamiliar environments tend to join other conspecific and, if choosing between two shoals, they exhibit a preference for the larger group [4-8]. This spontaneous preference for joining the larger shoal is commonly adopted to study fish ability to discriminate between quantities. In these tests the experimental apparatus is usually composed of three adjacent tanks. The central one, the ‘subject tank’, houses the test fish. At the two ends two ‘stimulus tanks’ (in which two shoals are inserted) face the subject tank. Subject is inserted in the subject tank and his/her behaviour is recorded for 10-15 minutes. Shoal preference is calculated as the time spent by the subject near the glass facing either of the stimulus tank.

Both mosquito-fish (*Gambusia holbrooki*) and guppies (*Poecilia reticulata*) proved able to discriminate between shoals differing by one unit up to 4 (1 vs. 2, 2 vs. 3 and 3 vs. 4) showing apparently the same effort (that is, fish accuracy was not affected by numerical ratio in the small number range). At the same time, fish could discriminate larger shoals provided that numerical ratio was at least 1:2 (i.e. 4 vs. 8). Performance above 4 units showed ratio-dependence and the capacity to discriminate between two quantities became increasingly accurate as the ratio between them increased [9]. Similar results have been reported in angel-fish (*Pterophyllum scalare*) [6-7]. As a reference, in a recent study [5] a group of undergraduate students was required to estimate the same numerical ratio presented to poeciliid fish. Participants had to estimate the larger of two groups of dots while prevented from verbal counting. Interestingly, humans and fish showed almost identical performance patterns for small and large quantities, suggesting that the evolutionary emergence of our quantity abilities may be more ancient than we have previously thought.

Use of number by poeciliid fish

Much debate has arisen over the exact mechanisms enabling animals to make such a discrimination. Since stimulus numerousness co-varies with non-numerical extent, such as the total area occupied by objects, the sum of their contour, their density and luminance, organisms can provide quantity judgments without necessarily being capable of numerical representation [10-11]. For instance, in a shoal choice test fish may select the larger shoal by using the overall space occupied by the groups instead of numbers. The assessment of numerical capacities in animals requires careful controls to exclude non-numerical cues being used in place of number.

One experimental strategy employed to exclude the use of continuous cues in spontaneous preference tests is the sequential presentation of items within each set, so that subjects can never have a global view of the entire contents of the sets. In order to choose the preferred or the reinforced set in these experiments animals have, therefore, to attend to each item and to build a representation of the contents of the set on the basis of the items that come sequentially into view. Then they have to repeat the process for the second set and, finally, to compare the two representations. For example, in Hauser and colleagues' study [12] rhesus monkeys observed experimenters that placed pieces of apple, one at a time, into each of two opaque containers. Monkeys had no opportunity to see the matched groups before selecting one of them and thus had to mentally add the number of food items inserted in each container.

We have recently adapted the item-by-item procedure in a shoal choice test using mosquito-fish as model [13]. The apparatus for shoal choice was modified by confining each stimulus fish in an adjacent and separate compartment. In this way subjects could choose between one large and one small group of companions but they could only see one fish at a time, thus preventing the possibility that they could use non numerical attributes of the shoal, such as the cumulative area occupied by fish, to estimate the larger set. Mosquito-fish proved to successfully discriminate the larger group of social companions also in this test, suggesting a spontaneous number representation.

To investigate whether animal species can process discrete (numerical) discrimination, extensive training procedures are also reported in literature. In a recent series of experiments we adopted one of these procedures consisting of training the subject to discriminate between sets containing different numbers of geometric figures. Mosquito-fish were placed in an unfamiliar tank and trained to discriminate between two doors in order to re-join their social group. Doors were associated with a pair of stimuli consisting of groups of figures differing in numerosity. These figures were controlled for non-numerical variables, therefore fish could solve the task by attending numerical information only. Fish proved be able to use numerical information both in the small (2 vs. 3) and in the large (4 vs. 8) number range [14-15]. Interestingly, their accuracy was not affected by the total set size: mosquito-fish can discriminate 4 vs. 8 as well as 100 vs. 200. On the contrary, numerical ratio affected the performance, and discriminating a 1:2 numerical ratio was easier than a 2:3 or 3:4 ratio. As reference we tested adult humans presenting the same stimuli: again, the performance of humans largely overlapped that of fish. This further supports the idea of similar non-verbal numerical systems shared among vertebrates.

Conclusions and future directions

Recent studies have demonstrated that numerical abilities not only predate verbal language but also have a very ancient evolutionary origin. Fish are able to spontaneously discriminate between quantities and use numerical information when continuous variables are controlled for. To date, two main procedures have been reported in literature: spontaneous shoal choice test and training procedure using social reward. Only recently we have developed a new training procedure [16] using food reward. Subjects are singly housed in rectangular tanks. At intervals, two stimuli (groups of figures differing in numerosities) are introduced at opposite ends of the tank and food is delivered near the stimulus to be reinforced. Time spent near positive stimulus in probe trials is taken as a measure of discrimination performance. To validate the method, we replicated two published studies that used operant conditioning to investigate the mechanisms of numerical discrimination in mosquito-fish: our data indicate a complete overlap of the results obtained using the two different methods. The novel procedure, however, proved to be less time-consuming and showed less limitations than operant conditioning. In this sense, in the next future we might investigate the full range of numerical abilities in poeciliid fish.

More generally, the new paradigm may also be well suited for automation. Stimulus delivery on a computer screen could be synchronized with automated tracking of the fish movements using one of the available programs, which could also serve for the automated measurement and analysis of visual choice. This might provide a system for high-throughput conditioning of fish in a manner similar to approaches already used with rodents and offer a powerful tool in many fish studies involving learning.

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