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Evaluating guppy, *Poecilia reticulata*, as a model for sex differences in cognition.

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ABSTRACT

Whether cognitive differences exist between men and women is a question probably as old as experimental psychology though still highly controversial. Despite the large number investigations, sex differences in performance emerge only in a few cases, such as in visuo-spatial or verbal abilities. However, it is not clear if these differences are biological or due to cultural influences, possibly because we know very little about their underlying mechanisms and evolutionary causes. Sex differences in cognition have been investigated also in few other species, such as monkeys, rodents and chickens. Rodents, in particular, represent a powerful model in the field of spatial abilities, allowing, among the others, complex hormonal manipulations, drugs tests and between-species comparative studies. Recent years have seen a massive increase in the use of fish as models for cognitive research, at the point that fish are predicted to replace classical vertebrates models soon. The existence of cognitive sex differences in fish has not been investigated. Paradoxically, experimenters quite often use indiscriminately fish of both sexes in their experiments, introducing a confound if cognitive sex differences exist.

In this thesis, I studied cognitive sex differences in the guppy, *Poecilia reticulata*, one of the most studied species in behavioural and cognitive research. Male and female guppies are characterized by considerable differences in ecology and behaviour. I compared the performance of males and females in a wide range of cognitive tasks, including visual discrimination learning, novel object recognition, rule learning, reversal learning, spatial navigation, spatial learning and quantity discrimination. Male and female guppies showed similar abilities in solving most of the tasks, with only two exceptions. In a reversal learning task, guppies were initially trained to obtain food by choosing a predetermined colour between two options, a task that the two sexes learned equally well; then, the reward contingency was reversed and subjects had to inhibit the learned preference to select the other stimulus. Females quickly started to choose the new rewarded colour while males persisted longer in selecting the previously rewarded colour. The second difference emerged in a quantity discrimination task. Guppies were required to select the larger between two groups of conspecifics, following a natural tendency of social fish to stay in larger shoals to reduce predation risks. At the beginning of the experiment, females were

much better than males at solving the task; this difference vanished after some minutes. The two observed sex differences are possibly the result of differential action of natural selection on the two sexes. Indeed, male guppies are thought to undergo selection for high persistence that helps in courting females intensively; females are likely to undergo strong selection for predator avoidance skills as predators preferentially target females. The absence of sex differences in the other cognitive abilities can be due to the absence of sex differences in selective pressures, or, alternatively, to the existence of constraints that prevent differentiation.

In many of the tasks in which males and females obtained similar scores, they were nonetheless observed to behave quite differently. In visual discrimination tasks and in a spatial learning task, males were faster than females in deciding which option to choose, suggesting greater impulsivity in males. In the novel object recognition task, males and females expressed equal ability in discriminating the two objects, yet males explored the novel object at the beginning of the test, females some time afterwards. In the spatial navigation task, males persisted longer than females in trying to reach a target behind a transparent barrier.

The general lack of sex differences in cognitive abilities in guppies aligns with the scarce evidence of sex differences in cognitive abilities in mammals. However, I provided evidence that minor differences in performance, such as in persistence, impulsivity and exploratory tendency, typically found in mammals and birds are widespread in fish as well.

Valutazione di *Poecilia reticulata* come modello animale per lo studio delle differenze sessuali nella cognizione.

RIASSUNTO

L’esistenza di differenze sessuali nella cognizione è uno dei temi più studiati fin dalla nascita della psicologia sperimentale, ma tuttora uno dei più dibattuti. Nonostante le innumerevoli ricerche effettuate, sono state rilevate differenze sessuali solamente in pochissimi compiti, come quelli che misurano le abilità verbali e le abilità spaziali. Non è del tutto chiaro però se queste differenze nella prestazione siano dovute a differenze biologiche tra i due sessi oppure a differenze culturali. In parte questo è dovuto al fatto non se ne conoscono i meccanismi prossimi e neppure le cause evolutive. Sono state rilevate differenze sessuali nella cognizione anche in un limitato numero di altre specie. In particolare, i roditori sono utilizzati come modello per lo studio delle differenze sessuali nella abilità spaziali, specialmente nelle ricerche che richiedono manipolazioni ormonali, comparazione tra specie, o sperimentazioni farmacologiche. Negli ultimi anni, i pesci stanno gradualmente sostituendo roditori e altri animali modello nella ricerca in ambito cognitivo. Tuttavia, l’esistenza di differenze sessuali nella cognizione nei pesci non è ancora stata studiata. Paradossalmente, in alcuni esperimenti vengono utilizzati indiscriminatamente pesci di entrambi i sessi, introducendo un potenziale fattore confondente.

In questa tesi ho studiato le differenze sessuali nella cognizione in *Poecilia reticulata*. Si tratta di una specie molto usata nelle ricerche cognitive, in cui maschi e femmine differiscono sostanzialmente per morfologia, ecologia, e comportamento. Ho comparato maschi e femmine di *P. reticulata* in diversi compiti cognitivi, tra cui apprendimento di discriminazioni visive, memoria, apprendimento di regole astratte, orientamento, apprendimento spaziale, e discriminazione di quantità. I maschi e le femmine di *P. reticulata* hanno ottenuto prestazioni simili nella maggior parte degli esperimenti, con solo due eccezioni. In un esperimento di *reversal learning*, i soggetti sono stati dapprima addestrati ad ottenere un rinforzo alimentare scegliendo un predeterminato colore tra due opzioni, un compito che entrambi i sessi hanno appreso con la stessa efficienza. Successivamente, il colore rinforzato è stato invertito e i soggetti dovevano quindi inibire

la tendenza a scegliere il colore precedentemente rinforzato e selezionare il colore che precedentemente non era rinforzato. Le femmine si sono adattate velocemente all'inversione della contingenza del rinforzo, mentre i maschi hanno persistito molto più a lungo a scegliere il colore precedentemente rinforzato. La seconda differenza sessuale è emersa in un compito di discriminazione di quantità. In questo esperimento, i soggetti dovevano discriminare il gruppo sociale più numeroso tra due opzioni, seguendo la tendenza spontanea di questa specie a unirsi al gruppo più numeroso per diluire il rischio individuale di predazione. Le femmine riconoscevano il gruppo maggiore fin dall'inizio dell'esperimento, mentre i maschi solo alcuni minuti dopo. Queste due differenze sessuali osservate sono probabilmente dovute a forti pressioni selettive che differiscono sostanzialmente nei due sessi. Si crede infatti che i maschi di *P. reticulata* siano selezionati per esprimere comportamenti persistenti che sono d'aiuto durante il corteggiamento. Le femmine di *P. reticulata*, invece, sono il bersaglio preferito dai predatori e si ritiene siano selezionate per quei tratti, come la discriminazione della numerosità del gruppo sociale, che aiutano nella difesa dai predatori.

In molti degli esperimenti, nonostante la prestazione praticamente uguale, maschi e femmine hanno però mostrato di comportarsi in modo differente. In compiti di apprendimento di discriminazioni visive e spaziali i maschi erano molto più veloci nello scegliere una delle due opzioni, suggerendo una maggiore impulsività in questo sesso. In un compito di memoria, entrambi i sessi mostravano comportamento esploratorio rivolto verso un nuovo oggetto, tuttavia i maschi lo esprimevano all'inizio del esperimento mentre le femmine diversi minuti dopo. In un compito di navigazione spaziale, i maschi persistevano più a lungo delle femmine nel tentare di passare attraverso una barriera trasparente per raggiungere un gruppo di conspecifici retrostante.

La generale mancanza di differenze sessuali nelle abilità cognitive in *P. reticulata* è sorprendente vista la diffusa presenza di differenze sessuali in ecologia e comportamento in questa specie, ma è tuttavia in accordo con la scarsità di differenze sessuali rilevate nei mammiferi. Tuttavia, questa tesi ha dimostrato come altre differenze minori nella prestazione nei compiti cognitivi che generalmente si osservano in mammiferi e uccelli, come le differenze comportamentali tra i sessi in persistenza, impulsività ed esplorazione, sono diffusi anche nei pesci.

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1. GENERAL INTRODUCTION:

CURRENT KNOWLEDGE ON COGNITIVE SEX DIFFERENCES IN VERTEBRATES

For a diversity of researchers, such as anatomists, geneticists, endocrinologists, and ethologists, the difference between males and females is an interesting field of investigation. This is particularly true for cognitive psychologists. Since the birth of psychology, a lot of efforts have been made to study whether cognitive differences exist between men and women. This interest is driven by the implications the results could have for society, such as regarding education or work. However, this interest is also due to the fact that the issue remains unclear even after almost one century of investigation. One of the most mysterious aspects of cognitive sex differences is why and how they have evolved. An approach to similar problems surrounding the evolution of cognition that is gaining popularity is to adopt a comparative approach by studying animal models.

Sex differences in nonhuman animals have been investigated intensively only in rodent models such as the rat and the mouse. The literature on other species is still limited and, arguably, much more investigation is needed to obtain clear conclusions. Moreover, the available knowledge has not been studied and interpreted as a whole across all species. The lack of research on these topics limits the use of the comparative approach for studying cognitive sex differences.

The research presented in this thesis is the first complete investigation on cognitive sex differences in a species diverse from human, rat, and mouse. The introduction of this thesis (chapter 1) is the first review of cognitive sex differences in vertebrates. Therefore, this introduction has two goals. The first goal is to provide the background for the experimental section of the thesis presented in the following chapter. The second goal of this introduction is to examine eventual issues in the research on this topic (chapter 1.3.1) and to identify similarities, differences and general trends of cognitive sex differences across the different vertebrate species (chapter 1.3.2). This latter purpose is key for the analysis of the result of the experimental section in the last chapter of the thesis.

1.1 Cognitive sex differences in humans

Humans were the first species studied in cognitive sex differences research, and the most studied so far. The great majority of studies have compared the scores of men and women on cognitive tasks to determine whether either of the two sexes obtains better performances. These differences in performance have been classically thought to derive from differences in cognitive abilities (Halpern, 2013). However, some studies have suggested that this approach has several limits. Performance is not fully described by the score. For example, some tasks may be solved by the different participants with different strategies, resulting nevertheless in equal scores. Alternatively, differences in performance may also arise from differences in the strategy adopted to solve the task, as one strategy may be intrinsically more efficient. As a consequence, several lines of investigations have considered other aspects of the cognitive performance of men and women, with special attention to the way the two sexes acquire, process, store, and act on information, independently from their ability (e.g., Shettleworth, 1999; Sih & Del Giudice, 2012). Different strategies for accomplishing these goals often result in different performances, indicating that one strategy could be more suitable than others for completing a specific task. These aspects of cognition that affect cognitive performance but are not indicators of cognitive ability are often referred as “cognitive style,” especially in comparative psychology (Shettleworth, 1999; Sih & Del Giudice, 2012).

Men and women have been compared on almost every possible cognitive task (Halpern, 2013). However, sex differences in performance have been consistently found only for a few types of tasks, such as verbal, spatial, and mathematical tasks. Sex differences have occasionally been found for other tasks, but they are not confirmed by following studies and therefore are less likely to be a real phenomenon.

1.1.1 Verbal tasks

In early psychological research, women were thought to perform better than men on verbal tasks. This sex difference has been inferred from literature of both experimental and clinical studies. Since it has been reported in many classical textbooks, sex differences in verbal tasks have become common knowledge (Wallentin, 2009). However, a careful reading of the whole body of literature reveals much evidence against the superiority of women in verbal tasks (Wallentin, 2009). Nowadays, many authors believe this sex difference was somewhat exaggerated (Hyde & Linn, 1988; Wallentin, 2009).

Perhaps the paradigm most used for studying sex differences in verbal abilities is the verbal fluency task (Wallentin, 2009). This task is indeed commonly used in both clinical and experimental psychology. In the verbal fluency task, subjects are asked to come up with as many words as possible in a minute. Words must satisfy a specific requirement. There are usually two conditions: in the “lexical” condition, words must start with a particular letter; in the “categorical” condition, words must represent objects from a specific category. For example, Weiss and colleagues (2003) found women to perform better than men in both the lexical and the categorical condition among a sample of 97 college students. The men and women observed in this study were not balanced in terms of age and education, however, introducing potential confounding variables. Some studies agree with the result of Weiss and colleagues (e.g., Crossley et al., 1997; Lezak, 1995), suggesting women have greater verbal abilities than men. However, many other studies have found very different results (Kempler et al., 1998; Mathuranath et al., 2003; Tombaugh et al., 1999; Van Der Elst et al., 2006). For example, in a study of 1300 subjects using the verbal fluency paradigm, Tombaugh and colleagues (1999) found no evidence of sex differences, suggesting sex does not predict performance on this task, when using large sample size and controlling for confounding variables such as age and education. Van Der Elst and colleagues (2006) and Kempler and colleagues (1998) found instead that men were superior in the professional naming and animal categories, suggesting that sex difference may be related to specific categories of words. A very interesting result on this issue came from a study of Weiss and colleagues (2006) that investigated the strategies adopted by men and women to solve verbal fluency tasks. Their results indicated that women tend to switch more often between different word categories than men, a strategy that results in generating more words on average (Weiss et al., 2006). This result indicated that the sex differences in verbal fluency tasks may be due not to women's having superior verbal ability, but rather to their use of a more efficient strategy to solve the task.

Other studies have found that women perform better on different verbal tasks, such as those in which participants are required to recall a determined set of words (Ruff et al., 1989; Trahan & Quintana, 1990). However, one of the stronger pieces of evidence claiming to support the existence of women's superiority in verbal abilities is provided by developmental studies. The importance of developmental studies is that an effect reported since childhood is less likely to suffer interference from confounding factors, such as education level. For example, in their famous work, Kramer and colleagues (1997) reported an investigation of sex differences in 800 children between the ages of 5 and 16. Participants underwent to five sessions to learn lists of 15 semantically related words, which they were required to recall 20 minutes later. At all ages, girls outperformed boys. This kind of paradigm is based on verbal learning rather than verbal knowledge. Interestingly, in Kramer and colleagues' (1997) study on measures of vocabulary knowledge, males outperformed females, suggesting that the observed differences are not due to this confounding factor. The sex difference observed in children with the verbal learning paradigm appears to be present also in adulthood (Kramer et al., 1988), and it is perhaps the most consistent with respect to other tasks such as the verbal fluency task. However, it has been underlined that for verbal learning experiments, sex differences can be due to the use of different memory strategies between men and women, rather than to sex related differences in verbal abilities. Women are indeed more likely than men to use a semantic clustering strategy to remember the words presented during training and this strategy could be more effective than the strategies adopted by men (Kramer et al., 1997).

Other developmental studies focused on first language acquisition. In one- to two-year-old children, for example, girls scored better than boys in both vocabulary production and comprehension (Feldman et al., 2000). This effect, although it was also consistently found in large a meta-analysis (Wallentin, 2009), is usually very small and explains only up to 2% of the variance. Furthermore, the differences in first-language acquisition vanished when older children were tested with this paradigm (Bornestein et al., 2004). The current interpretation of these results is that early language-acquisition differences arise from a general, non-specific developmental difference between the girls and boys, which is also measurable in domains other than verbal abilities (Wallentin, 2009).

Researchers who support the existence of greater verbal ability among women, often look for its adaptive value and have generated hypotheses that explain the evolution of these sex differences. The most important is perhaps the “hunter-gatherer” hypothesis, which suggests that sex differences have arisen because of labour subdivision (Kolb & Whishaw, 2001). In early human societies, men travelled broadly to hunt in small groups or even alone; women instead moves less compared to men, but women lived in larger social groups. Therefore, women, but not men, were selected for enhanced social skills, such as verbal abilities, to improve communication within the group.

Researchers who deny the existence of sex differences in verbal abilities claim instead that the better scores of women observed in some, but not all, experiments are mainly due to differences at the cultural level (Wallentin, 2009). Girls, for instance, may spend more time in reading books than boys. This last point of view may be the best fit to current knowledge, as large meta-analyses have tended to suggest that sex differences in verbal abilities do not exist (Wallentin, 2009) or that they existed in the past but are rapidly reducing (Hyde & Linn, 1988). In this case, we are not speaking of a biological difference, but rather of a cultural difference; gender difference is therefore more appropriate than sex difference.

1.1.2 Spatial tasks

The most studied sex differences in cognition are spatial tasks. The concept that men perform better than women is widely accepted, but the presence of many inconsistencies in the results have given rise to a debate among researchers (e.g., Caplan et al., 1985; Voyer et al., 1995).

Many different spatial tasks have been used to study sex differences. Perhaps, the one that has provided the most established result is the mental rotation task. Participants are required to identify the rotated versions of a target stimulus among distractors. Men usually achieve much better scores than women (Collins & Kimura, 1997; Tapley &

Bryden, 1977; Schoenfeld et al., 2015). Interestingly meta-analysis has clearly confirmed that man perform better on the mental rotation task, with sex explaining up to 40% of the variance in performance (Maeda & Yoon, 2013). Developmental researchers have modified the mental rotation task for infants using a habituation procedure and found that male superiority in the mental rotation task is already present in three- to five-month-old children (Moore & Johnson, 2008; Quinn & Liben, 2008). This consistent sex difference in the mental rotation task is considered strong evidence that men have better spatial abilities than women.

The large use of the mental rotation task may be due to its simplicity. Yet, it is clear that this task presents some methodological issues. Real movement in the environment may require different visual computations than recognition of rotated objects. It is therefore difficult to understand which aspects of spatial cognition differ between men and women (Moffat et al., 1998). Nowadays, computerized techniques allow presentation of virtual environments to participants and thus facilitate the study of more complex and realistic spatial problems. For example, participants might be asked to learn a route in a virtual maze. Men substantially outperformed women on these tasks (Astur et al., 1998; Astur et al., 2004; Moffat et al., 1998), supporting the results obtained with the mental rotation task..

According to this evidence, many authors believe that men have better spatial abilities compared to women. Several evolutionary explanations have been proposed for this sex difference in humans (reviewed in Jones et al., 2003). However, these hypotheses still lack of experimental tests, or, when testes are present, they tend to exclude hypotheses (Jones et al., 2003). For example, the hunter-gatherer hypothesis claims that the division of labour between the two sexes has caused men to range more than women for hunting and to be often involved in tracking and following animal movements, which results in a positive selection for spatial abilities in men (Jones et al., 2003; Silverman & Eals, 1992). Alternatively, the “range size” hypothesis suggests that since humans are a mildly polygamous species, men may have ranged further than women to enhance mating opportunities and men therefore were selected for enhanced spatial abilities (Gaulin, 1995). Because the predictions are similar, it is impossible to test or to disentangle these two possibilities in our species.

It should be said that sex differences in spatial abilities have also been investigated with a large number of other tasks. Meta-analyses have consistently showed that men and women reached overall equal performance in these other tasks (Linn & Petersen, 1985; Voyer et al, 1995). Therefore, some authors have pointed out the possibility that the sex differences observed in mental rotation and virtual maze tasks are not indications of sex differences in spatial abilities, but rather indicate sex differences in other factors that determine performance in only these specific tasks (Caplan et al., 1985).

Further support for the possibility that men’s superior spatial performance is limited to some specific tasks comes from a paradigm that consistently yields to better performance among women. In the object location memory task participants are usually exposed to a set of objects in a determinate position, such as in a grid of squares. Then the objects are removed, and participants are required to indicate their original position. The ability to perform this task is thought to depend on multiple processes. Each object could be located on the basis of its precise position, as well as on the basis of its relative position compared to the other objects. There is much evidence that women perform better on the object location memory task, (Eals and Silverman, 1994; James & Kimura, 1997; Postma et al., 1998; Silverman et al., 2007; Silverman & Eals, 1992; Spiers et al., 2008). Moreover, a recent meta-analysis also agreed that for participants above the age of 13, females usually achieve larger scores than males (Vojer et al., 2007). These results have suggested that women possess better spatial abilities underlying the performance on this task. Therefore, the men's superiority in spatial abilities appears not to be a general effect but is related only to specific processing components. The evolutionary explanation for this sex related difference in object location memory has been proposed on the basis of the hunter-gatherer hypothesis (Silverman & Eals, 1992). This hypothesis sustains that subdivision of labour in the early evolutionary history of the species caused men and women to adapt to different cognitive challenges by evolving different cognitive skills (Kolb & Whishaw, 2001). In particular, women obtained food sources by gathering, while men did so by hunting. Women's foraging strategy is thought to require different spatial competences than hunting. The spatial skills required for females are the ability to learn and memorize the location of fixed food sources (e.g., edible plants) in the midst of complex vegetation (Silverman & Eals, 1992). Moreover, an efficient strategy to remember the positon of fixed food sources would be to represent the configuration of objects in a spatial array (Silverman & Eals, 1992). Even for the object location memory task, in which sex differences appear quite robust, there are alternative explanations. For example, James and Kimura (1997) suggested that women perform better on object location memory tasks because they have better memory for objects *per se*.

Possibly, the largest critique to the existence of sex differences in spatial abilities is the fact that, as previously observed for verbal abilities, the two sexes quite often exploit different strategies to solve spatial tasks. When navigating in a maze, men tend to use geometric characteristics of the environment, while women tend to use landmarks and therefore focus on the objects in the environment (Galea & Kimura, 1993; Sandstrom et al., 1988; Saucier et al., 2002). In another experiment to study this phenomenon, participants were required to choose an object based on its characteristics or by its location (Jones & Healy, 2006). Here, women used the visual characteristics of the object to solve the task, while men used the location (Jones & Healy, 2006). Since the two sexes use different strategies to solve the same problems, it is difficult, if not impossible, to understand whether one sex has greater spatial abilities. Indeed, if one of the two strategies is somewhat more efficient, one sex would achieve better performance independently from spatial abilities. The sex differences favouring men in spatial tasks such as mental rotation may therefore be due to sex differences in the ‘style’ used by either sex to solve the task rather than to sex differences in spatial abilities. At the same level, women may perform better on object location memory tasks because they use a strategy that is more attuned to remembering the exact location of an object.

Lastly, in these spatial tasks, the magnitude of sex difference has decreased in recent years (Voyer et al., 1995). This suggests that cultural differences could have affected in the early reports that men scored better on spatial tasks.

1.1.3 Mathematical tasks

There is a third potential cognitive sex difference in humans’ mathematical abilities. There is evidence that men outperform women in solving mathematical problems. However, there is also much contrasting evidences, and it is possible that the sex difference in performance is not due to biological differences between the two sexes but rather to cultural differences.

One of the earlier findings suggesting that men possess superior mathematical abilities is the differential representation of the two sexes in high-level careers in mathematics and science. Since most of the employees in these fields are men, men were thought to have better intrinsic aptitude to mathematics (Spelke, 2005). This deduction, of course, lacked scientific validity and testing (Spelke, 2005). Following studies tried to compare the performance of men and women on mathematical tests more rigorously using a standardized battery of tests. Most of these studies exploited the scores of scholastic aptitude tests and demonstrated an advantage of boys over girls (e.g., Benbow & Stanley, 1980; Benbow & Stanley, 1983; Gallagher et al., 2000; Leahey & Guo, 2001). These results have been broadly generalized and are thought to be representative of the entire population.

Assuming that men have an advantage in mathematical tasks, how can we explain its evolution? Indeed, it is quite difficult to imagine that selection has favoured men's ability to solve equations, for example. In this perspective, some authors have suggested that this sex difference is an indirect effect of selection for enhanced spatial abilities (Geary, 1996). As a result of selection for spatial navigation, males are thought to have better skills for geometrical problem solving (Geary, 1996).

One of the problems with studies based on scholastic aptitude tests is that they provide data from a subsample of the population. This subsample cannot be considered representative. Indeed, meta-analyses have revealed that in samples from the general population, males have no or only a negligible advantage in mathematical tasks (Friedman, 1989; Hyde et al., 1990). Moreover, among children from elementary and middle schools, girls outperform boys (Hyde et al., 1990). Since the only difference favouring men arises from samples of high schools and colleges (Hyde et al., 1990), it seems that the men's superiority in mathematical tests exists only in a selective subsample of the population. In the light of this evidence, the hypothesis that men have better cognitive abilities in this field looks less convincing, and a number of alternative explanations have been proposed.

Sex differences in scholastic tasks may be the result of cultural differences, and therefore gender differences should be considered rather than sex differences in mathematical abilities. For example, in cross-national comparison, gender equity is one of the most powerful predictors of sex differences in mathematical tasks (Else-Quest et al., 2010). The fact that the magnitude of sex difference in mathematical tasks has been declining over the years, similarly to the trend observed for gender inequity, also indirectly supports the gender difference interpretation (Hyde et al., 1989). Another source of sex differences in mathematical scores is gender stereotypes. Spencer and colleagues (1999) showed that in tasks that usually produce sex differences, women achieve the same performance as men if the task is described to the participants as not producing sex differences. Lastly, as in verbal and spatial tasks, the possibly exists that men have better mathematical abilities because they have a different, more suitable approach to the problems (Gallagher et al., 2000).

1.1.4 Human cognition: sex differences or sex similarities?

The three fields previously described (i.e., verbal, spatial, and mathematical tasks) provide the largest and most reliable evidence of sex differences in human cognition. It is interesting and surprising that only three sex differences have emerged among the number of cognitive tasks in which men and women have been compared (Halpern, 2013). Even in those contexts, the differences between the two sexes are usually quite small and there is a broad overlap between the performance of men and women. Frequently, follow-up studies have failed to achieve replication of positive results, and meta-analyses have often supported the absence of sex differences. Looking at the overall picture, similarity, rather than difference, seems to describe cognition between men and women. It is possible that researchers have given too much emphasis to the few differences and ultimately exaggerated them.

Aside from these issues about the existence and size of sex differences in cognitive performance, even when they appear quite clear, as in the case of sex difference in the mental rotation task, their nature is still far from being understood. Some authors have claimed that sex differences in performance derive from sex differences in cognitive abilities. This conclusion is challenged by a number of issues. The first one is the confounds of gender differences due to culture. Indeed, it is not clear whether these differences are biological or due to cultural influences and therefore gender rather than sex differences. It is possible that the duality between sex differences and gender differences is little understood because very little is known about the underlying mechanisms and evolutionary causes of the phenomenon. The second main problem is that most of these sex differences in performance could be more parsimoniously explained by the use of different strategies to solve the task. In such case, there would be sex differences in terms of not cognitive abilities but other cognitive aspects. Some authors may have ascribed these kinds of differences to cognitive style rather than cognitive ability (Shettleworth, 1999).

1.2 Cognitive sex differences in nonhuman species

Cognition is expected to respond to selective pressures as other traits do and, consequently, to evolve adaptively (Sherry et al., 2006). As proposed by several psychologists, although never demonstrated, human sex differences in cognition are likely to have evolved because of sex differences in ecology and behaviour (Gaulin, 1995; Jones et al., 2003; Kolb & Whishaw, 2001). In the evolutionary history of human species, these differences in ecology and behaviour caused men and women to face different environmental challenges. Selection might have favoured the evolution of sex-specific cognitive phenotypes. For example, women may have been embedded in more complex social systems compared to men, and therefore they may have faced the problem of communicating with others more often (Kolb & Whishaw, 2001). The requirement for social communication skills might have driven the evolution of enhanced verbal abilities in women compared to men (Kolb & Whishaw, 2001). Men, in contrast, were more likely than women to take long trips, such as for hunting purposes (Gaulin, 1995; Jones et al., 2003). The need to solve spatial navigations problems might have caused the evolution of enhanced spatial abilities in men (Gaulin, 1995; Jones et al., 2003). Although the possibility that sex differences in cognition are due to behavioural and ecological sex differences is interesting, empirical tests of this hypothesis remain difficult, if not impossible, in humans (Kimura, 2004).

Compared to other species, human sex differences in behaviour and ecology are considered to be mild (Gaulin & FitzGerald, 1986). Many other animals show much more extreme sex differences in these aspects (reviewed in Magurran & Garcia, 2000; Selander, 1966; Shine, 1989). If cognitive sex differences evolve because of sex differences in behaviour and ecology, the interesting possibility exists that cognitive sex differences are present in many other species, perhaps to a larger extent than in humans. Accordingly, animal models have been advocated for the study of sex differences in cognition, as well as their neurobiological bases and evolutionary origins.

The comparative approach based on animal models also offers two advantages compared to research in humans. The first is the absence of cultural confounds. The second advantage is that nowadays translational research on animal models is the basis of biomedical experimentation. Therefore, understanding sex differences in animal models might allow future utilization of research on those cognitive diseases with sex-specific characteristics, such as Alzheimer’s disease, and for developing sex-specific medications.

Unfortunately, very few attempts to investigate sex differences outside humans have been made. The only exception is represented by rodent models of learning and memory (rats and mice), in which sex differences in cognition have been investigated quite intensively.

1.2.1 Rodents: sex differences in spatial tasks

Over the past two decades, rats and mice have been broadly adopted as models in learning and memory research. Most of the tasks adopted for this purpose required the animals to learn and remember how to navigate in a maze. Early paradigms consisted of simple T-mazes in which the subjects had to choose a predetermined arm of the maze to get a reward, usually a piece of food. In these experiments, male rodents substantially outperformed females (e.g., Joseph & Gallagher, 1980). However, some authors advocated that behavioural sex differences in locomotor and exploratory activity could account for these results (Beatty, 1979). Later paradigms were supposed to overcome the problem of sex differences in behavioural traits (Jonasson, 2005).

One of the innovative spatial paradigms often used in rats is the Morris water maze task (Morris, 1984). The subject is inserted in a large circular pool and has to find a visible or invisible platform that allows it to escape the water. After repeated trials, the subject is expected to learn to navigate to the platform by using the features of the pool and the room surrounding the pool. Male rats usually show better learning scores than females in the Morris water maze task (Jonasson, 2005; Perrot-Sinal et al., 1996; Saucier et al., 2008). Interestingly, in mice a male advantage over females has been observed (Upchurch & Wehner, 1988), but there are also several reports of the opposite results, with females performing better than males (Jonasson, 2005). One possible explanation is that the paradigms were developed for rats and therefore lead to odd results in mice (Whishaw & Tomie, 1996). In rats, these results have been questioned by successive observations. For example, there is evidence that female performance is impaired because of the acute stress effect of the forced swimming in the Morris water maze (Beiko et al., 2004). Indeed, long habituation with the apparatus before the experiment diminishes or eliminates sex differences in performance (Beiko et al., 2004; Perrot-Sinal et al., 1996).

Another task often adopted to study sex difference in rodents is the radial maze (Olton, 1987). The subject is inserted in a circular arena from which eight arms depart. At the far end of each arm there is a hidden feeder. After visiting the feeder in one arm and consuming its food, the subject is expected to visit a new arm. The accuracy in visiting new arms and in avoiding the arms previously visited is used as a measure of spatial abilities. The radial maze is thought to also involve aspects of memory other than spatial. For example, visits to previously visited location are thought to mirror reference and working memory errors. Although there are differences related to strain and experimental protocols, males have a general advantage in the radial maze compared to females for both rats (Jonasson, 2005; Roof, 1993; Seymoure et al., 1996) and mice (LaBuda et al., 2002; Jonasson, 2005).

Many other versions of mazes for rats, such as the Tolman sunburst maze, have found better performance in males (Dawson, 1972; Joseph et al., 1978). It is quite clear that in rats, males have an advantage in different types of spatial tasks, suggesting that males have greater spatial ability. In mice the situation is less clear. However, there are also alternative explanations. For example, in rats the hippocampus maturates faster in males than in females (Bucci et al., 1995). Thus, it is possible that the male advantage in spatial tasks is not due to sex differences in spatial abilities but rather to the different maturation rate of the hippocampus between male and female rats. Indeed, if tested at the age of six months, when the hippocampus is completely maturated in both sexes, male and female rats showed no sex differences in the Morris water maze (Bucci et al., 1995).

As observed in humans, in rodents there is also evidence that the strategies through which males and females solve spatial tasks may be diverse. Tropp and Markus (2001) trained rats to choose a predetermined arm of a four-arm maze indicated by both a large white panel outside the maze and by local inserts inside the maze. After the rats learned the task, distant and local cues were rotated 90° clockwise and counterclockwise, respectively. Males chose the arm indicated by the outside panel, suggesting a preferential use of distant cues. Females appeared to use both cues (Tropp & Markus, 2001). In the Morris water maze task, female rats appeared to navigate on the basis of cues both close to the target (Sava and Markus, 2005) and in the room surrounding the pool (Roof & Stein, 1999; Sava and Markus, 2005) according to their oestrous state (Sava and Markus, 2005); males instead tend not to use proximate cues. In mice there is evidence of differential navigation strategies as well: males use only extra-maze cues to orientate, whereas females also use landmarks within the maze (Bettis and Jacobs, 2009). Lastly, a similar sex difference in cue utilization may also exist in kangaroo rats (*Dipodomys merriami* and *D. microps*), as females' spatial learning is impaired by the deletion of local landmarks, but males' is not (Barkley and Jacobs, 2007). These sex differences may be due to sex differences in cognitive style in rodents. It is possible that the navigation strategy adopted by males is more efficient for solving the spatial learning tasks used in rodents. If this is correct, male advantage in spatial learning tasks might be determined by sex differences in cognitive style rather than ability. Supporting this possibility is the finding that female rats are better than males in object location memory (Saucier et al., 2008). It is worth noting that the results of research on sex differences in spatial navigation tasks, object location memory, and navigation strategies are extremely similar to results obtained in humans.

The large number of species of this order and the presence of closely related species with differences in ecology allows testing of evolutionary hypotheses, a possibility completely lacking in human psychology. The pioneering works of Gaulin and FitzGerald (1986) tested the range size hypothesis, according to which male superiority in spatial tasks evolves as a consequence of the sex differences in range size typical of polygamous mating systems. The two researchers compared the spatial learning performance of males and females in two species of congeneric rodents, *Microtus pennsylvanicus* and *M. pinetorum*. In the former species, males are polygamous and range in a much larger territory compared to females; in the latter species, males and females form monogamous pairs and share the same territory. Consistently with the prediction of the range size hypothesis, male *M. pennsylvanicus* showed better learning performance in a maze task compared to females (Gaulin & FitzGerald, 1986). In *M. pinetorum*, the two sexes showed the same performance (Gaulin & FitzGerald, 1986). This result has also been confirmed in other congeneric species (Gaulin & FitzGerald, 1989; Gaulin et al., 1990), perhaps proving the most convincing evidence of adaptive evolution of sex differences in cognition.

1.2.2. Rodents: sex differences in other cognitive tasks

Since rodents have been used as models in several fields of cognitive research, the literature has also provided some indications of sex differences in nonspatial tasks. This evidence is less abundant, however. One of these differences regards the ability to discriminate and recognize objects. Research in this field usually exploits the novel object recognition task. The subject is first exposed once to an object for familiarization; then, the subject is presented with both the familiar and a novel object. Novel object recognition is measured as the preference to explore the novel object over the familiar object. In this task, female advantage has been demonstrated in mice and rats (Ghi et al., 1999; Soutcliffe et al., 2007; Bettis & Jacobs, 2012; Bettis & Jacobs, 2013). In mice, some authors have suggested that this sex difference regards discrimination abilities. Indeed, sex differences in performance are present only if the familiar and the novel object are very similar (Bettis & Jacobs, 2012). However, one experiment also found a reversed pattern in mice, with males, but not females, showing novel object recognition up to 24 hours after familiarization (Frick and Gresack, 2003). Sex differences in novel object recognition are not necessary explained by sex differences in cognitive ability. Also, in this case it is likely that the differences regard aspects of cognitive style, such as attention to object features, or also personality type (Bettis & Jacobs, 2012).

Evidence from many tasks has revealed another general sex difference in rodents that may be related to cognitive flexibility and persistence. In a reversal learning task, Guillamón and colleagues (1986) trained male and female rats to enter a predetermined arm in a T-maze that could be identified because the two arms had different colours. Then, the target arm was flipped. Male and female rats showed equal proficiency in learning the initial discrimination. Yet, once the contingency was reverted, females adapted to choosing the current rewarding arm much faster than males did. Thus, males persisted longer than females in choosing the previously rewarding arm (Guillamón et al., 1986). A similar lack of flexibility in males has also been shown in other tasks. For example, in a classical shuttle box, male rats showed more persistence than females in pressing the wrong lever (Van Halen et al., 1987). According to several authors, differences in cognitive flexibility and persistence are likely to reflect sex differences in cognitive style rather than ability (Sih & Del Giudice, 2012).

Lastly, one study investigated sex differences in quantity discrimination in meadow voles (*Microtus pennsylvanicus*). This species, like many other terrestrial mammals, deposits scent marks along paths. Sometimes these marks are over-marked by conspecifics. Voles can discriminate the number of successive over-marks deposited by an individual

(Ferkin et al., 2005). The study by Ferkin and colleagues (2005) found that female voles were better than males in accomplishing this task.

1.2.3 Nonrodent mammals

Although broad investigation of sex differences in cognition have been made only in humans and rodents, there are sporadic reports in other mammals.

A better male performance in some tasks of spatial memory has been reported for rhesus monkeys (*Macaca mulatta*; Lacreuse et al., 2005). This sex difference already occurs in young monkeys, but it tends to decrease with age (Lacreuse et al., 1999). Interestingly, in this species males and females perform similarly on a large number of other tasks in which spatial abilities are not involved (Lacreuse et al., 1999), a pattern very similar to the one observed in humans. In a congeneric species, there is also evidence of greater cognitive flexibility in females in the reversal learning task (Ha et al., 2011).

Recently, the range size hypothesis has also been confirmed in two species of the order *carnivora*. Males perform better than females in spatial tasks among giant pandas (*Ailuropoda melanoleuca*), a polygamous species, but not in Asian small-clawed otters (*Amblonyx cinerea*), a monogamous species (Perdue et al., 2011).

Lastly, a sex difference favouring females in object recognition has been found in dogs (Müller et al., 2011). In their experiment, Müller and colleagues (2011) exposed dogs to an object for familiarization. After temporary occlusion, they exposed the dog either to the same object or a larger copy of the object. Females, but not males, responded to the increased size with increasing looking time (Müller et al., 2011).

1.2.4 Birds

From an evolutionary perspective, the most interesting studies of cognitive sex differences in birds are related to spatial abilities. In the green-backed firecrown hummingbird (*Sephanoides sephanoides*), only males are territorial. Males are therefore required to accurately keep track of food sources and to defend them. Females instead opportunistically intrude on male territories to feed. In this species, males remembered nectar location better than females in a standardized field experiment (González-Gómez et al., 2014). In two-nest parasitic cowbirds (*Molothrus bonariensis* and *M. ater*), there is a reverse pattern of spatial-abilities requirements. Females search for host nests before reproduction and return to them several days later to lay eggs. Females are therefore expected to undergo selection for enhanced spatial abilities, a pattern reversed compared to the one usually found in polygamous rodents. Astié and colleagues (1998) compared male and female cowbirds in a spatial memory task and found greater accuracy among females. This finding has been recently confirmed with different procedures that better control for the use of nonspatial cues in the task (Astié et al., 2015; Guigueno et al., 2014).

Other studies have investigated utilization of cues during discrimination learning tasks. In domestic chickens (*Gallus gallus domesticuus*), there is evidence of sex-specific strategies to learn discrimination. Vallortigara (1996) trained domestic chicks to discriminate between two objects that differ by both colour and position. In a probe trial, he showed that males had learned to discriminate the target object based on its position, females on its colour (Vallortigara, 1996). This is strikingly similar to the sex differences in spatial navigation strategies observed in rodents and humans. By contrast, in the monogamous *Parus major*, males and females rely on spatial location rather than local cues (Hodgson et al., 2005). Also in hummingbirds (*Selasphorus rufus*, *Hylocharis leucotis* and *Eugenes fulgens*), despite the more complex spatial use of males, there is evidence that both males and females use spatial location cues for spatial orientation (Hurly & Healy, 1996; Tello-Ramos et al., 2014). The mating system of these hummingbirds is not completely clear.

In domestic chicken (*Gallus gallus domesticus*), there is also evidence of reduced cognitive flexibility and increased persistence in males (Rogers, 1974), although this has not been tested with a reversal learning task. After starting to respond to a stimulus, males persist in their response longer than females (Rogers, 1974). Males are also less likely to be distracted by other perceptual stimuli (Rogers, 1974). In sharp contrast, investigations in two monogamous species (*Corvus corax* and *Parus major*) did not find any evidence of sex differences in cognitive flexibility with the reversal learning task (Range et al., 2006; Titulaer et al., 2012). In a third monogamous species (*Taeniopygia guttata*), males instead outperform females in reversal learning (Brust et al., 2013).

1.2.5 Reptiles and anurans

Outside mammals and birds, there are very few studies of sex differences in cognition. In the polygamous lizard *Eulamprus quoyii,* a sex difference favoring males in spatial learning tasks has been observed (Carazo et al., 2014). In lizards, males are usually required to move in a larger territory compared to females and also to process more complex spatial information (Carazo et al., 2014). This result seems therefore to support the range size hypothesis as well.

In the study of Uller and colleagues (2003), male and female salamanders were compared for their ability to discriminate between tubes containing different quantities of live prey. The species investigated, *Plethodon cinereus*, exhibits social monogamy (Gillette et al., 2000; Lang et al., 2000). The two sexes showed the same accuracy in this quantity discrimination task (Uller et al., 2003).

1.2.6 Fish

In the literature there are only three reports of sex differences in fish. Both are side-findings of experiments developed for other purposes. The first study examined social learning abilities, in which female guppies outperform males (Reader & Laland, 2000). The second study found instead that male redtail splitfin (*Xenotoca eiseni*) are somewhat more efficient than females on a spatial task when using geometrical features (Sovrano et al., 2003). These two studies indicated that sex differences in cognitive abilities and cognitive style are present in fish too. Another study investigated sex differences in acquiring a simple conditioning paradigm in the crimson spotted rainbowfish (*Melanotaenia duboulayi*; Bibost & Brown, 2014). Subjects were conditioned to associate the appearance of a red light with a food reward. Across the seven days of training, males and females showed the same increase in performance, suggesting the absence of a sex difference in this task (Bibost & Brown, 2014).

1.3 Trend in research on cognitive sex differences in vertebrates

1.3.1 Issues of cognitive sex differences research

The review of the current knowledge on cognitive sex differences has revealed several issues.

(*i*) Experiments in this field have been conducted in very few model species (humans, rats, mice). Data on other species are scarce.

(*ii*) The three species most investigated (humans, rats, mice) are closely related (e.g., they are all mammals, and two out of three are rodents). This has also caused a bias in the distribution of investigated species throughout the vertebrate phylogenetic tree. Sufficient literature is available for mammals and to some extent birds, but not for lower vertebrates such as fish, amphibians, and reptiles.

(*iii*) In nonhuman animals, sex differences have been studied in a limited number of cognitive tasks (e.g., spatial learning, reversal learning). Part of this issue could be due to the legacy of human literature, in which most of the works regard spatial abilities. On the other hand, it is possible that the lack of methodologies for studying cognition in animals has caused this bias. In any case, to fully understand cognitive sex differences there is arguably a need to investigate many more tasks.

These issues might impose severe limits to research on cognitive sex differences for many reasons. In particular, issues *i* and *ii* are detrimental for comparative research aimed at understanding the evolution of cognitive sex differences in vertebrates. Evolutionary psychologists are interested in understanding how and when sex differences in cognition have evolved in vertebrates. Are cognitive sex differences shared characteristics of vertebrates' nervous systems? Are cognitive sex differences present only after a determined phylogenetic event, such as the differentiation of mammals? Have cognitive sex differences evolved only in a few species or groups because of specific selective pressures? To answer these questions, data on many more species are required. In particular, it is important to include all vertebrates, from mammals to fish, whereas the present data represent only a few mammalian species. The answers to these questions are the starting point to understand the presence of cognitive sex differences in humans too.

Issue *ii* is also detrimental for research aimed at understanding the proximate mechanisms underlying cognitive sex differences. Are sex differences in cognition due to sex differences in hormone production? Are cognitive sex differences related to anatomical sex differences in brain structures? Is there sex-specific genetic variation in cognition?

Although human research has made progress in these fields, the use of animal models remains an important strategy of investigation. Indeed, the study of brain morphology or the manipulation of hormones is possible in animals to a wider extent than in humans, partly because of ethical motives. Additionally, more powerful methodologies for studying genetics are available in animals, such as selective breeding and artificial selection. All of these methodologies are simpler and cheaper to apply in lower vertebrates such as fish (Kalueff et al., 2015).

1.3.2 Conclusions on cognitive sex differences in vertebrates

Aside from the issues above, the review of the existing literature also provides some interesting observations. The major conclusions can be summarized as follows.

(*i*) Sex differences in cognitive tasks are present in all vertebrate classes (table 1).

(*ii*) It is not clear whether sex differences in cognitive tasks arise because of sex differences in cognitive abilities or other factors, such as differences in cognitive style and in the strategies adopted to solve a task.

(*iii*) Sex differences in cognitive tasks that are undoubtedly related to sex differences in cognitive abilities are scarce or perhaps inexistent.

(*vi*) Sex differences in cognitive aspects other than abilities, such as cognitive style, are widespread across all vertebrates.

(*v*) Sex differences in cognition are often related to those situations in which males and females substantially differ in their behaviour and ecology, such as in use of space for polygamous species. This suggests that male and female cognition responds to selective pressures as other traits do and, consequently, evolve adaptively (Sherry et al., 2006).

(*vi*) Sex differences in cognition are more likely to occur in polygamous species (table 1). This can be due to the fact that ecological and behavioural differences are larger in polygamous species because the reproductive roles of males and females are more diverse in such mating system.

(*vii*) Cognitive sex differences are sometimes astonishingly similar between species that are phylogenetically distant (table 1). For example, males outperform females on spatial tasks, such as mazes, in humans, macaques, rats, mice, and two *carnivora*, a lizard and a fish. Females perform better on tasks that require object encoding and use this strategy for solving spatial tasks in humans, rats, chickens, and perhaps fish. Females have enhanced cognitive flexibility and are less persistent than males in rats, macaques, and chickens. This suggests that, irrespective of phylogeny, sex-specific selective pressures on cognition that arise from polygamous mating systems are constant, and so are the resulting adaptations.

Table 1: Main cognitive sex differences in vertebrates. Presence and absence of sex differences are indicated with + and – respectively. **P** and **M** indicate species with polygamous and monogamous mating system, respectively. Polygamous species are also grey highlighted. Almost all sex differences have been observed in polygamous species.

			Spatial learning	Object location	Spatial strategy	Object encoding	Reversal learning	Quantitative task
Mammals	Humans	P	+	+	+	+		+
	Rodents	P	+	+	+	+	+	+
		M	-					
	Other mammals	P	+			+		
		M	-					
Birds		P			+		+	
		M			-			
Reptiles		P	+					
Amphibians								-
Fish		P	+					

1.4 Fish: a rising model for cognitive research

Small fish such as the zebrafish (*Danio rerio*) and the guppy (*Poecilia reticulata*) are rapidly becoming new popular model organisms in many research fields, especially in biomedicine. Fish exhibit homology to humans with regard to genome and physiology (Kalueff et al., 2014; Stewart et al., 2014). Moreover, fish and humans share all major brain structures, neurotransmitters, receptors, and hormones (Kalueff et al., 2014; Stewart et al., 2014). For these reasons, fish are currently used for studying normal and pathological behaviours, in addition to cognitive functions and disorders, and they are used for screening and testing new drugs (Kalueff et al., 2014; Stewart et al., 2014). The importance of fish in these researches is growing exponentially: since 2010, there increase in publication number have been larger for fish than for classical animal models such as rodents (Stewart et al., 2014). Another factor that may have contributed to the recent boom in fish research is the reduced cost of experiments on fish compared to other vertebrates (Kalueff et al., 2015).

Outside the biomedical field, fish are similarly gaining importance for the study of cognition. There are two main reasons for this phenomenon. The first reason is the key position occupied by fish in the vertebrate phylogenetic tree. Being at the root of the vertebrate lineage, fish offer the possibility to investigate the evolution of cognitive functions shared among vertebrates (Bshary et al., 2002; Bshary & Brown, 2014; Brown et al., 2008). The second reason is the wide evolutionary radiation that has made fish the larger vertebrate group. In order to test the adaptive value of cognitive traits, it is important to compare closely related species that differ in their ecology (Bshary & Brown, 2014). This approach is simpler in groups showing large radiation, such as fish (Bshary et al., 2002; Bshary & Brown, 2014; Brown et al., 2008). As suggested by issues *i* and *ii* (chapter

1.3.1), filling in the lack of knowledge on cognitive sex differences in fish might be important to understanding the evolution of this phenomenon in vertebrates.

For the reasons described above, it would be useful to obtain more information about the existence of cognitive sex differences in fish. Interest in the study of cognitive sex differences in fish also exists for practical reasons. For example, most of the research on fish cognition is carried out using indiscriminately male and female subjects or in only one sex, introducing a confounding factor if sex differences exist. Moreover, many cognitive diseases and neurological impairments, such as Alzheimer's disease, have sex-specific occurrence and effects (Li & Singh, 2014; Young & Pfaff, 2014). This requires knowledge of whether similar sex differences occur in fish that are used as models for translational research in these fields to avoid errors during experiments. This also necessitates the development of medicaments specific to the sex of the patient (Li & Singh, 2014; Young & Pfaff, 2014). If fish express sex differences in cognitive ability and style similar to those present in humans, they might became an important resource for translational research with this purpose.

2. EXPERIMENTAL SECTION:

STUDY OF COGNITIVE SEX DIFFERENCES IN *POECILIA RETICULATA*

2.1 Aims of the research

The research presented in this thesis is aimed to investigate the existence of cognitive sex differences in the guppy (*Poecilia reticulata*), a fish species commonly adopted in cognitive studies. I compared the performance of male and female guppies in many cognitive tasks that required, at least in part, different skills to be solved.

A further goal of this thesis is to compare the results obtained in these experiments on guppies with the conclusions that emerged from the review of literature on other vertebrates (chapter 1.3.2). By analysing similarities and differences between guppies and other vertebrates, this thesis aimed to understand whether sex differences in cognition follows a general trend. This last comparison allowed to understand whether fish are suitable as models for sex difference in cognition.

Since this is the first research aimed to investigate in deep cognitive sex differences in a fish species, the literature did not provide useful indications for choosing the tasks that are more suitable. I selected the tasks to compare male and female guppies following two main criteria. The tasks should be commonly adopted in fish research and/or should investigate cognitive performance for sex differences may occur in other vertebrates. A second issue was the difficulty to predict the results of the experiments and to formulate hypothesises on the outcomes. I therefore decided to formulate predictions only in those few cases in which ecological and behavioural sex differences between male and female guppies (*v*, chapter 1.3.2) clearly suggest the possible outcome of the experiment. In the other experiments, I adopted an explorative approach and I did not formulate predictions.

Since previous works on other species have suggested that sex differences in cognitive tasks are not only related to cognitive abilities (chapter 1), whenever possible I also measured a number of variables to characterize the behaviour and cognitive style of male and female guppies during the tasks.

I organized the experimental part of the thesis in four main chapters. In each chapter, I collected the experiments aimed to investigate guppy sex differences in a specific cognitive domain. The first chapter (chapter 2.3) regards sex differences in visual discrimination learning; the second chapter (chapter 2.4) regards sex differences in oddity discrimination learning and in reversal learning, two tasks that require abstract rule learning; the third chapter (chapter 2.5) regards sex differences in spatial learning tasks; the last chapter (chapter 2.6) regards sex differences in quantity discrimination tasks.

2.2 The model species: *Poecilia reticulata*

The study of fish cognition and behaviour involves very few species. The guppy (*Poecilia reticulata*) is possibly one of the most studied species together with the zebrafish and, in the past years, with the goldfish. Other fish, such as the redtail splitfin, the archerfish, or some cichlids species, have been used but with less extent. The guppy has attracted the interest of researchers for the study of morphological, ecological, and behavioural sex differences (Griffiths & Magurran, 1998; Houde, 1997; Magurran, 2005; Magurran & Garcia, 2000). In these contexts, male and female guppies showed many differences (Griffiths & Magurran, 1998; Houde, 1997; Magurran, 2005; Magurran & Garcia, 2000). According to the hypothesis that sex differences in cognition arise because of sex differences in ecology and behaviour (*iv*, chapter 1.3.2), guppies are likely to show some degree of cognitive sex differentiation. I therefore chose the guppy as subject species for this study.

2.2.1 Biological notes on *Poecilia reticulata*

Phylum: Chordata
Class: Actinopterygii
Order: Cyprinodontiformes
Family: Poeciliidae
Genus: *Poecilia*
Species: *Poecilia reticulate*

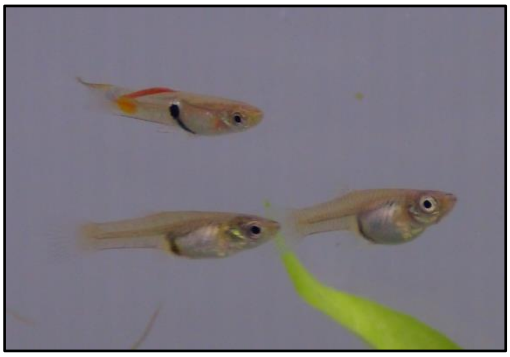

Figure 1: Male (above) and females (below) *P. reticulata*.

The guppy is a freshwater fish native to South America. Original distribution area encompass Trinidad, Tobago, Venezuela, Guyana and Surinami (Magurran, 2005). However, the guppy has been introduced in each continent, apart from Antarctica, for mosquito control and has shown a large adaptability to new environments. The main requirement for its life is the presence of warm water (22-26 °C; Magurran, 2005). The guppy is tolerant to a wide salinity range, but its presence in brackish waters is scarce. In its original distribution area, guppy typically lives in small streams with slow water speed and abundant vegetation, but it can be found also in large rivers (Magurran, 2005).

Sexual maturation occurs two months after birth. The guppy is characterized by large sexual dimorphism (figure 01). Females grow up to 5 cm, a size that is almost twice the male size. Body shape is also different between the two sexes, being females more rounded than males. Female body is grey perhaps to reduce conspicuousness. In sharp contrast, males show a bright colouration based on yellow-orange, black and iridescent spots (Magurran, 2005).

The peculiarity of guppies reproduction is the internal fertilization. Males own a modified anal fin which allowed the transfer of sperms to females (Houde, 1997; Magurran, 2005). One month after fertilization females give birth to a batch of fully developed and independent guppies. Reproduction continues through the year. Males have two strategy to achieve copulation. The first strategy is to obtain consensual mating by courting females (Houde, 1997). The second strategy consists in obtaining coercive copulations via sneaky behaviour (Houde, 1997). Males are involved in sexual behaviour almost all day long, while females devote most of their time budget to foraging (Magurran & Seghers, 1994). Indeed, female fertility increases with the increase in body size (Houde, 1997). The frantic males sexual activity is a possible cause of the spatial sexual segregation observed in this species. Females tend to live deeper and more open water to reduce the disturbance from males (Darden & Croft, 2008).

Research interest in the guppy was born because of its peculiar ecology. However, nowadays the guppy is intensively used as model species in many research fields, such as evolutionary biology, ecology, reproduction, toxicology, genetics, behaviour, and cognition (Brown et al., 2008; Magurran, 2005; Walsh-Monteiro et al., 2014; Wilson et al., 2014).

Because of its wide adaptability, the peculiar reproductive behaviour and the bright male colouration, the guppy is breed for ornamental purpose. Nowadays, dozens of different strains are available from pet sellers. Although domestic strains have more complex colouration and habituate faster to human presence, there is evidence that other typical behaviours of this species are conserved after domestication (e.g., Swaney et al., 2015).

2.2.2 Guppies used in the experiments

In the experiments of this thesis, I used two different strains of guppies maintained in the laboratory at Department of General Psychology, University of Padova. The first was a wild guppies strain. These guppies were descendants (approximately 30 generations) of feral guppies collected in 2002 from the lower Tacarigua River, Trinidad (figure 01). This population is known to be exposed to high predation pressure. I made a great effort to maintain these guppies in an enriched environment resembling wild condition and to avoid any external interference such as interaction with humans. Wild guppies were maintained in 100 x 70 x 54 cm grey plastic tanks with a 1:1 sex ratio and were free to breed. Feeding was the only exposure to humans these wild guppies experienced before experiments.

Some of my experimental procedures, especially trainings, required fish to interact frequently with the experimenter for long periods of time. In the preliminary experiments, I observed that wild guppies did not accustomate to such intense interaction with humans. This could cause guppies to express non-natural behaviours during the experiments (i.e., freezing and hiding) or even to refuse to participate to the task. This lack of habituation could also have detrimental effects on wild guppies wellbeing. For these reasons, I performed some experiments with a second strain of guppies. These guppies were descendant of approximately 200 domestic guppies (“snakeskin cobra green”; figure 02) bought from a local pet shop in 2012. Domestic guppies habituated easily to interaction with the experimenter. I bred domestic guppies in 60 x 40 x 35 cm glass aquaria. Each aquarium contained 20 individuals of both sexes. Another advantage of domestic guppies was that sexual size dimorphism is reduced compared to wild guppies, reducing the possibility that this factor affected the experiments.

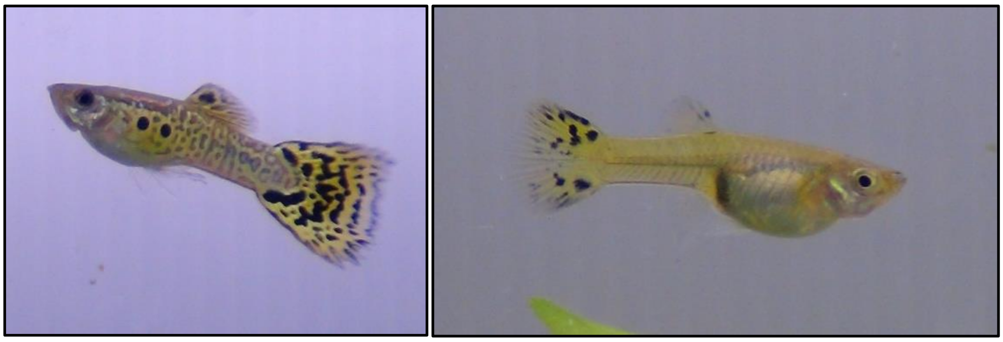

Figure 2: Male (left) and female (right) domestic guppy.

The maintenance tanks of both guppy strains were provided with gravel bottom, abundant plants, and water filters. Water temperature was kept nearly constant (26 ± 1 °C), and 36-w fluorescent lamps supplied illumination from 7:30 to 19:30. Fish were fed three times per day with commercial food flakes (Fioccomix, Super Hi Group, Ovada, Italy) and live *Artemia salina* nauplii.

During the experiments, guppies were 5 to 8-months old. Subjects were randomly selected from maintenance tanks. I did not use pregnant females. Each subject participated to only one experiment. In many of the experiments, visual inspection of the subjects suggested the existence of a size differences between males and females. In these cases, I measured the subjects after completion of the experiments and I considered their length in the analysis to reduce the possibility of confounding effects. Following a well-established procedure, I anesthetised the subject in an MS-222 bath (Sigma–Aldrich, St Louis, Missouri, USA), and then I photographed it to measure its standard length from the digital image thanks to a computer software (ImageJ).

2.2.3 Ethical note

My experiments consisted of observations of fish behaviour without manipulation. In training procedures, fish spontaneously participated in the experiments, otherwise they were substituted. If the subject was housed for long time in the experimental tank (for example in training experiments), the tank was provided with natural plants, bottom gravel and social companions to minimise differences from maintenance tanks. In experiments in which subjects were tested in an unfamiliar tank, such as in the shoal size discrimination task, the durance of the observation was limited to 30 min; then the subject was released in a maintenance tank. None of the subjects expressed distress during the experiment. In one experiment, one female died during an interval between two tests. However, this female did not show any evidence disease suggesting its dead occurred because of natural causes. At the end of the experiment, subjects were released in maintenance tanks identical to the ones previously described and kept for breeding purpose. Experiments comply with the law of the country (Italy) in which they were performed (Decreto legislativo 4 marzo 2014, n. 26). The experimental procedures have been approved by Università di Padova Ethical Committee.

2.3 Visual discrimination learning

2.3.1 Introduction

In this first experimental chapter, I examined sex differences in the ability to perform visual discriminations. The capacity of learning visual discriminations is possibly one of the most studied topic in animal cognition (Shettleworth, 1999).

Most of the experiments in this field are based on training procedures. In a series of trials, animals are required to discriminate between two figures that differ in shape, size or orientation (Baldwin et al., 1981; Fuss et al., 2014; Sutherland, 1969). During each trial of the training, the animal is presented with the two figures. If the animal selects the correct figure, it receive a reward, usually a piece of food. The two figures to be discriminated are usually presented with a monitor or drawn on white cards. The great majority of species can learn visual discriminations, perhaps because this capability is key in many ecological situations (Shettleworth, 1999). For example, animals are often required to learn to discriminate between preys, predators, conspecifics and potential mates. Fish have proven able to solve these tasks as well (Siebeck et al., 2009; Mackintosh & Sutherland, 1963; Mark & Maxwell, 1969; Sutherland & Bowman, 1969), but often they perform poorly in the discrimination of figures with different orientation (Gierszewski et al. 2013; Mackintosh & Sutherland, 1963). This difficulty can be due to the fact that discrimination of figures with different orientation possibly involve a large suite of abilities, such as for example, left-right spatial discrimination (Tapley & Bryden, 1977). Experiment 1 and 2 of this section exploited a training procedure to test the existence of sex differences in the ability to learn the discrimination between two different figures or between the same figure with different orientation, respectively. The training procedure I adopted in these experiments was new. I developed it following a method often used in mammals, birds and reptiles (Amy et al., 2012; Astié et al., 1998; Leal & Powell, 2012). In each trial, the subject is presented with two small discs with different characteristic, for example a different image. Under one of the discs, there is a food reward that the fish can reach by dislodging the disc. To find the food reward the subject had to learn the difference between the two discs. In experiment 1 subjects had initially to learn to discriminate a bar from an S-shaped segment drawn on the discs and, subsequently, subjects had to learn to discriminate between a triangle and a square. In experiment 2, subjects were required to discriminate an E-shaped figure from an identical figure that was horizontally flipped. In humans, there is broad evidence of male-advantage in discriminating rotated 2D objects, a task similar to experiment 2 (e.g. Collins and Kimura, 1997; Quinn & Liben, 2008).

The training procedure of experiment 1 and 2 allowed a simple presentation of stimuli as stimuli were two-dimensional black figures drawn on a white background. However, some authors have suggested that two-dimensional stimuli are not salient for animals and this fact reduces discrimination performance (O'Hara et al., 2015). I therefore conducted experiment 3 to study the discrimination of real objects. The most used paradigm to study object discrimination learning is the novel object recognition task. The novel object recognition task is a non-rewarded paradigm based on spontaneous exploratory behaviour. It consists of two phases and does not require training (Bevins & Besheer, 2006; Ennanceur & Delacour, 1988). During the first phase, subjects undergo a one-trial familiarization with an object. After an established time interval, subjects were simultaneously exposed to the familiar object and to a novel object. During this second phase, subjects are expected to interact mainly with the novel object because of exploratory behaviour. By measuring the preference for the novel object over the familiar one, it is possible to assess the ability of discriminating between the two objects (Ennanceur & Delacour, 1988). In novel object recognition task, a female advantage has been found in mice and rats (Ghi et al., 1999; Sutcliffe et al., 2007; Bettis & Jacobs, 2012; Bettis & Jacobs, 2013).

2.3.2 Experiment 1: Shape discrimination

In this experiment, guppies were trained to discriminate between a bar and an S-shaped segment and, subsequently, between a triangle and a square.

Materials and methods

Subjects: I used 10 male and 10 female domestic guppies.

Experimental apparatus: Experiment was performed in glass aquaria (60 x 40 x 35 cm) filled with 30 cm of water (figure 3). The bottom of the aquaria was made of natural gravel. By using green plastic material, each aquaria was divided in a front main compartment (30 x 40 cm) and a start box (10 x 8 cm). A transparent guillotine door controlled the connection between the main compartment and the start box. Outside the trials, the guillotine door was lifted allowing the subject to freely swim between the compartments. A grid prevented the subject to reach the sector behind the start box, where abundant natural vegetation and filters were housed. In the main compartment, a green plate (20 x 15 cm) perforated with 48 holes (Ø 1 cm, depth 0.3 cm) was placed horizontally on the gravel substratum. Each experimental apparatus housed one subject for the entire length of the experiment.

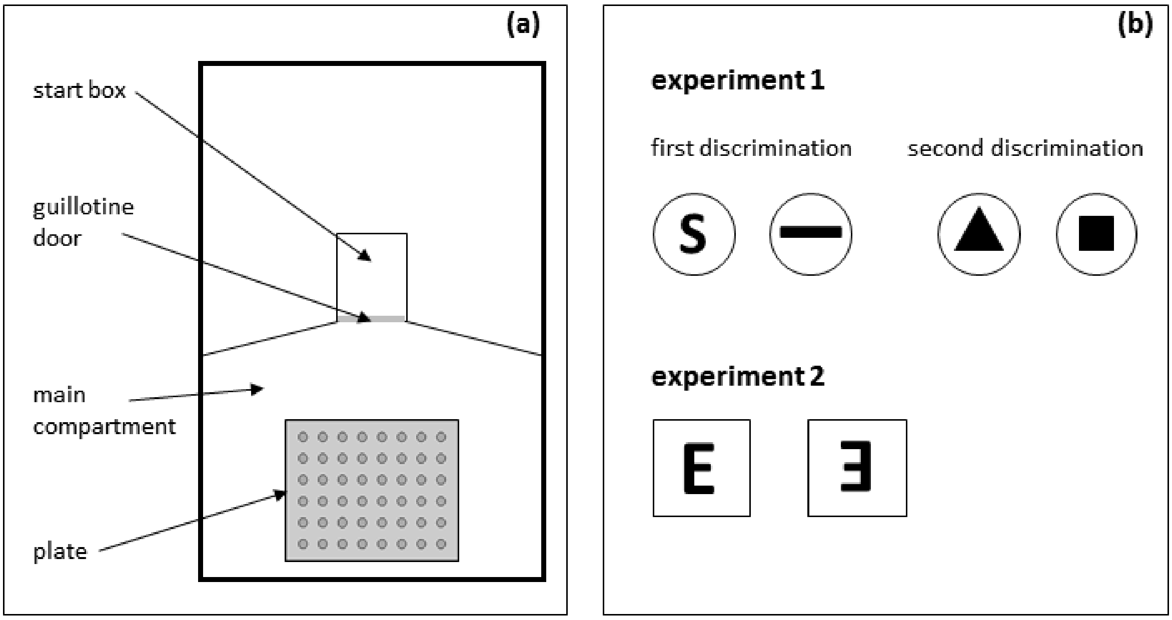

Figure 3: (a) Aerial view of the apparatus and (b) representation of the stimuli used in experiments 1 and 2 for studying visual discrimination learning.

General description of the experiment: During each trial of the experiment, some holes of the plate were covered with two small plastic discs, which subjects had previously learned to dislodge (see below). The two discs were white and they had a different shape drawn in black (figure 03). One of the figures, counterbalanced between subjects, indicated the presence of a food reward in the hole under the disc. Subject underwent 10 trials in each day of the experiment. During the initial six days (60 trials), subjects had to learn to discriminate a bar from an S-shaped segment (figure 03); from day 7 to day 12 (60 trials), subjects had to learn to discriminate between a triangle and a square (figure 03).

Description of trials: At the beginning of each trial, the subject was gently guided into the start box by inserting a transparent panel into the tank. Then, the experimenter closed the guillotine door and positioned a green plastic panel in front of the corridor to ensure the subject could not see the main compartment. The two discs were displaced on the holes of the plate according to a pseudo-random, pre-set scheme. The food reward, consisting of a small portion of commercial food flakes, was placed into the hole under the reinforced disc using a plastic Pasteur pipette. The experimenter added water scented with food to the apparatus to stimulate the guppies in starting to search food and to prevent the use of olfactory cues to solve the task. After that, the experimenter removed the green panel, allowing the subject to observe the task from the corridor for 10 s, and, lastly, opened the guillotine door, leaving the subject to enter the main compartment and dislodge the discs. The first disc dislodged by the subject was considered an indication of its choice to measure accuracy in the discrimination. Latency to dislodge a disc after entering the main compartment was recorded using a digital chronometer and, rounded to the nearest second, considered to be the decision speed as in previous literature on fish (Mamuneas et al. 2015; Wang et al. 2015). The experimenter allowed the subject five minutes to dislodge a disc; after that, the trial was considered null and repeated later. If a subject performed two consecutive null trials, the session was interrupted and the experiment continued in the following session. After dislodging an incorrect disc, the subject was allowed five minutes to find the rewarded disc.

Description of the experimental phases: The experiment was made up of five consecutive phases. Phases 1–3 consisted of the habituation to the apparatus and to the procedure. In phase 4, I evaluated discrimination learning performance. Phase 5 was a control test for the use of olfactory cues.

(1) In phase 1, subjects were habituated to the apparatus. Two subjects, one male and one female, were moved from a maintenance tank to a tank identical to the experimental apparatus and left for three days to become accustomed to the new environment. Food was delivered as in the maintenance tanks.

(2) In phase 2, subjects were habituated to the procedure. From here on, no food was delivered to the subject outside the experiment to ensure motivation to participate in the trials. This phase lasted three days. Three times per day the subjects were led to the start box according to the procedure of the trial and their food ration was delivered in six, randomly chosen, holes of the plate. The subjects were then allowed to enter the main compartment and fed. On the third day of this phase, six yellow-coloured discs were placed so as to partially cover the holes with food, in a way that the subjects could reach the food by peaking and moving the discs. At the end of the third day, the two subjects were moved individually to an experimental tank. The guppy is a social species and it is reported to habituate faster to new tanks in presence of many social companions (Miletto Petrazzini et al., 2015); thus, I provided five small conspecifics in each experimental tank. To avoid any interference with the subject performance, social companions were moved in another tank during the occurrence of trials.

(3) After one day of habituation to the experimental tank, the subject was trained to dislodge the discs. Over two days I administered 16 trials in which only one yellow disc was present in the plate. This disc partially covered a hole with a food reward. The gap was progressively reduced across the trials, from approximately 75% of the hole to complete occlusion. The subjects that at the end of this phase were able to dislodge a completely occluded disc started the experiment, while the others were discarded and replaced with another one of the same sex. I discarded two males and three females. One additional female stopped participating in the experiment after the third olfactory control cue trial; thus, its performance in this last phase was not analysed.

(4) In this phase, lasting 12 days, I evaluated guppies' discrimination learning performance. The experiments were conducted six day per week. Each day, the subject performed 10 trials subdivided in two blocks (five trials each) separated by almost five hours.

(5) Here I controlled whether subjects learned to find the food reward by using olfactory cues. This phase consisted of 15 trials in which two identical yellow discs were placed on the plate. One of them, randomly chosen, hid the food. If the subjects learned to find the food reward using olfactory cues, they were expected to perform better than chance in this phase. I performed this phase the day after the end of phase 4.

Statistical analysis: Analysis was performed in R version 3.0.2 (The R Foundation for Statistical Computing, Vienna, Austria, http://www.r-project.org). For each subjects, the overall accuracy was calculated as proportion of correct choices over all training and it was always arcsine square root transformed before conducting parametric analysis (Sokal & Rolf, 1995). I also calculated daily accuracy for each day of training separately to perform the analysis with the mixed-effect model. Overall decision time was calculated as average latency to dislodge the disc over all training. I also calculated a daily decision time as average latency to dislodge the disc for each day of training. Decision time was log transformed to deal with a right-skewed distribution. I used independent-sample *t* test and Pearson's correlation test to study sex differences in standard length and relationship between standard length, overall accuracy and overall decision speed, respectively. To assess whether subjects learned the task I compared the number of correct choices over all training with chance level (50% of correct choices) with binomial test. This analysis provided also an indication of within-sex individual differences. In each experiment, the daily accuracy and the daily decision speed were analysed with linear mixed-effects models (LMMs) fitted with the 'lme' function, from the 'nlme' R package. In all models, day of training, sex and discrimination were fitted as fixed effects. Subject ID was fitted as a random effect. Statistical significance of the effects in the models was assessed with *F*-tests. If guppies learned the task, I would expect a significant effect of the day of training in the LMM, with increasing trend. When this occurred, I also run a one-sample *t* test to compare the overall accuracy was compared with chance level (50%). In case of no significant effect of sex, I used the Bayesian information criteria of the LMMs with and without the effect of sex to approximate a Bayes factor (BF_{01}, Schwarz, 1978), which is helpful to estimate the relative strength of the evidence for the two models without the confounding of sample size (Dienes, 2014). For example, $BF_{01} = 5$ indicates that the model without the effect of sex is five times more likely to explain my data that the model with the effect of sex. The existence of a speed–accuracy trade-off was studied using Pearson's correlation. Lastly, the proportion of correct choices in the olfactory cue control test (arcsine square root transformed) was compared with chance level (50%) using a one-sample *t* test. In the text, $M \pm SD$ is given.

Results

Males and females had a comparable standard length (32.20 ± 1.93 mm and 32.40 ± 3.17 mm, respectively; independent-samples *t* test: *t*(18) = .170, *p* = .867). There was not a significant correlation between standard length and overall accuracy (Pearson's *r*(18) = -.066, *p* = .781) or between standard length and overall decision speed (Pearson's *r*(18) = -.266, *p* = .258).

Analysis of individual performance indicated five males and five females showed a statistically significant preference for the reinforced stimulus (more than 59.17% correct choices, binomial test). The LMM revealed daily accuracy was not different between the two discriminations (*F*(1,198) = 3.568, *p* = .060). Day of training did have a significant effect in the model (*F*(5,198) = 4.251, *p* = .001), as the accuracy increased linearly with the day of training (polynomial trend analysis: *p* = .043; figure 4). Also, subjects chose the correct disc in 60.15 ± 5.67% of the trials, an overall accuracy significantly greater than chance (one-sample *t* test: *t*(19) = 7.895, *p* < .001).

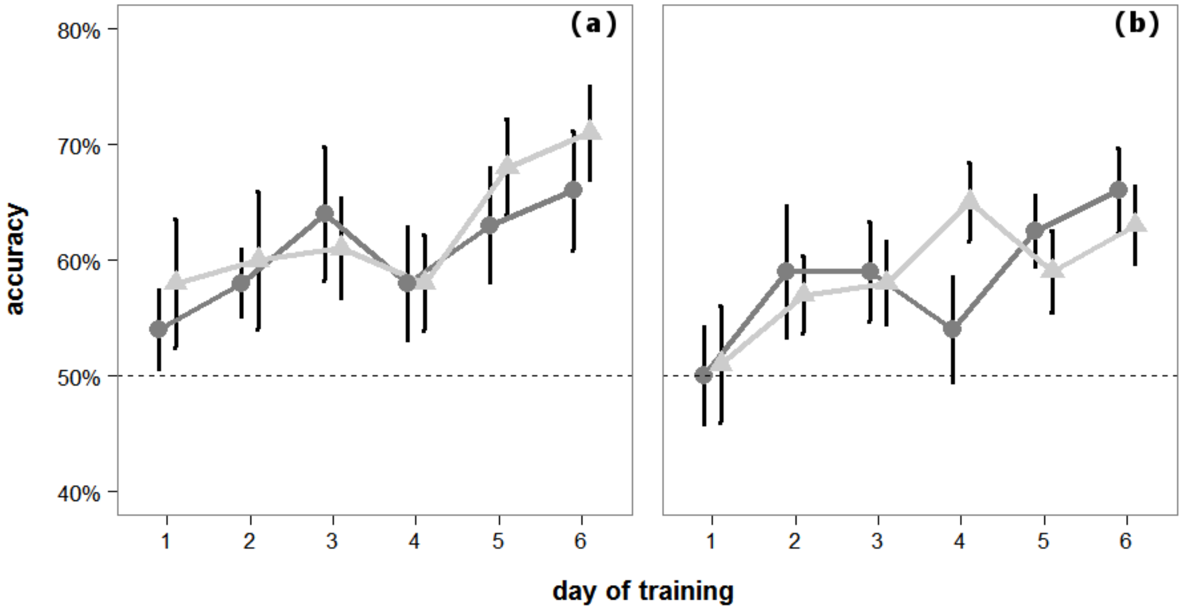

Figure 4: Accuracy of males (grey) and females (dark) in the **(a)** first and the **(b)** second discrimination of experiment 1 (shape discrimination). Data points represent *M* ± *SE* percentage of correct choices. Dashed line is chance performance.

Sex had not significant effect in the model (males' accuracy: 60.83 ± 4.98%; females' accuracy: 59.47 ± 6.47%; $F(1,18) = .099$, $p = .757$), and there was no significant sex by day of training interaction ($F(5,198) = .079$, $p = .779$; figure 4). The other interactions in the LMM were not significant. Bayesian analysis support the absence of sex differences in these data ($BF_{01} = 198.46$).

The overall decision speed was 11.84 ± 6.99 s. The LMM on daily decision speed revealed no effect of discrimination ($F(1,198) = .080$, $p = .778$), but decision speed varied significantly across days of training ($F(5,198) = 4.278$, $p = .001$). Moreover, the days of training by discrimination interaction was significant ($F(5,198) = 5.739$, $p < .001$). Males appeared to be faster than females at making decisions in all days of the test (male' speed: 8.88 ± 2.84 s; females' speed: 14.79 ± 8.72 s; figure 5), but this effect was not significant ($F(1,18) = 3.602$, $p = .074$). Sex by day interaction was not significant ($F(5,198) = 0.303$, $p = .912$), nor were the other interactions in the model. There was no significant correlation between overall accuracy and overall decision speed (Pearson's $r(18) = -.032$, $p = .895$). A sex-separated analysis confirmed the absence of correlation (males: $r(8) = -.081$, $p = .823$; females: $r(8) = .065$, $p = .858$).

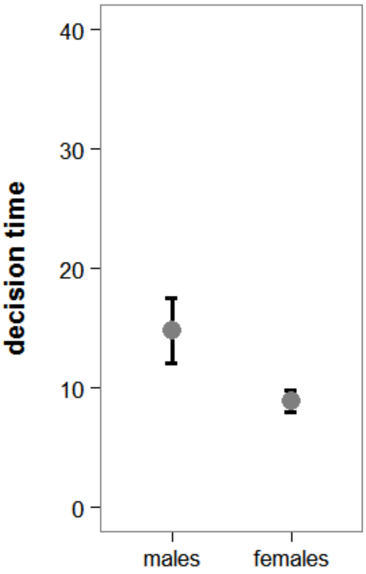

Figure 5: Average decision speed (M ± SE seconds) of males and females guppies in experiment 1 (shape discrimination).

In the olfactory cue control test, subjects did not choose the discs hiding the reward more than chance (50.87 ± 10.71%; one-sample t test: $t(18) = .355$, $p = .727$).

2.3.3 Experiment 2: Mirror image discrimination

In this experiment, guppies were trained to discriminate an E-shaped figure from an identical figure that was horizontally flipped.

Materials and methods

This experiment followed the procedure of experiment 1 of this section (chapter 2.3.2). The stimuli, an E-shaped figure and its horizontal mirror image, were presented by mean of two white vertical cards (4 x 4 cm) placed behind two identical yellow discs (figure 3b). I used the cards to ensure the orientation of the stimuli was fixed, irrespective of the position of the subject. The length of the discrimination learning phase (phase 4, described in chapter 2.3.2) was extended to 10 days (100 trials) because previous studies suggested mirror-image discrimination is very difficult to learn for fish (Gierszewski et al. 2013; Mackintosh & Sutherland, 1963). The experimental subjects were 10 male and 10 female domestic guppies. Two males were discarded and replaced before the beginning of the experiment because they were not able to dislodge the disc. One additional female ceased to participate in the experiments on the ninth day of the training; thus, its performance was evaluated only up to that point. Data analysis followed the procedure described for experiment 1 of this section (chapter 2.3.2).

Results

Males and females had a similar standard length (32.30 ± 1.89 mm and 31.20 ± 1.69 mm, respectively; $t(18) = 1.374$, $p = .186$). There was no significant correlation between standard length and overall accuracy ($r(18) = -.168$, $p = .478$) or between standard length and overall decision speed ($r(18) = -.427$, $p = .061$).

Analysis of individual performance found only one male and one female significantly learned the task (more than 60% correct choices). The LMM revealed daily accuracy was not significantly affected by day of training (*F*(9,161) = .811, *p* = .607; figure 6a), suggesting guppies did not learn the task. Sex had not significant effect in the model (males' accuracy: 52.90 ± 5.38%; females' accuracy: 52.58 ± 14.20%; *F*(1,18) = .078, *p* = .783), and the sex by day of training interaction was no significant (*F*(9,161) = .968, *p* = .468; figure 6a). Bayesian analysis support the absence of sex differences in these data (BF_{01} = 219.04).

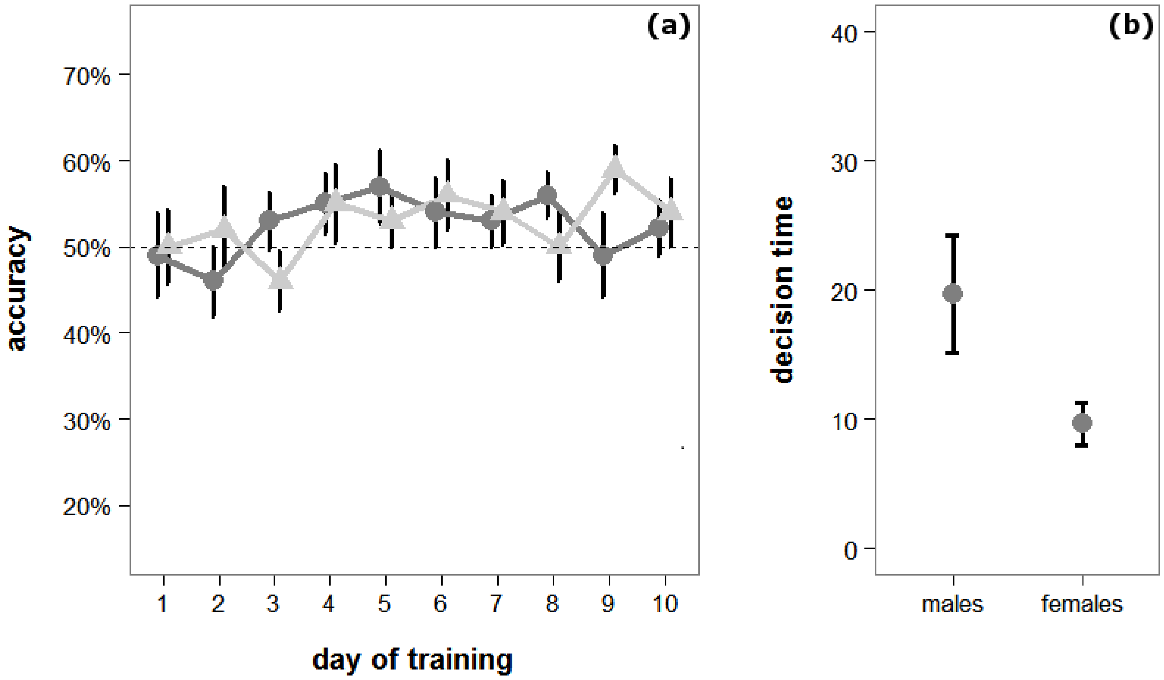

Figure 6: **(a)** Accuracy of males (grey) and females (dark) in experiment 2 (mirror image discrimination); data points represent *M* ± *SE* percentage of correct choices; dashed line is chance performance. **(b)** Average decision speed (*M* ± *SE* seconds) of males and females guppies in experiment 2.

The overall decision speed was 14.65 ± 11.61 s, but the LMM revealed daily accuracy changed across days of training (*F*(9,161) = 5.962, *p* < .001). Sex had a significant effect in the model (*F*(1,18) = 15.062, *p* = .037; figure 6b): males had a faster decision speed than females (9.63 ± 5.15 s and 19.68 ± 14.20 s, respectively). No significant sex by day of training interaction was found (*F*(9,161) = 1.746, *p* = .083). There was no significant correlation between overall accuracy and overall decision speed (*r*(18) = .277, *p* = .237). Since the sex difference in decision speed could potentially have affected the results of the correlation test, we also ran a sex-separated analysis. Again there was not statistically significant evidence of correlation (males: *r*(8) = .553, *p* = .098; females: *r*(8) = .122, *p* = .738).

In the olfactory cue control test, subjects did not choose the discs hiding the reward more than chance (50.53 ± 9.77%; *t*(18) = .216, *p* = .831).

2.3.4 Experiment 3: Novel object recognition

In this experiment I compared the performance of male and female guppies in a novel object recognition task.

Materials and methods

Subjects: The experimental subjects were 16 male and 16 female wild guppies.

Stimuli and experimental tanks: I used two plastic objects as stimuli in this experiment, a white parallelepiped (4 × 4 × 2 cm) and a blue pyramid (base: 4 × 3 cm, height: 7 cm; figure 7a). I used four identical copies of each object. A transparent plastic rod was fixed to each object to allow insertion into the experimental tanks, 10 cm under the water's surface. The familiarization phase was performed in 50 × 20 × 32 cm tanks (figure 7b) filled with 25 cm of water. The bottom of each tank was made of natural gravel; one half of the tank housed a water filter and natural vegetation (Java moss). The other half of the tank was used to present the object (hereafter "familiar") during familiarization. The longer walls of the tank were covered with green plastic, and fluorescent lamps over the middle part of the tank provided illumination. The test phase was performed in a two-chamber apparatus built with grey plastic material inside a 50 × 20 × 32 cm tank (figure

7c). Above the central corridor (14 × 6 cm) that connected the two chambers, two fluorescent lamps, oriented toward the chambers, provided illumination. I used four identical test tanks in order to simultaneously observe four subjects. Before the test, the two objects were inserted in the two chambers: the familiar object and the complementary (hereafter "novel") object, with a balanced position across subjects. A digital video camera mounted one meter above the tanks recorded the experiments.

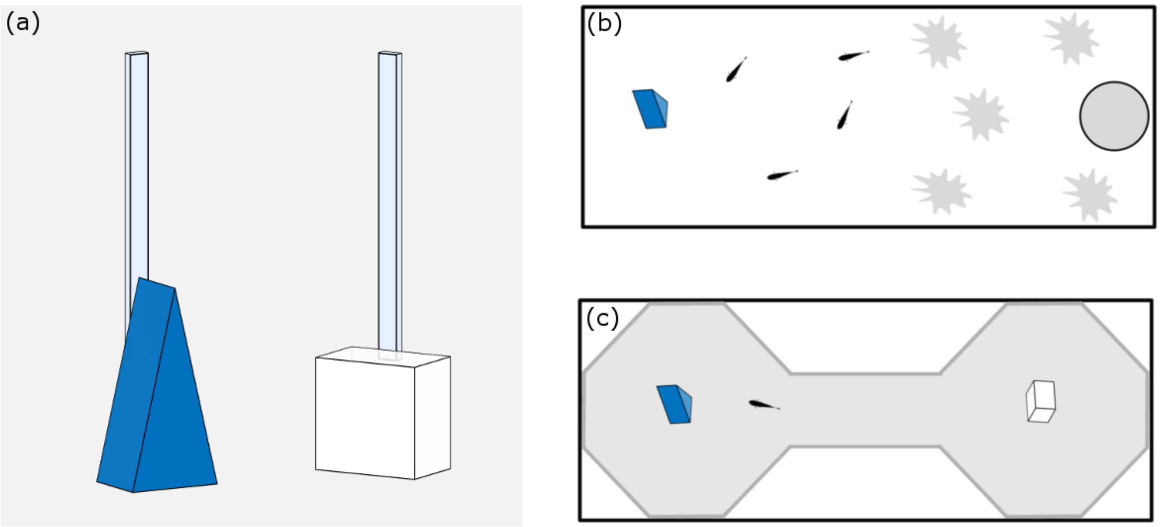

Figure 7: Schematic representation of stimuli and apparatuses: **(a)** blue pyramid and the white cube used as stimuli; **(b)** familiarization tank, half occupied by plants and water filter; **(c)** two-chambers tank used to asses NOR ability in the test phase.

Procedure: As the guppy is a social species, I exposed the subjects to the familiarization phase in groups of four (two males and two females) to avoid social deprivation. Overall, I observed eight groups of subjects (16 males and 16 females). Six days before familiarization, I moved each group of four subjects from the maintenance tanks to a familiarization tank to habituate. On the seventh day, between 11:00 and 14:00, I gently inserted the predetermined familiar object into the empty half of the tank for familiarization. To control for innate preference, half of the groups familiarized with the cube and half with the pyramid. Groups were randomly assigned to one of two familiarization time lengths (one hour or three hours). At the end of the familiarization, the object was removed from the tank and the subjects were left undisturbed. The test phase took place after an interval of one day for half the groups, and six days for the remaining groups. The duration of the familiarization and the interval were balanced between subjects. Each subject was netted from the familiarization tank and moved into a small plastic jar filled with water. The jar was then gently inserted in the central corridor of the test tank to let the subject swim out of the jar. Subject behaviour was recorded for 12 minutes. During habituation and interval, subjects were fed three times a day, as they had been in the maintenance tanks. On the familiarization day, subjects were fed in excess twice, one in the morning and one in the evening.

Data collection and analysis: I observed subjects' behaviour from the video recordings. Using a computer software (Ciclic Timer), I measured the proportion of time subjects spent in each of two chambers and in the central corridor during each two minute interval in the experiment. In each block of two minutes, the preference for the novel object was computed as time spent in the chamber with the novel object divided by the sum of the time spent in the chamber with the familiar object and the time spent in the chamber with the novel object. I performed an arcsine square root transformation on the preference index before analysis (Sokal & Rohlf, 1995). To test if the preference for the novel object was greater than chance, I used a one-sample t test against the mean of a random choice (0.5). The preference for the novel object and on the proportion of time subjects spent in the central corridor were then studied with linear mixed-effects models (LMMs; using the 'lme' function) fitted with blocks of minutes, sex, familiarization and interval length as fixed effects and with the subject's ID as random effect to account for repeated measures. As all interactions of second and third order were not significant ($p > 0.1$), I fitted a reduced model with first-order interaction only. To compare males' and females' preferences for the novel object in the periods when both expressed a significant preference, I used an independent-sample *t* test. One female (in the group with 1-hour familiarization and a 1-day interval) naturally died during the interval between familiarization and the test phase.

Results

Overall, subjects expressed a significant preference for the novel object (54.59 ± 9.11 %, one-sample *t*-test: *t*(30) = 2.771, *p* = .010), showing they achieved recognition.

In the LMM on the preference for the novel object, I found no significant effect for the block of minutes (*F*(5,135) = 0.900, *p* = .483), sex (*F*(1,24) = .051, *p* = .824, BF_{01} = 129.08), familiarization length (*F*(1,24) = 3.761, *p* = .064), or interval length (*F*(1,24) = 0.185, *p* = .671). However, there was a significant interaction between sex and block of minutes (*F*(5,135) = 2.664, *p* = .025; figure 8a): graphical inspection of figure 8a suggested males expressed a preference for the novel object at the beginning of the experiment, which subsequently decreased with time; females expressed a preference for the novel object only in the second half of the experiment. No other significant interactions were found in the model.

To better understand the sex by block of minutes interaction, I performed further analysis for the first and second halves of the experiment (minutes 1 to 6 and 7 to 12, respectively). In the first period of the experiment, males significantly preferred the novel object (56.79 ± 11.90 %, one-sample t-test: *t*(15) = 2.220, *p* = .042), but females did not (51.87 ± 9.45 %, *t*(14) = .785, *p* = .446). In sharp contrast, in the second period of the experiment, I observed the inverted situation: females significantly preferred the novel object (57.62 ± 12.88 %, *t*(14) = 2.240, *p* = .042), but males did not (52.10 ± 11.90 %, *t*(15) = 1.035, *p* = .317). A direct comparison between the males' preference in the first period and females' preference in the second period revealed no difference between the two sexes in terms of NOR ability (independent sample t-test: *t*(29) = .190, *p* = .851, BF_{01} = 5.8 x 10^{12}; figure 8b).

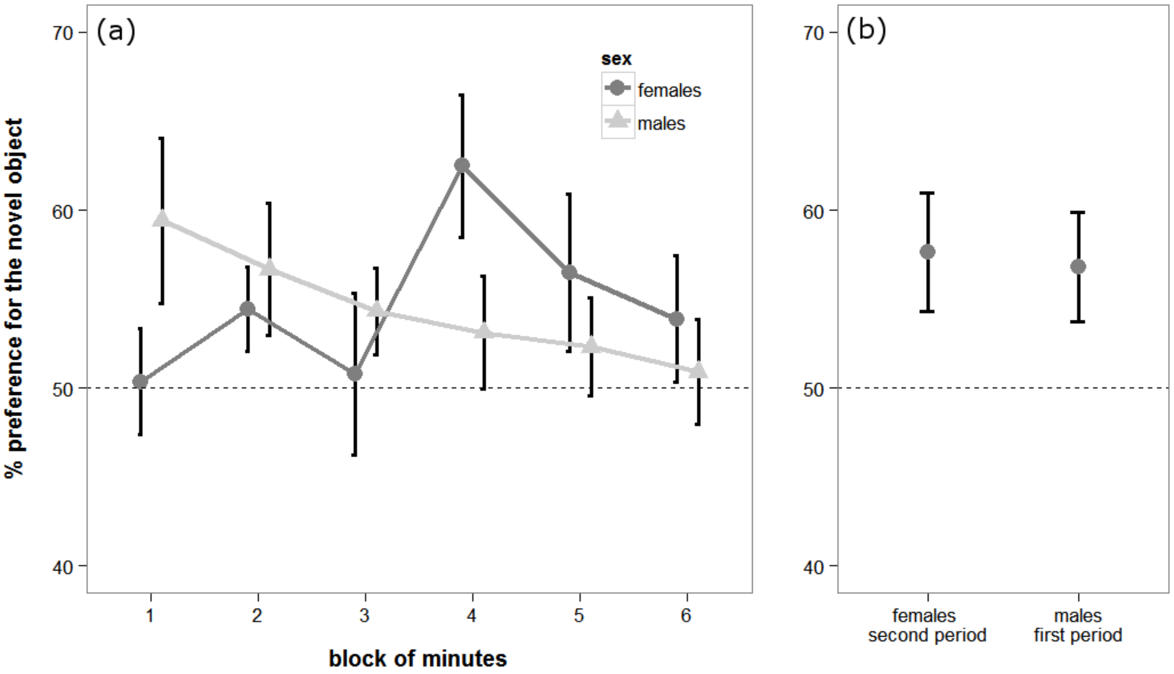

Figure 8: Comparison of NOR performance of male and female guppies: **(a)** during the full length of the experiment (12 minutes subdivided in six blocks); **(b)** in the two periods of the experiment in which the two sexes expressed a significant preference for the novel object (males: minutes 1 to 6; females: minutes 7 to 12). Data points represent *M* ± *SE* preference for the novel object.

To explore sex differences in object exploration, I also analysed time spent in the central corridor. On average, subjects spent 19.90 ± 6.15 % of the time in the central corridor. The LMM on this variable revealed that proportion of time spent in the central corridor significantly decreased as the experiment went on (*F*(5,135) = 6.643, *p* < .001; figure 9). In the model, there was also a significant effect of sex: females spent more time in the central corridor than males did (females: 22.02 ± 6.61 %; males: 17.92 ± 6.12 %; *F*(1,24) = 4.564, *p* = .043; figure 9). Moreover, subjects that were tested after a 6-day interval spent significantly more time in the central corridor than did those tested after a 1-day interval (1 day: 16.83 ± 5.48 %; 6 days: 22.78 ± 5.41 %; *F*(1,24) = 9.525, *p* = .005). There was no significant effect of familiarization length (*F*(1,24) = .419, *p* = .524), nor there was any significant interaction.

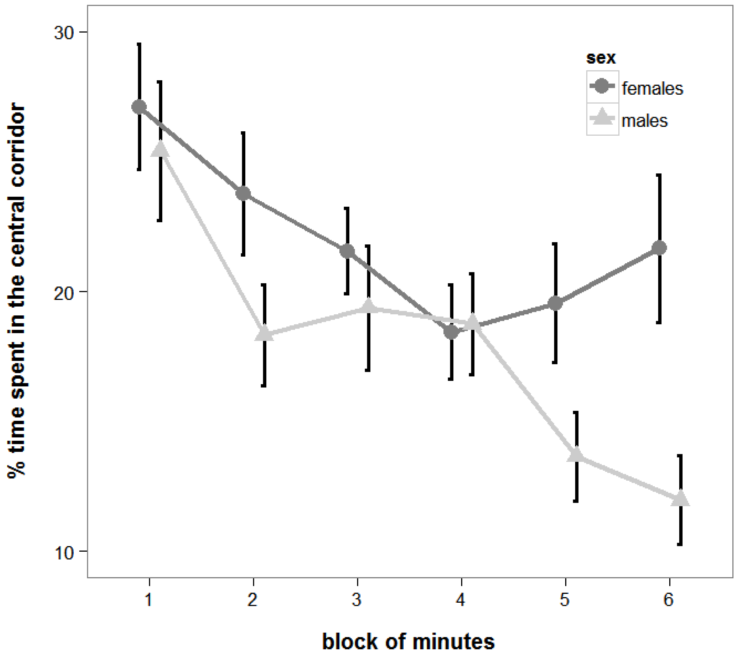

Figure 9: Proportion of time (*M* ± *SE*) male and female guppies spent in the central corridor during the test phase of experiment 3 (novel object recognition) subdivided in the six two-minutes blocks. The smaller is the proportion of time spent in the central corridor the higher is the attraction for the objects.

2.3.5 Discussion

Ability to solve the tasks

Overall, in these three experiments I found little evidence that male and female guppies differ in the ability to learn visual discriminations. In experiment 1 and 2, I administered guppies three discriminations of different difficulty with a training procedures. In experiment 1, guppies firstly learned the discrimination between two stimuli that differed for many features (S vs bar), a type of task several fish species can accomplish (Agrillo et al., 2012a; Hemmings & Matthews, 1963; Siebeck et al., 2009). Then, guppies learned the discrimination between two figures that differed only for their geometric shape (triangle vs square), an ability that has been reported for few fish species only (Siebeck et al., 2009). In both discriminations of experiment 1, guppies showed a steady increase in accuracy and reached a performance around 60% of correct responses after only three days. The average accuracy and the increase in accuracy over days were essentially equal for male and female guppies.

In experiment 2, I trained guppies to discriminate between an E-shaped figure and its horizontal mirror image. This task appears to be very difficult for fish (Gierszewski et al., 2013; Mackintosh & Sutherland, 1963) and perhaps also for other vertebrates (Bradshaw et al., 1976; Riopelle et al., 1964; Todrin & Blough, 1983; but see Hopkins et al. 1993). I used this task because its difficulty could disclose subtle differences between the sexes. Guppies did not discriminated the two rotated figures and their performance was lower than in the two shape discriminations of experiment 1. Indeed, I observed no evident increase in accuracy over days despite I performed four additional days of training. Individual analysis suggested that only two subjects (one male and one female) significantly learned the task. The ability to perform left–right discriminations, that perhaps is more important in horizontal mirror image discriminations, may be related to cerebral lateralisation (Chiandetti & Vallortigara, 2009). Among poeciliids, approximately 10 % of the individuals are strongly lateralized (Facchin et al., 1999). According to this picture it is possible that in general guppies could not solve the mirror image discrimination, but only the most-lateralised individuals were able to solve the discrimination. As in experiment 1, here I found no differences in the accuracy of males and females. However, the difficulty of the discrimination of experiment 2 and the consequent floor effect on performance could have masked an existing sex difference.

In experiment 3, I compared novel object recognition ability of male and female guppies. In sharp contrast to the two previous experiments of this section, here subjects were not exposed to training but I exploited spontaneous one-trial learning behaviour. Guppies learned the features of an object in the one-trial familiarization. In the subsequent test phase, guppies were able to discriminate between the familiar object and the novel object, as revealed by the preference for interacting with the latter. My experiment is the first attempt to study novel object recognition in adult guppies. The neophilic response of subjects toward the novel object is similar to those exhibited by other species in the NOR task (rats: Ennaceur & Delacour, 1988; mice: Bettis & Jacobs, 2012; and zebrafish: Lucon-Xiccato & Dadda, 2014), allowing proper utilization of this paradigm in adult guppies as well. However, a study reported juvenile guppies avoid the novel object in a similar task (Miletto Petrazzini et al., 2012). This difference is likely to be due by behavioural differences between juvenile and adult guppies (Lucon-Xiccato & Dadda, 2014). Interestingly, subjects showed no performance reduction in the 6-day interval relative to the 1-day interval, whereas a previous study have reported zebrafish performance drops after 96 hours (Braida et al., 2014). Aside the possibility of between-species differences in novel object recognition ability, it is possible that the low mnemonic performance of zebrafish was due to the use of two-dimensional stimuli instead of real objects, as reported for other species (O'Hara et al., 2015). Interestingly, I also found no difference in NOR performance due to the familiarization length, but the familiarization lengths adopted in this study (30 and 60 minutes) were much longer than the ones commonly adopted in rodents (Ennaceur & Delacour, 1988), and subjects probably had enough time to acquire information on object features in both conditions.

The direct comparison between males' and females' performance in experiment 3 did not reveal any sex difference in the ability to accomplish novel object recognition. However, the analysis of the temporal pattern of the preference for the novel object revealed a confounds to this analysis. Males' preference for the novel object was higher at the beginning of the experiment but rapidly decreased in the following minutes. Conversely, females expressed a preference for the novel object only in the second period of the experiment, and females performance rapidly decreased in the remaining minutes as well. When presented with novel objects, the exploratory behaviour of fish occurs very quickly, and, subsequently, fish interact randomly with familiar and novel objects (Lucon-

Xiccato & Dadda, 2014). The temporal pattern observed in my experiment apparently agree with previous experiments, as guppies preferred the novel object for very few minutes and subsequently reduced their interest. However, the two sexes showed this ephemeral preference with different timing. The first analysis that compared males and females across overall experiment length could cause erroneous results, as it included periods of time in which only one sex was exploring the novel object. I therefore performed a further analysis and compared the two sexes in the periods of the experiment in which they expressed a significant preference for the novel object. I found males' preference for the novel object in the first period of the experiment equal to females' preference in the second period of the experiment. Therefore, there is no evidence of sex difference in novel object recognition ability in guppies.

Altogether, results of the three experiments of this section reveal a general absence of sex differences in guppy discrimination learning ability. This has been confirmed with both procedures based on training and procedure that exploits spontaneous one-trial learning. Bayesian analysis provided "very strong" to "decisive" evidence (Jeffreys, 1998) that a model without the effect of sex better represented my data and that the lack of significance was unlikely to be explained by sample size. Perhaps, none of the learning abilities involved in discriminating between the stimuli of the three experiments are relevant for those situations in which male and female guppies experience different selective pressures. Alternatively, it is possible that I studied basic mechanisms of learning and discrimination; thus, there might be robust genetic and developmental constraints preventing sexual differentiation. Another work agrees with the possibility that basic learning ability does not differ between the two sexes in fish. Bibost and Brown (2014) studied the ability of the *Melanotaenia duboulayi* in learning a stimulus-reward association, finding no evidence of sex differences. Regarding discrimination, it is worth noting that the cognitive functions underlying visual perception and shape recognition are probably based on phylogenetically ancient systems. Indeed, the mechanisms that allowed the representation of an object present in the visual field are fundamentally the same from fish to humans (Gori et al., 2014; Sovrano & Bisazza, 2008). In addition, the same mechanisms of shape recognition are used in a number of different contexts, such as spatial orientation, foraging and predator recognition. Even if in one of these contexts males and females experience different selective pressures, the pressures imposed in the other contexts are likely to constraint the evolution of sex differences.

Regarding experiment 3, it is interesting that I have failed to find, in fish, the sex differences in object encoding often reported in mammals. Since other authors have failed the same goal when studying birds (Hodgson & Healy, 2005; Tello-Ramos et al., 2014), sex differences appear not to be a shared characteristic of vertebrates' systems for object encoding. Sex differences in this field may have evolved in mammals and some bird species (Vallortigara, 1996) as a consequence of the specific ecological requirements of the species rather than inherited from a common ancestor.

Other sex differences in performance

A very different result emerged considering other aspects of the performance of male and female guppies that are not measures of discrimination learning abilities. In experiment 2, the latency of males to choose a disc after entering the experimental compartment was significantly shorter that of females (see also results of oddity discrimination learning task, chapter 2.4.2). In experiment 1, I found a similar pattern although here the effect of sex only approached statistical significance. This difference is not likely to be due to sex difference in swimming speed. Indeed, guppies could easily swim the distance between start box and discs (15 cm) ten times faster or more (approx. 1

s; Karino et al., 2006) than the latency we observed in our experiments (18 s on average). In addition, female guppies are known to swim faster than males (Karino et al., 2006). Observation made during the experiment revealed that guppies spent most of this time inspecting the stimuli. Therefore, the sex difference I found in my experiments is likely to reflect the time a fish required to take its decision. A faster male decision speed in cognitive task has been reported in another fish species (Mamuneas et al., 2015) and may reflect the existence of different “cognitive styles” in the two sexes (Shettleworth, 1999). Decision speed does not appear to be the only sex difference in cognitive style in guppies. Previously, Reader and Laland (2000) found females having greater innovation tendency than in males. Males pay a cost to their reduced innovation tendency, being less ready to modify their behaviour to novel environmental situations (Reader and Laland, 2000). I did not detect a similar cost in my study as there was no speed-accuracy trade-offs in males. This may be due to methodological reasons as my experiments were not designed to detect costs and benefits of rapid decisions. For instance, in my procedure guppies could see the task from behind the guillotine door for some seconds, and both individuals with fast and slow decision speed could have sufficient sampling time to process the information.

How can we explain this sex differences in decision speed? Perhaps, it arise from the sex difference in time budget typical of this species. Female guppies devote most of their time to foraging; by contrasts, male guppies are most of the time involved in sexual activity (up to one sexual act per minute from sunrise to dawn) and can devote only 20% of their time to foraging (Magurran & Seghers, 1994). Because of this time constrain, males might have been selected to make sudden foraging decisions. On the other hand, if a single unified system for decisions exists in animals, it is possible that comparative and decisional processes have been selected in contexts other than foraging, such in mate selection. Male guppies court or try sneak copulatory attempts with virtually each female they find (Magurran & Seghers, 1994). Even in presence of several potential mates, males do not exhibit delay in mating behaviour. In sharp contrasts, females careful evaluate many potential mates, basing on multiple indicators, a process that may require hours. Lastly, an alternative explanation is that sex difference in decision speed in guppies is a by-product of other selective pressures, not directly related to cognition. In sticklebacks, *Gasterosteus aculeatus*, bolder individuals (usually males) are faster than shyer ones (usually females) in choosing which arm to enter in a T-maze to obtain a food reward (Mamuneas et al., 2015). Links between personality traits and cognitive style have been reported also in other species (e.g., Carazo et al., 2014; Titulaer et al., 2012). Previous studies let suspect a diffuse covariation between individual differences in behaviour and cognition (Sih & Del Giudice, 2012). In the guppy, males are in general bolder than females (Harris et al., 2010; Irving & Brown, 2013). The sex difference in decision speed in my experiments may be the ultimate consequence of the sex difference in personality.

In experiment 3, aside from the lack of sex difference in novel object recognition ability, it is worth noting that males and females behaved quite differently during the task. The preference for the novel object was indeed expressed with different timing. This difference is possibly due by sex differences in behavioural traits. In guppies, compared to females, males are notably bolder (Harris et al., 2010; Irving & Brown, 2013) and more active (Irving & Brown, 2013). These personality characteristics indicate that male guppies are more likely to approach and explore unknown objects as previously found in closely related species (Brown et al., 2007). This remarkable sex difference in personality traits could explain my results: male guppies perhaps appear more prone to object exploration and therefore approached the novel objects earlier than females did. My analysis on the proportion of time guppies spent in the central corridor of the test apparatus supported this interpretation. Guppies spent more time in the corridor at the beginning of the experiment, suggesting that fish initially prefer to avoid the two objects. Further, proportion of time spent in the central corridor decreased quickly. However, males spent less time in the central corridor than females did, as males were more attracted by the two objects. Future studies on novel object recognition in fish should carefully consider the sex of the subjects to avoid this factor confounds the results.

2.4 Oddity concept learning and reversal learning

2.4.1 Introduction

Since some characteristics of the environment are often predictable, animals can benefit from learning predictive relationships (Zentall et al., 2014). Sometimes these relationships are abstracted and consisted in grouping objects or events in distinct classes on the basis of previous experience and forming therefore a concept (Zentall et al., 2014). Concept learning have been intensively studied in human, but the literature provides wide evidence that other animals can learn concepts (reviewed in Zentall et al., 2014). One task often used to study this capability is the oddity concept learning task. It consist in a training experiment in which the animal is presented with sets made of several identical objects and one odd object. To obtain a food reward the animal had to learn to select the odd object. Since the set of objects changes over different trials, the animal could learn the concept “choose the odd object” to maximize reward intake (Hille et al., 2006). In experiment 1 of this section, I used a training procedure to study oddity concept learning in male and female guppies. Guppies were presented with sets of five objects. One of the objects differed in colour from the others. This object hide the presence of a food reward. The pairs of colours (the odd colour and the colour of the other objects) were changed daily, therefore guppies could maximize their performance by learning the concept “choose the odd colour” (Hille et al., 2006).

In other circumstances instead, some attributes of the environment can change rapidly. Here, animals are reward for being flexible and for adapting rapidly to the novel situation. A task often adopted to study cognitive flexibility is the reversal learning, in which the animal is initially trained to choose a predetermined stimulus among two options and, after learning the association and reaching the learning criterion, it is required to choose the other stimulus (Guillamón et al., 1986; Leal & Powell, 2012). The subject had therefore to learn to ignore the stimulus previously reinforced. There is evidence that fish can accomplish this task, but less efficiently compared to birds and mammals (Engelhardt et al., 1973; Warren, 1960; Wodinsky & Bitterman, 1957). In experiment 2, guppies were tested in a reversal learning task using the discrimination of two colours. In a subsample of subjects I repeated four times the alternation of training to criterion and contingency reversal. Rats and pigeons improve their performance over successive reversals, an evidence that they learn the concept of the alternation of reward contingency (Bullock & Bitterman, 1962; Pubols, 1957). Results from experiments on macaques, rats, and domestic fowl suggest that females are more flexible than males adapt faster to the reversal of the contingency (Guillamón et al., 1986; Ha et al., 2011; Rogers, 1974).

Both the tasks of this chapter required subjects to discriminate among colours. This allow to make and hypotheses about the outcome of the experiments. Female guppies are note for their ability to evaluate mate attractiveness basing on subtle differences in colour, hue and intensity of the colour spots on males body (Houde, 1997; Kodric-Brown, 1989; Long & Houde, 1989). These features are memorized by females for long time in order to make future mating decisions (Dugatkin et al., 1992; Eakley & Houde, 2004). The high cognitive requirements for mate choice suggests selection should favour the evolution of enhanced ability for discerning, learning, memorizing and discriminating colours in females. I therefore expected females should perform better than males in experiment 1 and the first phase (colour discrimination) of experiment 2.

2.4.2 Experiment 1: oddity discrimination

In this experiment, guppies were trained to choose the object with the odd colour in a set of five stimuli, four of which had the same colour. Each day of the training I changed the colour combination to allow guppies to learn the general concept "choose the odd colour".

Materials and methods

In this experiment, I used the same training paradigm described for experiment 1 and experiment 2 of the previous section (detailed description in chapter 2.3.2). Guppies were trained to dislodge a disc on a perforated plate to get a food reward. During each trial of the discrimination phase (phase 4, chapter 2.3.2), five discs were displayed in a row. Four discs were of the same colour, while one was of a different colour and concealed the food reward. This experiment lasted six days (60 trials), and each day the subject was administered a different pair of colours to discriminate (figure 10). I trained 10 male and 10 female domestic guppies. According to the previous experiments (chapter 2.3.2), subjects that did not learn to dislodge the discs before the experiment (phase 3) were discarded and substituted with new subjects of the same sex. Four males and one female were discarded in this experiment.

Statistical analysis have been performed following experiment 1 and 2 of the previous sections (details are described in chapter 2.3.2). The analysis of accuracy was more complex to study also the strategy adopted by subjects to solve the task (e.g., if they learned the concept “choose the different” or if they recurrently learn the colour discrimination presented each day). I used the ‘glmer’ function from the ‘lme4’ R package to fit the response of the subjects in each trial (correct or incorrect) in a generalised linear mixed-effects model (GLMM) with logit link function and binomial error distribution. I fitted day of training, trials of the day and sex as fixed effects and subject ID as a random effect. I asses statistical significance with Wald χ^2 test. I also analysed individual differences in the learning strategy. For each subject, I computed an improvement rate across trials within days and an improvement rate across days. The first was the Spearman ρ of the correlation between the proportion of correct choices in each trial across the days of training and the ordinal position of the trial (from 1 to 10); the second was the Spearman ρ of the correlation between the proportion of correct choices on each day and the ordinal position of the day (from 1 to 6).

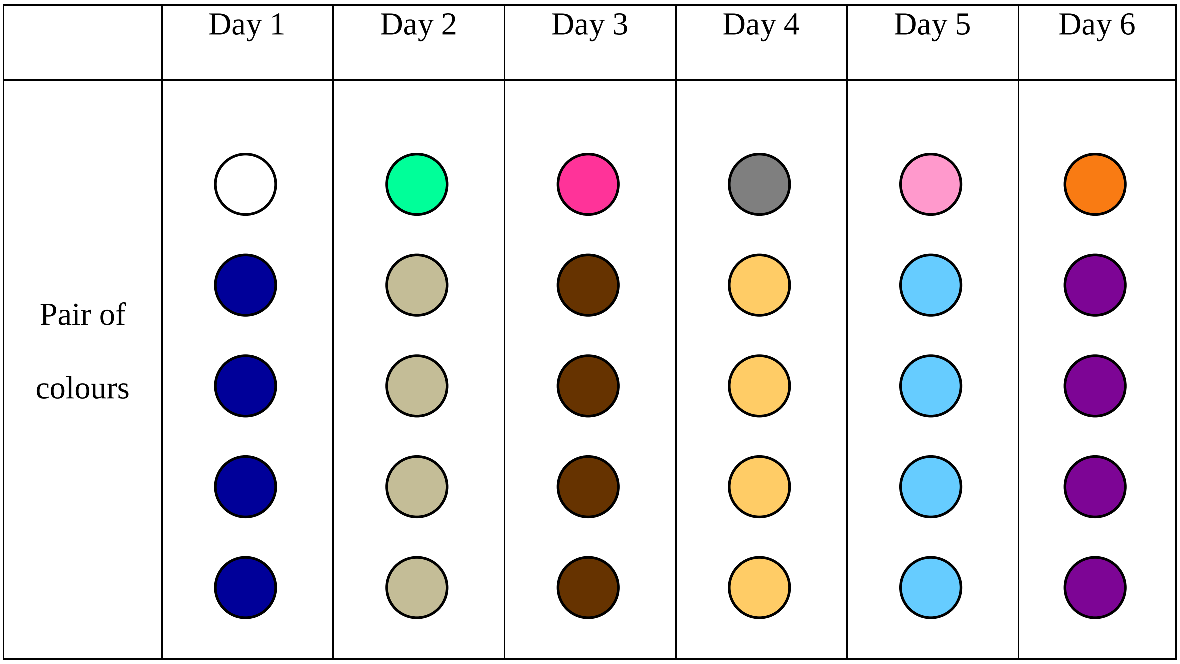

Figure 10: Pairs of colours used in the oddity discrimination learning (experiment 1). Guppies had to choose the odd colour to find the reward. The rewarded colour was flipped for half of the subjects.

Results

Males and females had comparable standard length (30.60 ± 3.13 mm and 32.90 ± 2.33 mm, respectively; $t(18) = 1.862$, $p = .079$). There was no significant correlation between standard length and overall accuracy ($r(18) = .361$, $p = .118$) or between standard length and overall decision speed ($r(18) = -.107$, $p = .652$).

Analysis of individual performance found nine males and seven females significantly learned the task (more than 30% correct choices, binomial test). Overall, subjects chose the correct disc in 40.50 ± 11.21 % of the trials, an overall accuracy that was significantly greater than chance ($t(19) = 8.579$, $p < .001$).

My oddity concept learning task could potentially be solved using two different strategies: the first consisted of recurrently learning which colour concealed the food reward each day; the second consisted of learning the general concept that the food reward was always concealed under the odd colour. I predicted that if guppies used recurrent learning they would increase their performance across trials within each day of training; conversely, if guppies adopted concept learning, they would increase their performance across the days of training. The two sex could adopt differently the two strategy. In the GLMM to explore these possibilities, day of training had a significant effect (Wald $\chi^2(5) = 28.622$, $p < .001$; figure 11a, but there was no significant increase (linear trend: $p = .331$). Trial within day had not significant trial (Wald $\chi^2(9) = 12.777$, $p = .173$; linear trend: $p = .771$) or sex (males' accuracy: 43.17 ± 11.09 %; females' accuracy: 37.83 ± 11.25 %; Wald $\chi^2(1) = 1.141$, $p = .995$; figure 11a). Bayesian analysis also revealed absence of sex differences better explain our data ($BF_{01} = 18.501$). Sex by day of training interaction was not significant (Wald ($\chi^2(5) = 3.387$, $p = .547$; figure 11a), revealing the two sexes exploited the same strategy to solve the task. There were no other significant interactions in the model. These analysis suggested males and females adopted the same strategy to solve the task, yet it was not clear which one. A possible explanation is that some individuals adopted recurrent learning, whereas some others adopted concept learning, irrespective of the sex. Indeed, I found a negative correlation between the improvement rates (Spearman's rank correlation: ρ = -.506, p = .023). Thirteen subjects (six males and seven females) appeared to increase their performance across trials within day, as predicted for the use of recurrent learning strategy; seven subjects (four males and three females) appeared to increase their performance across the days of training, as predicted for the use of concept learning strategy.

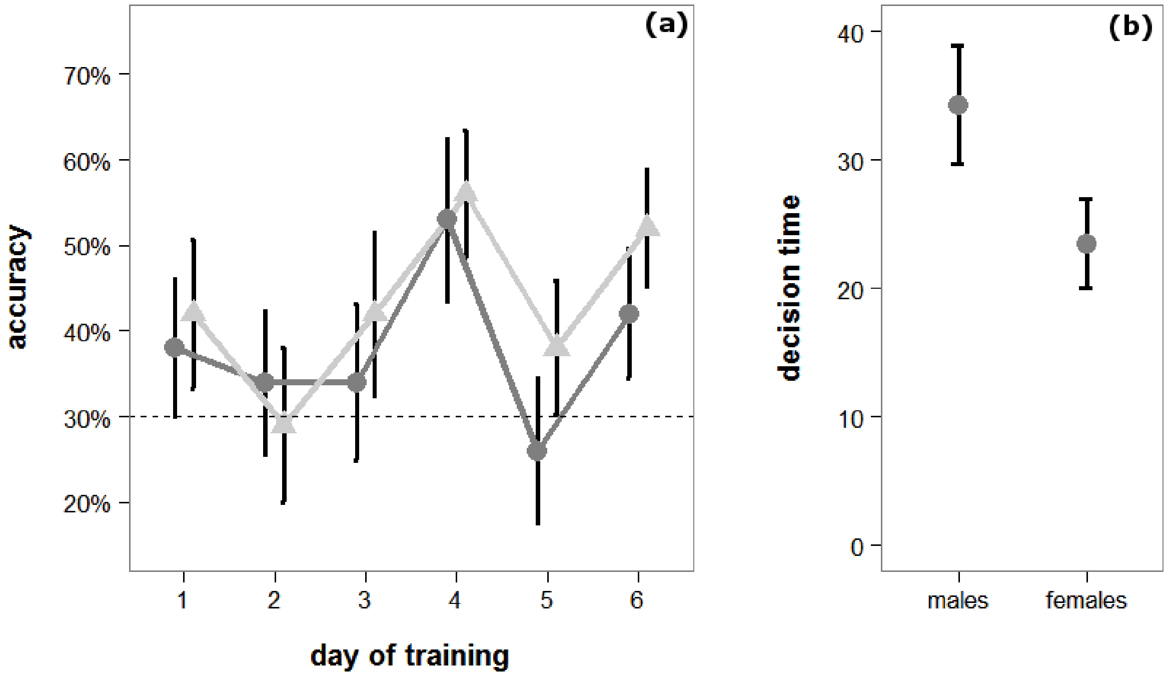

Figure 11: **(a)** Accuracy of males (grey) and females (dark) in experiment 1 (oddity discrimination learning); data points represent *M* ± *SE* percentage of correct choices; dashed line is chance performance. **(b)** Average decision speed (*M* ± *SE* seconds) of males and females guppies in experiment 1.

The significant effect of day of training in the two models on the accuracy without any evidence of a linear trend could be explained by the fact that the colour discriminations presented in some days of training were more difficult to achieve by the subjects. A separated analysis for each day of training (corresponding to the different pairs of colour) revealed subjects achieved a significant performance in day 1 (40.00 ± 25.75 %; one-sample *t* test: $t(19) = 3.279$, $p = .004$), day 4 (54.50 ± 26.25 %; $t(19) = 5.276$, $p < .001$) and day 6 (47.00 ± 22.73 %; $t(19) = 5.265$, $p < .001$), as well as an almost significant performance in day 3 (38.00 ± 28.94 %; $t(19) = 2.063$, $p = 0.053$). By contrast, subjects did not significantly solve the task in day 2 (31.50 ± 26.80 %; $t(19) = .698$, $p = .494$) or in day 5 (32.00 ± 25.87 %; $t(19) = 1.131$, $p = .272$). Comparing within each pair the performance of subjects trained with one colour as positive and subjects with the other colour as positive, I found a significant difference in day 1 (independent-sample *t* test: $t(18) = 7.452$, $p < .001$), day 2 ($t(18) = 4.187$, $p < .001$) and day 5 ($t(18) = 3.543$, $p = .002$) but not in the other days. Therefore, it seems reasonably that the low performance of the subjects in some discriminations was due, at least in part, to the preference for one of the two colours in some pairs.

The overall decision speed was 28.86 ± 13.75 s, but the LMM revealed daily decision speed decreased significantly across days of training ($F(5,90) = 4.260$, $p = .002$). Sex had a significant effect in the model ($F(1,18) = 5.517$, $p = .031$; figure 11b): males had a faster decision speed compared to females (23.46 ± 11.05 s and 34.27 ± 14.55 s, respectively). No significant sex by day of training interaction was found ($F(5,90) = 1.000$, $p = .423$). Overall, there was no significant correlation between accuracy and decision speed ($r(18) = -.154$, $p = .518$). In addition, no correlation between accuracy and overall decision speed was found in the sex-separated analysis (males: $r(8) = -.337$, $p = .341$; females: $r(8) = .246$, $p = .493$).

In the olfactory cue control test, subjects did not choose the discs hiding the reward more than chance (20.33 ± 9.04 %; $t(19) = .430$, $p = .672$).

2.4.3 Experiment 2: Reversal learning

Guppies were initially trained on a simple red *versus* yellow colour discrimination; the subjects were rewarded with food when they chose the correct colour among the two options. After learning the task, the reward contingency was reversed and the subjects should the selected the previously unrewarded colour.

Materials and methods

Subjects: I used 14 male and 14 female guppies matched for body length.

Experimental apparatus: The apparatus was the same adopted in previous training experiments (described in chapter 2.3.2).

Pre-experiment procedure: Compared to previous training experiments, the procedure was slightly modified in order to evaluate performance of the subjects by mean of a learning criterion. Subjects underwent the habituation to the apparatus and to the procedure as described for previous experiments (chapter 2.3.2). The training to dislodge the discs (phase 3, chapter 2.3.2) was slightly different. To train the subject to dislodge the discs, in the first nine trials (cued trials), two discs (one yellow and one red) partially covered two randomly chosen holes of the plate, leaving a gap that was progressively reduced. The initially rewarded disc concealed a small piece of food. These nine cued trials were not included in the analysis.

Experimental procedure: At the beginning of the experiment, the subject was trained on the discrimination of the two colours, red and yellow. I administered six trials per day. Trials followed the procedure previously described (chapter 2.2.3). In the experimental trials, two holes in the plate were completely covered with the two plastic discs. The position of the two discs was chosen according to a pseudo-random pre-set scheme. A small piece of crumbled food flake was concealed under the predetermined disc. The initial rewarded colour was counterbalanced across subjects and was the same of training to dislodge the disc. The subject was allowed to observe the plate for 30 s from behind the transparent guillotine door before entering the experimental compartment and dislodge the discs. I recorded the first disc dislodged as indication of the choice, but correction was allowed within five minutes. The learning criterion was eight correct responses out of 10 consecutive trials. This weak learning criterion was selected to avoid overtraining before the reversal learning (Warren, 1960). The procedure here adopted caused subjects having a different number of observed trials. For example, faster learning subjects would perform fewer trials compared to slow learners. Because of this differences in number of observations I did not measure decision speed in this experiment. After the fish reached the learning criterion in the discrimination learning, it was admitted to the discrimination reversal learning. In the discrimination reversal, the procedure and the learning criterion were the same except the contingency of the reward was reversed between the two discs. In a subsample of 16 fish (eight males and eight females), after reaching the criterion of the first reversal, the contingency of reinforcement was reversed again and the subject continued this procedure until it completed a series of four reversals. To ensure that subjects had not learnt using olfactory cues, I performed a control test at the end of the serial reversal on eight randomly chosen subjects (four males and four females). These subjects underwent given 24 further trials with the same procedure as before, except I presented two discs of the same colour with one, randomly chosen, hiding food.

Statistical analysis: Learning was computed as the number of errors to criterion. Due to a floor effect (64 % of subjects made 0 errors), colour discrimination learning was analysed as a binary variable (0 = no error, 1 = one or more errors) using a generalized linear model (GLM) with binomial error distribution and logit link function (sex and reinforced colour as factors and body length as a covariate). The number of errors in discrimination reversal learning and in serial reversal learning was square root transformed and analysed, respectively, using ANCOVA and a linear mixed model (LMM). The ANCOVA was fitted with sex and reinforced colour as factors and body length as a covariate. The LMM was fitted with sex and reversal as fixed effects and subject ID as a random effect to account for repeated measures. The proportion of correct choices in the olfactory cues control test was arcsine square root transformed and analysed using one sample t-test.

Results

Colour discrimination learning: Colour discrimination was learnt by all 28 subjects in 9.18 ± 2.50 trials and with 0.68 ± 1.19 average errors. Eighteen subjects (64 %) made no errors; five subjects (two males and three females) made one error; three subjects (one male and two females) made two errors; one male made three errors; and one female made five errors. There was no significant effect of sex (GLM: $\chi^2(1,23) = .288$, $p = .592$; figure 12), body length ($\chi^2(1,23) = .760$, $p = .383$), or reinforced colour ($\chi^2(1,23) = 1.314$, $p = .252$), and no interaction was significant.

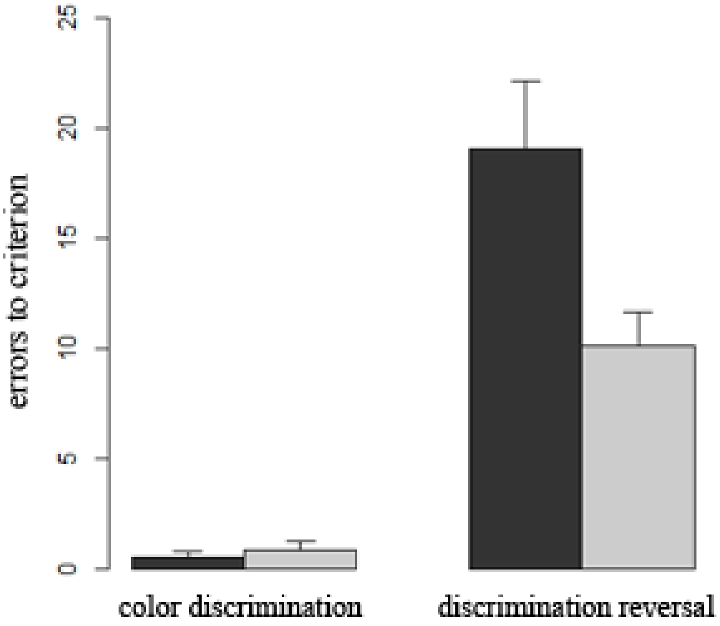

Figure 12: Number of errors (*M* ± *SE*) made by male (dark bars) and female (grey bars) guppies in colour discrimination learning and in discrimination reversal learning of experiment 2 (reversal learning).

Discrimination reversal learning: In the first reversal, subjects made 14.61 ± 10.01 errors to reach the learning criterion. Males made more errors than females. The ANCOVA revealed a significant effect of sex (*F*(1,23) = 6.861, *p* = 0.015; figure 12) but no significant effect of size (*F*(1,23) = 3.343, *p* = 0.080), reinforced colour (*F*(1,23) = 2.955, *p* = 0.099), or interaction.

Serial reversal learning: No effect was significant (LMM: sex: *F*(1,14) = 1.197, *p* = 0.292; reversal: *F*(3,42) = 1.312, *p* = 0.283), but there was a significant sex by reversal interaction (*F*(3,42) = 4.869, *p* = 0.005; figure 13). When analysed separately, males improved their performance in subsequent reversals (*F*(3,21) = 3.326, *p* = 0.039), while variation in female performance was not significant (*F*(3,21) = 2.723, *p* = 0.070).

Olfactory cue control test: Control tests indicated that subjects were not using an olfactory cue: disc hiding food was selected in 46.35 ± 7.86 % of the trials, a proportion not significantly different from chance (one-sample *t* test: *t*(7) = 1.133, *p* = 0.231).

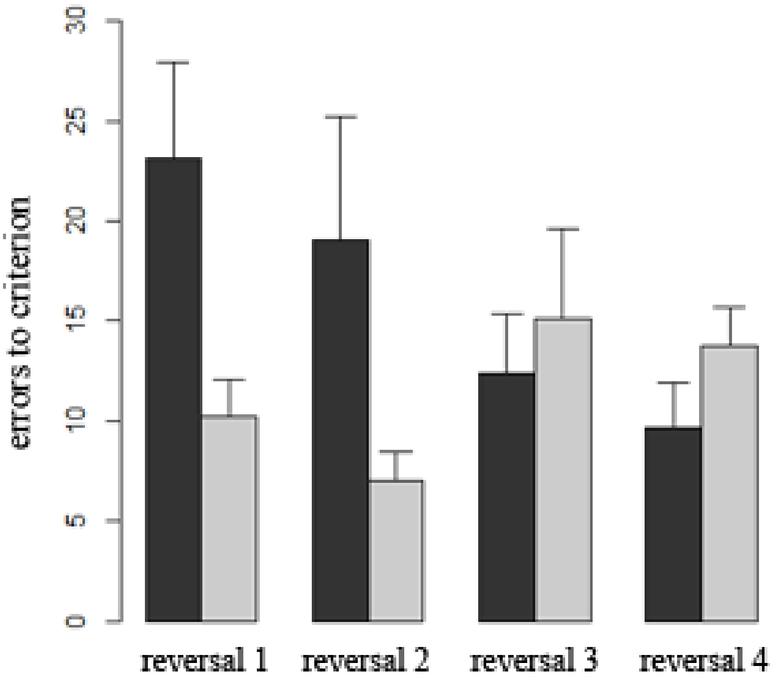

Figure 13: Number of errors (*M* ± *SE*) made by male (dark bars) and female (grey bars) guppies in serial reversal learning of experiment 2.

2.4.5 Discussion

In experiment 1 of this chapter, guppies performed a task based on oddity concept learning (Hille et al., 2006). Guppies learned this task as they overall choose the correct disc more than chance. There was no evidence of sex difference in the accuracy. This is supported by Bayesian analysis that indicated our data are approximately 20 times to have occur in absence of sex difference. I failed in finding clear evidence of oddity concept learning (improvement of the performance across days of training). This can be due to the fact that I presented the same pair of colours throughout each day and I changed the pair daily. With this design, the task could be solved either with a strategy different to learning the concept (i.e., always choosing the odd disc). Indeed, fish could recurrently learn the different colour discrimination presented each day. An analysis of individual performance suggested that around one-third of the subjects used concept learning, whereas the remaining subjects recurrently learned the new discrimination each day. Although these results are promising in suggesting that guppies can learn oddity concept, further investigations are required. Males and females were equally split between the two groups that adopted different strategies. This suggests that, in this species, individual within-sex differences in learning strategies encompass differences between the sexes.

In experiment 2, both sexes rapidly learned the initial colour discrimination. After the nine cued trials, the vast majority of subjects reached the learning criterion without any further error. The acquisition of colour discrimination appears to be faster than previously found in fish (e.g., Parker et al., 2012) and in other vertebrates (e.g., Range et al., 2006). The remarkable performance of guppies in learning a colour discrimination task might be related to the use of a procedure that mimics guppies' natural foraging habits. These fish tend to search for small orange fruits that drop from the forest canopy into the rivers (Rodd et al., 2002). In contrast to their rapid initial learning, subjects made a larger number of errors after reversal of the reward contingency. I found a striking sex difference in this second phase. Males showed a greater persistence of the previously learned response and made twice the errors of females before learning the reversed colour-food association. Males and females might differ in several aspects associated with performance in reversal learning, such as feeding motivation, physical strength, or general learning ability. However, these factors are unlikely to explain the differences in reversal learning as I found no sex difference in the initial colour discrimination learning. Moreover, experiments of previous section (chapter 2.3) suggested the absence of sex differences in the ability to learn visual discriminations. In my study, males, but not females, gradually reduced the number of errors in successive reversals. After two reversals males' performance appeared to be close to females' performance. Unfortunately, it is not possible to determine whether this effect is due to cognitive sex differences (i.e. males are better than females in learning the serial repetition of reversals) or simply to a floor effect, given that females made less than 10 errors to criterion already in the first reversal.

I expected a better performance of females in experiment 1 and in the initial colour discrimination of experiment 2. Female guppies base mate choice on colour ornaments present on males' body; several studies have revealed a fine ability of females in estimating the size and hue of the different colour spots as well as their capacity to remember and compare the quality of different males (Dugatkin et al., 1992; Eakley & Houde, 2004; Kodric-Brown, 1989; Long & Houde, 1989). One might therefore expect females being selected for enhanced colour discrimination learning abilities. Yet, my two experiments revealed not female advantage in this task. A recent study has shown males probably need the same capacity as females in estimating male body coloration. Indeed, males are able to exploit female preference by associating with dull males (Gasparini et al.

2013). The ability to learn colour discriminations could therefore be equally selected in both male and female guppies.

The results of the second phase of experiment 2, instead, agree with previous data obtained in polygamous mammals and birds in which females showed greater cognitive flexibility. Females are indeed more ready to change their response when a learned rule becomes inappropriate (Guillamón et al., 1986; Ha et al., 2011; Rogers, 1974). The evolutionary explanation for this sex difference in cognitive flexibility remains speculative. One possibility is that it is related to the different social and parental roles of males and females. Some authors (Laland & Reader, 1999) have suggested that the evolution of flexibility may be promoted by high parental investment and complex social life and males and female guppies differ markedly in these two traits (Croft et al., 2004; Houde, 1997). Alternatively the greater females cognitive flexibility may a consequence of the different roles of the two sexes in mating competition and mate choice (Allan et al., 2012; Gaulin & FitzGerald, 1986). Theoretical and empirical studies show that the evolution of male persistence traits to overcome female resistance is favoured in polygamous species by mean of sexual selection (Rowe et al., 2005). It is not known if differences in cognition that evolved in one context (e.g. reproductive strategies) may affect other behaviours (e.g. foraging). However, the possibility exists that sex differences in cognitive flexibility arise from selection for different mating strategies in males and females. A critical prediction of this hypothesis is that the sex difference in flexibility should be large in polygamous species, such as the guppy, and reduced or absent in monogamous species. Currently there are not sufficient data in the literature to test this hypothesis, but, interestingly, recent studies found no evidences of a greater female cognitive flexibility in three monogamous avian species (Brust et al., 2013; Range et al., 2006; Titulaer et al., 2012).

As in experiments of previous section (chapter 2.3), in my oddity concept learning task males were substantially faster than females in decide which disc to dislodge. This sex differences in decision speed appears therefore to be robust in guppies and to exist in very different tasks.

2.5. Spatial learning

2.5.1 Introduction

Several life-history processes, such as foraging, reproduction, and predator avoidance, require animals to navigate through space (Odling-Smee & Braithwaite, 2003). Spatial abilities have attracted the interested of a large number of researchers. As a consequence, most of the investigations on cognitive sex differences have focused on spatial abilities. In this field, a male superiority has been commonly found in several polygamous mammals (e.g., Collins & Kimura, 1997; Jonasson, 2005). Existing literature provides several examples of spatial tasks available for fish (e.g., Aoki et al., 2015; Brown & Braithwaite, 2005; Girvan & Braithwaite, 1998; Sovrano et al., 2003). In this section, I am reporting the results of three experiments based on previously developed paradigms. In each experiment, guppies had to solve a spatial problem to reach a reward. Guppies were observed repeatedly in the task to measure the improvement in performance as a measure of learning. The reward was a shoal of conspecifics. The guppy is a social species and individuals in the wild form shoal of different size (Griffiths & Magurran, 1998). This social behaviour is thought to be an antipredator strategies and it is therefore expressed mainly in hazardous situations (Magurran & Seghers, 1991). Indeed, in large groups the individual risk of being caught is drastically diluted (Krause & Ruxton, 2002). To exploit this social tendency, I tested subjects in a novel environment with no shelters, a situations in which guppies show a strong antipredator response and try to join available shoals. In an adjacent tank there was a visible shoal of conspecifics. The subject could reach the shoal by completing the spatial task.

In experiment 1a, I studied the ability of learning to navigate around an obstacle to reach a target, a behaviour often referred as detour (Bisazza et al., 1997a; Bisazza et al., 1997b; Regolin et al., 1995). Most of the fish species adopts detour behaviour (Bisazza et al., 2000). I used either a transparent obstacle (experiment 1a) that facilitate the sight of the stimuli and an obstacle made of net grid (experiment 1b).

In experiment 2, I studied the performance of male and female guppies in a Y maze, a very common paradigm for spatial abilities investigations in fish and other vertebrates (Aoki et al., 2015; Conrad et al., 2003; Patterson-Kane et al., 1997; Takahashi et al., 2010). One of the two arms of the maze conducted to the reward shoal, while the other conducted to a dead end. Here, I also tried to investigate the strategies adopted by the guppies to learn the task with three different experimental conditions. In the first condition, the rewarded arm was always on the same side (left or right) and there was not landmark; in the second condition, the rewarded arm switched alternately between left and right in each trial and it was indicated by a landmark; in the third condition, the rewarded arm was always on the same side and it was also indicated by a landmark.

In experiment 3, I used a more complex maze paradigm used to study spatial learning in fish (Girvan & Braithwaite, 1998). In each trial, guppies had to choose between four different routes, and one of them allowed to reach the reward shoal.

One of the many ecological sex differences in guppies (described in chapter 2.2.1) regards space utilization. Males inhabit shallow water rich of vegetation and therefore their habitat is characterized by elevated spatial complexity; females often prefer deep open waters (Darden & Croft, 2008). Moreover, males are much more likely than females to migrate in different parts of the habitat (Croft et al., 2003). These sex differences in spatial ecology allowed to formulate a prediction on the outcomes of my experiments on spatial abilities. I expected the sex with larger and more complex space use, the male, to be selected for enhanced spatial abilities and therefore to perform better in my spatial learning experiments.

2.5.2 Experiment 1: Detour behaviour

In this experiment, I studied how male and female guppies navigate around an obstacle to reach a visible target, a shoal of conspecifics. I performed two versions of this experiment. In experiment 1a, the obstacle was a transparent barrier; in experiment 1b, I added a grid to the transparent barrier.

Materials and methods

Subjects: In experiment 1a, I observed 12 males and 12 females of the wild guppy strain. I observed the same number of subjects in experiment 1b.

Apparatus: I built the apparatus following the large number of studies investigating detour behaviour of guppies and other poeciliid fish (e.g., Bisazza et al.,1997a; Bisazza 1997b; figure 14). The apparatus consisted of two tanks. The first one (“experimental tank”), is an 80 x 40 x 35 cm glass aquarium in which I built a white start box (10 x 10 cm) that housed the subject at the beginning of the experiment. A short corridor (10 x 5 cm) led from the start box to the central arena of the experimental tank. In the opposite side of the experimental tank, I built a goal zone (15 x 40) with green plastic that simulated the colour of natural vegetation. A second, smaller (50 x 20 x 35 cm), glass aquarium (“stimulus tank”) with the target shoal was placed adjacent to the goal zone. Form the start box, the subject could see the stimulus shoal through the transparent glass wall. To enter the goal zone, the subject had to pass thought a one-way corridor made of transparent plastic that prevent the subject to move back to the central arena. Outside the goal zone, the walls and bottom of the experimental tank were covered by white plastic. In the middle of the central arena, at 20 cm from the start box, I placed a barrier that the subject had to detour to reach the goal zone. The barrier was a 15 cm transparent plastic panel. Two lateral sides (5 cm) made with green plastics prevented guppies from accidentally detouring the barrier by simply moving along it. To detour the barrier, the subject has to go backward and to move far from the stimulus shoal. In experiment 1b, a grid net (1 x 1 mm holes) covered entirely the transparent side of the barrier. Subjects could detour the barrier from both right and left side.

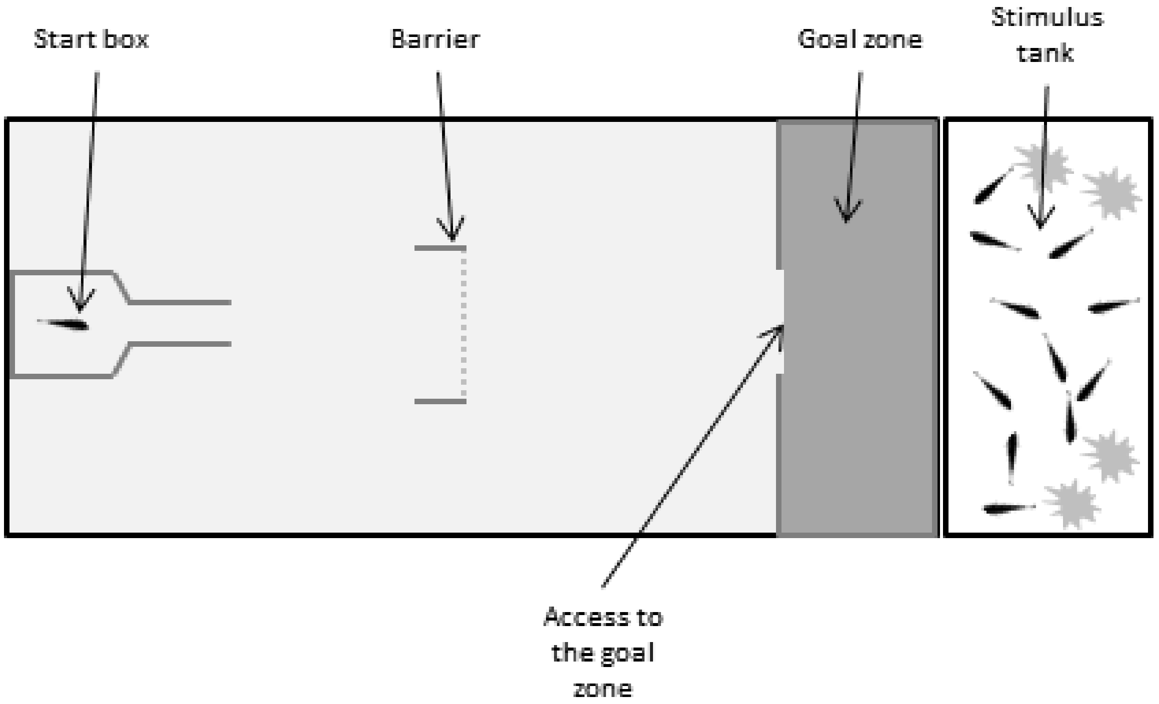

Figure 14: Aerial view of the apparatus adopted for experiment 1 (detour behaviour). From the start box the subject could see the stimulus shoal. To reach the goal zone, the subject had to detour around the barrier.

The stimulus tank was provided with natural gravel, many natural plants, a water filter and two 15-w fluorescent lamps. The background wall of the stimulus tank was white to improve stimuli' visibility. I used 12 male and 12 female guppies as stimuli. These guppies were housed in the stimulus tank since almost three days before the experiment. Two transparent partitions confined 6 males and 6 females in the middle (10 cm) of the stimulus tank to ensure they were visible from the subject in the start box. A sliding panel between the two tanks regulated the sight of the stimulus tank during the different phases of the experiment (see procedure below). The entire apparatus was placed in a dark room, and the experimental tank was illuminated indirectly from the stimulus tank. With this set up, I ensured stimuli were clearly illuminated and therefore visible by the subject. A camera recorded the experiments from above.

Procedures: Each subject was observed in the detour task five times to study improvement in successive trials due to learning. To start the experiment, I inserted the sliding panel between the two tanks. Then, I gently netted the subject from the maintenance tank and moved it into the start box. This latter procedure was made by inserting the net in the start box oriented toward the opposite direction of the stimuli, and allowing the guppy to exit spontaneously. After ensuring that the subjects exited the net, I removed the net and the sliding panel between the two tanks. The subject now see the stimulus tank and the trials started. I observed the trial from a monitor connected to the camera. When the subject entered the goal zone, I let it undisturbed 5 min with the shoal as reward. After the reward, I inserted the sliding panel between the two tanks and, after 2 more min, I netted the subject and moved it back to the start box for a novel trial. Each subject performed five consecutive trials, since pilot study revealed very often guppies lose social motivation in longer experiments. I discarded subjects taking longer than 20 min to complete the trial (4 males and 5 females). I assumed these guppies were somewhat not motivated to join the stimulus shoal. Discarded subjects have been substituted with subjects of the same sex. From the recordings, I measured the time to solve the task, i.e. the time took by the subject to enter the goal box after exiting the start box.

Statistical analysis: For each experiment, I built a linear mixed-effect model (LMM) on the log(time to solve the task). I fitted trial and sex as fixed effects, and subject ID as random effect. In case of significant effect of trial or sex by trial interaction, I performed a trend analysis. Approximate Bayes factor (BF_{01}) was calculated as in previous experiments. In the text data are expressed as $M \pm SD$.

Results

Experiment 1a: I found a significant effect of trial in the LMM ($F(4,88) = 9.253$, $p < .001$). Time to solve the task decreased linearly from the first to the fifth trial ($p = .013$), indicating subjects improved their performance across trials. Moreover, I found a significant effect of sex, as males took significantly longer than females to navigate around the transparent barrier ($F(1,22) = 18.293$, $p < .001$; figure 15a). There was not trial by sex significant interaction ($F(4,88) = .641$, $p = .635$).

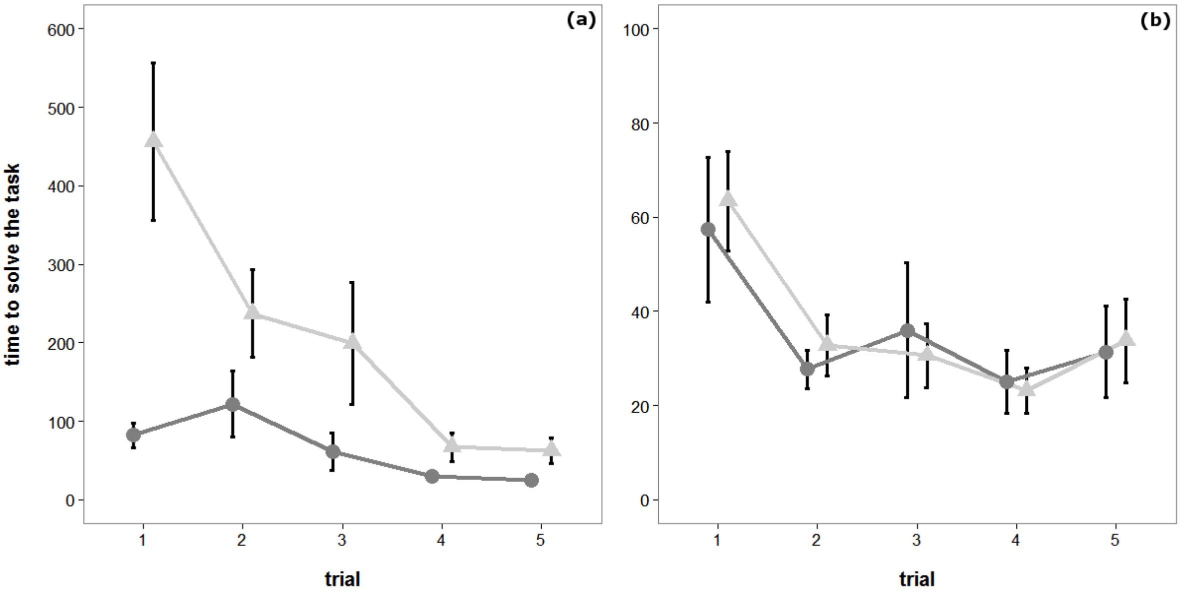

Figure 15: Time to solve the task ($M \pm SE$) of males (grey) and females (dark) in **(a)** experiment 1a and **(b)** experiment 1b (detour behaviour). Note that the two panels have different scale.

Experiment 1b: I found a significant effect of trial in the LMM ($F(4,88) = 4.908$, $p = .001$), indicating time to solve the task significantly decreased following a linear trend ($p = .020$). There was not significant effect of sex ($F(1,22) = .548$, $p = .467$, $BF_{01} = 17.56$) nor significant trial by sex interaction ($F(4,88) = .205$, $p = .935$; figure 15b).

2.5.3 Experiment 2: Y maze

In the Y maze, the subject was required to choose the correct arm in order to reach the reward, a shoal of conspecifics. By using three different conditions, I also studied spatial navigation strategies of male and female guppies to look for sex differences.

Materials and methods

Subjects: I tested 12 males and 12 females of the wild guppy strain in each condition, resulting in 36 males and 36 females overall.

Apparatus: The apparatus is formed by two tanks. The experimental tank was an 80 x 40 x 35 cm glass aquarium in which I built a Y maze using white plastic (figure 16). In one side of the experimental tank there was the start box; in the opposite side there was a goal zone as described for experiment 1 of this section. The stimulus tank adjacent to the goal zone is identical to the one described for experiment 1 of this section (chapter 2.5.2) and so are the stimulus guppies. A 10 x 10 cm hole with a transparent plastic and a grid (as in experiment 1b), allowed the subject in the start box to see the stimulus shoal. One of the two arms conducted to goal zone; the other arm conducted to a dead end closed by mean of a grid. The shape of the two arms prevented the subject to see which arm lead to the dead end before entering. As a visual cue I used a 1 x 10 cm blue plastic rectangle fixed in vertical position by a weight pasted on its base. Other details of the apparatus are identical to the one described for previous experiments.

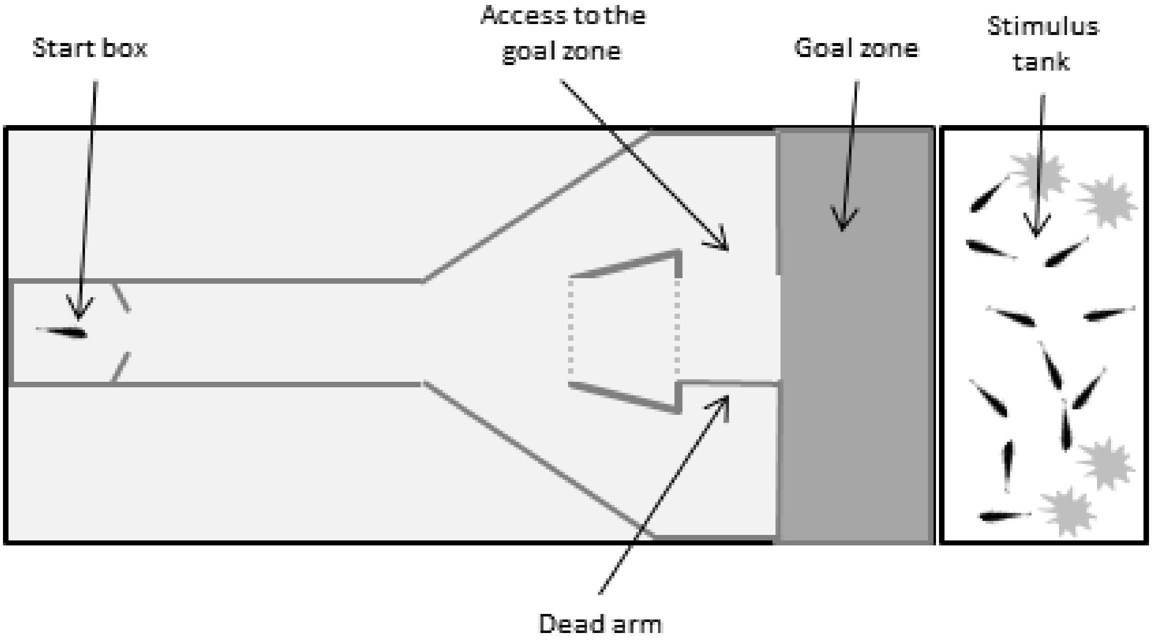

Figure 16: Aerial view of the apparatus adopted for experiment 2 (Y maze). From the start box the subject could see the stimulus shoal. To reach the goal zone, the subject had to choose the correct arm of the maze.

Procedures: The procedure followed the one adopted in experiment 1 (described in chapter 2.5.2). Yet, pilot study revealed with this apparatus guppies consistently reach the goal zone up to 6 consecutive times, perhaps because the narrow corridor encourages them to swim forward. Thus, I decided to perform six trials for each subject. The position of the rewarded arm, right or left, was alternated between subjects. In the first condition ("side condition"), the rewarded arm was always in the same right-left position for each subject; there was no visual cue available to identify the rewarded arm. In the second condition ("landmark condition"), the rewarded arm was switched alternately between the two arms across the six trials (i.e., left-right-left-right-left-right for half of the subjects and right-left-right-left-right-left for the remaining subjects); the visual cue indicated which arm was rewarded in each trail. In the third condition ("side + landmark condition"), the rewarded arm was always in the same right-left position and it was also indicated by the landmark. In the second condition, the subject was moved in a small jar for few seconds between each trial in order to allow the experimenter to change the position of the grid and the visual cue. From the recordings, I measured, as indication of learning, the time to solve the task as in experiment 1. As an indication of the accuracy in discriminating the two arms, I recorded the first arm chosen by the subject in each trial (rewarded or nonrewarded). I also recorded decision speed, measured as the latency to enter the first chosen arm after the subject exited the start box. I discarded and substituted eight males and eight females that did not complete the trial within the cut off time (20 min).

Statistical analysis: To test whether guppies significantly choose the rewarded arm, I used one-sample t test on the accuracy, proportion of correct choice (after arcsine square root transformation; Sokal & Rohlf, 1995), against a chance performance (0.5 of correct choices). In this analysis I did not considered the choice in the first trial as subjects are expected to choose randomly. I studied more in details the accuracy with a generalized linear mixed-effects model (GLMM) with binomial errors structures and logit link function (Crawley, 2007). Here, I fitted trial, sex, condition as fixed effects, and subject ID as random effect. LMMs generally followed as described in the previous experiments (chapter 2.5.2). Other details of the analysis are described in chapter 2.5.2.

Results

In trials 2 to 6, subjects accuracy in choosing the rewarded arm was 53.61 ± 27.85 %. Since this accuracy did not differ from chance ($t(71) = 1.288$, $p = .202$), guppies apparently were not able to solve the simple Y maze task. Analysis of choices' accuracy with the GLMM confirmed the absence of learning. There was not increase in the likelihood of choosing the correct arm across the six trials ($\chi(6) = 3.881$, $p = .693$). Sex had not significant effect in the model ($\chi(1) = .209$, $p = .648$, $BF_{01} = 19.76$), as well as the interactions involving sex (figure 17a). This suggest the absence of sex differences in this task. I found a significant effect of reward side ($\chi(1) = 6.997$, $p = .008$), and a significant reward side by condition interaction ($\chi(2) = 10.139$, $p = .006$). To understand this interaction, I built three simpler models, one for each condition. For the landmark condition and in the side condition, there were not significant effects in the model (all *p* values > .1). Conversely, in the side + landmark condition there was a significant effect of reward side ($\chi(1) = 10.148$, $p = .001$). Subjects tend to choose at first the correct arm more than chance when the right arm was rewarded (69.44 ± 29.16 %; $t(11) = 1.901$, $p = .084$). Subjects chose the correct arm less than chance when the left arm was rewarded (31.94 ± 15.01 %; $t(11) = 4.092$, $p = .002$). Thus, subjects expressed a spontaneous right turning bias. The LMM on the time to solve the task revealed roughly the scenario of above.

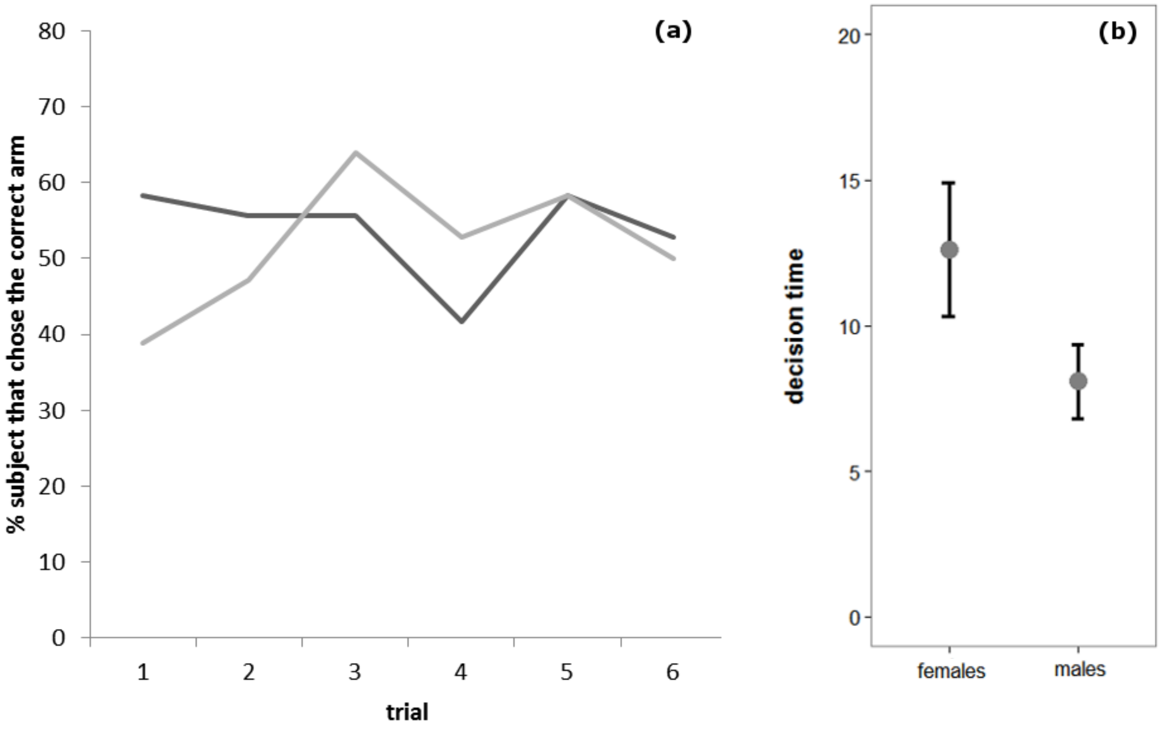

Figure 17: **(a)** Percentage of males (dark) and females (grey) that chose the correct arm in experiment 2 (Y maze). **(b)** Average decision speed (*M* ± *SE* seconds) of males and females guppies in experiment 2.

There was not improvement in the performance, i.e. reduction of the time need to solve the task ($F(5,294) = 1.552$, $p = .174$). Sex had not significant effect in the model ($F(1,66)$ =

.021, $p = .884$, $BF_{01} = 46.31$). Interactions involving sex term were not significant. I found only a significant reward side by condition interactions ($F(2,294) = 4.882$, $p = .008$). By analysing separately each condition with three simpler models, I found only a reward side significant effect in the model for the side + landmark condition ($F(1,20) = 6.438$, $p = .020$). Subjects were faster to reach the goal box in the trial when the right arm was rewarded (70.69 ± 125.10 s) compared to the opposite situation (89.07 ± 117.39 s).

The analysis of the decision speed showed, instead, a significant effect of sex ($F(1,66) = 4.589$, $p = .036$), as males are much faster than females in deciding which arm to enter (figure 17b). Other effects and interactions were not significant.

2.5.4 Experiment 3: Complex maze

In experiment 3, I used a more complex maze paradigm. Guppies had to choice between two routes in an open arena to move forward. In each single trial, guppies faced twice this choice before reaching the reward, a shoal of conspecifics. Thus, there were overall four possible routes, but only one led to the reward.

Materials and methods

Subjects: I tested 24 males and 24 females of the wild guppies strain.

Apparatus: In this apparatus, two transversal walls divided the arena between the start box and the goal zone, forming three sectors (figure 18). Subject in the start box could see the stimulus shoal by mean of a hole (10 x 10 cm) covered with transparent plastic and grey net grid in the middle of the two walls. Each of the transversal walls was provided with two doors (4 x 5 cm) that allowed to access to the next sector of the maze. Only one of the routes was correct, the other was blocked as in experiment 2 (chapter 2.5.3). In the opposite wall, the correct route was the one in the opposite side (figure 18). A visual landmark (a little artificial plant) indicated the correct route. The bottom of the second sector was covered with light yellow plastic to allow subject note the difference from the first sector. Other details of the apparatus resembled the previous ones (described in chapters 2.5.2 and 2.5.3).

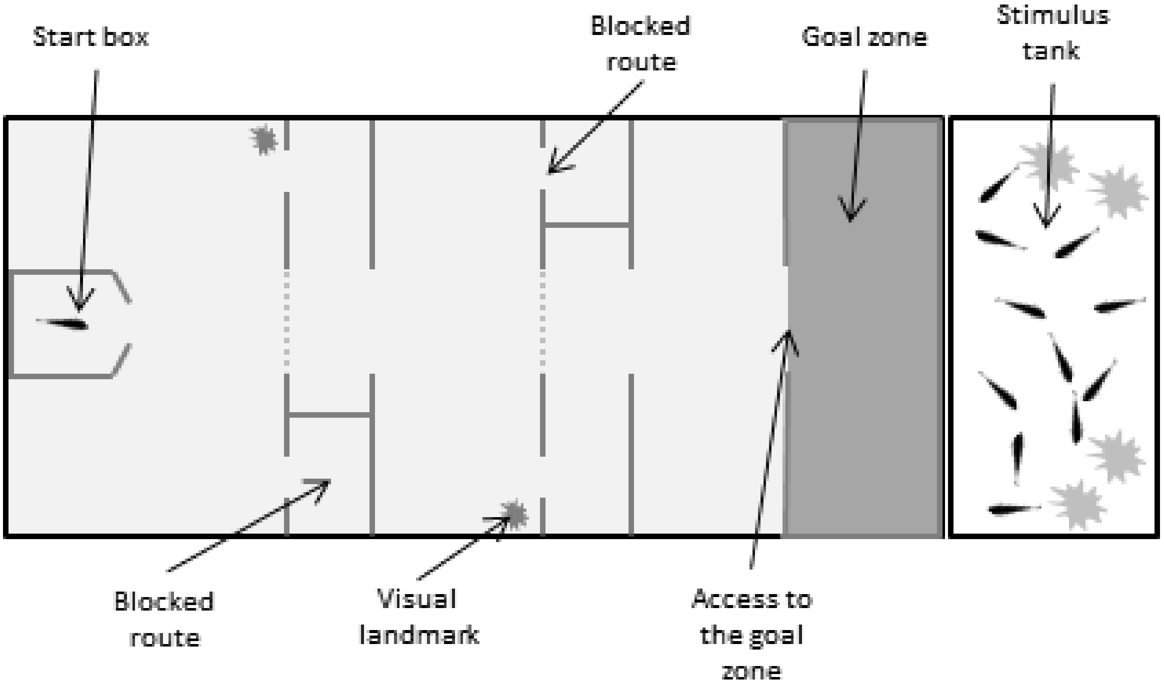

Figure 18: Aerial view of the apparatus adopted for experiment 4 (complex maze). From the start box the subject could see the stimulus shoal. To reach the goal zone, the subject had to choose the correct route.

Procedures: The procedure is the one adopted in previous experiments of this chapter (described in chapters 2.5.2 and 2.5.3). I performed five trials for each subject. I discarded and substituted two males and three females that did not complete the trial within the cut off time (20 min). As in the previous experiment, I measured decision speed, time solve the task, and choice accuracy in the choice from the recordings.

Statistical analysis: I conducted statistical analysis as in experiment 2 (described in chapter 2.5.3).

Results

In trials 2 to 5, subjects accuracy in route choice was 61.71 ± 20.21 %. This accuracy was significantly greater than chance ($t(47) = 3.862$, $p < .001$); thus, guppies proved able to learn the solution of the maze. The analysis on choice accuracy with GLMM revealed no effect of trial or reward side ($\chi(4) = 6.339$, $p = .175$ and $\chi(1) = 0.365$, $p = .546$, respectively). Sex had a significant effect in the model instead ($\chi(1) = 4.225$, $p = .040$), as males were more accurate than females (figure 19). Indeed, in trials 2 to 5, a sex-separated analysis revealed male accuracy was significantly greater than chance (68.75 ± 16.48 %; $t(23) = 4.754$, $p < .001$), whereas female accuracy did not differ from chance (54.69 ± 21.43 %; $t(23) = 1,047$, $p = .306$). However, I found also a significant sex by trial interaction ($\chi(4) = 11.706$, $p = .020$). None of the other interactions was significant. To explore the significant sex by trial interaction, I built two sex-separated GLMMs fitted with trial and reward side as fixed effects. I found that female accuracy in route choice did not change across the trials ($\chi(4) = 3.676$, $p = .451$; no other significant effects in the model). Conversely, male accuracy significantly change across trials ($\chi(4) = 13.811$, $p = .008$; no other significant effects in the model).

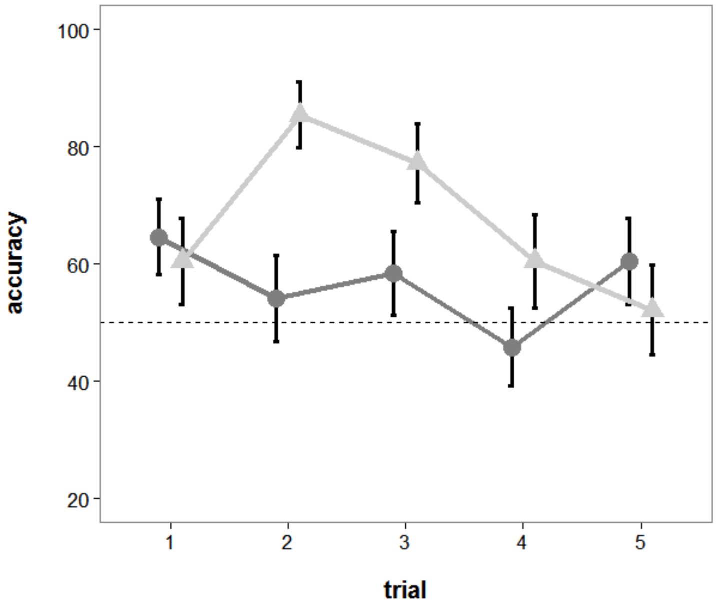

Figure 19: Accuracy of males (grey) and females (dark) in experiment 3 (complex maze). Data points represent *M* ± *SE* percentage of correct choices. Dashed line is chance performance.

Graphical inspection of figure 19 suggested a rather unexpected patter of change in male accuracy. Males apparently increased in accuracy from trial 1 to trial 2, and subsequently they decreased in accuracy from trial 2 to 5. Analysis on males' data supported this hypothesis. In a GLMM fitted on data of trial 1 and trial 2 only, there was a significant effect of trial ($\chi(1) = 6.840$, $p = .010$), indicating male accuracy increased. In a model fitted on data of trial 2 to trial 5 there was a significant effect of trial as well ($\chi(3) = 14.265$, $p = .003$), but here trend analysis indicated a significant linear decreasing of the accuracy ($p < .001$).

The LMM model to study time to solve the task found a significant effect of trial ($F(4,176) = 6.813$, $p < .001$; figure 20a). However, trend analysis revealed there was not clear linear decreasing across trials for this variable ($p = .492$), but rather a quadratic trend ($p = .008$; figure 20a). This pattern of response possibly suggests habitation to the apparatus or increase of exploratory behaviour. Sex had not significant effect on the time to solve the task ($F(1,44) = 2.016$, $p = .163$). There was no other significant effect or significant interaction. This suggested the sex differences in spatial cognition I found was related to the accuracy males and females expressed to solving the task, but not to the average time.

The model on decision speed revealed a significant effect of trial ($F(4,176) = 6.349$, $p < .001$). Subjects' decision speed decreased in a linear fashion ($p = .004$). Sex had no significant effect in the model ($F(1,44) = .106$, $p = .747$). Yet, I found a significant sex by trial interaction ($F(4,176) = 3.745$, $p = .009$; ; figure 20b). By splitting data of the two sex and building two separated models, I tried to understand the meaning of this interaction. Female decision speed significantly decreased across trials ($F(4,88) = 5.971$, $p < .001$) following a linear trend ($p = .007$), although inspection of figure 20b suggest that possibly female decision speed tended to increase in the last trial. Males changed their decision speed across trials as well ($F(4,88) = 3.666$, $p = .008$), but their trend was cubic ($p = .010$; figure 20b) rather than linear ($p = .716$). No other significant terms were found in these two models. Other terms in the initial model on decision time were not significant.

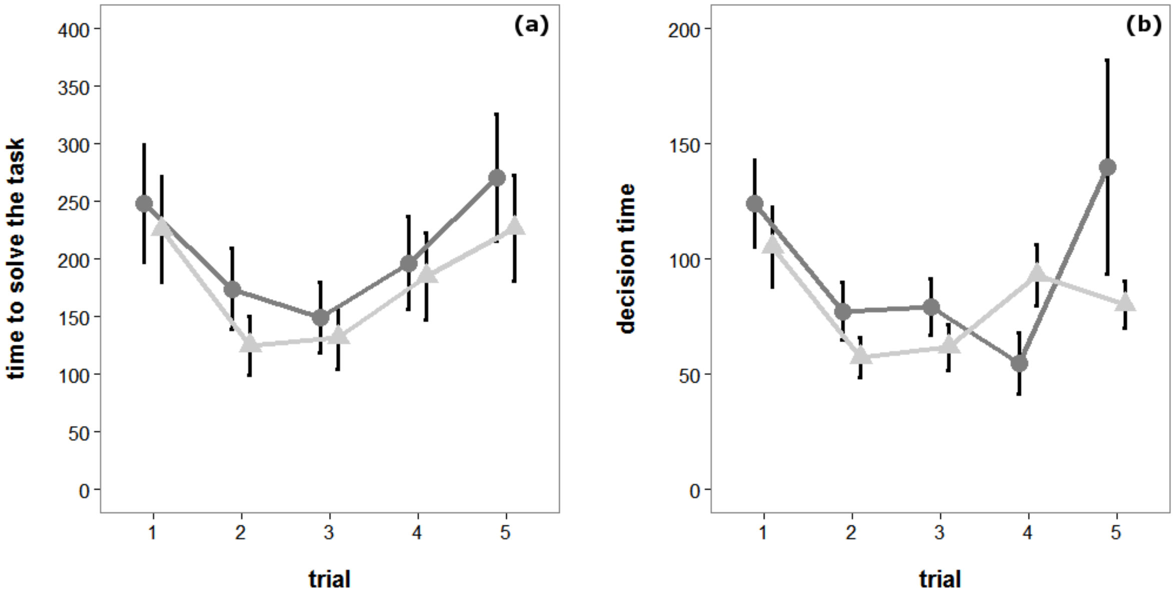

Figure 20: **(a)** Average time to solve the task (*M* ± *SE* seconds) of males (grey) and females (dark) in experiment 3 (complex maze). **(b)** Average decision speed (*M* ± *SE* seconds) of males (grey) and females (dark) guppies in experiment 3.

2.5.5 Discussion

In experiment 1a and 1b, guppies proved able to rapidly learn to navigate around the obstacle. This is revealed by the marked decrease observed for the time to reach the target shoal. In experiment 1a, I found a striking sex difference: male time to reach the reward was, on average, three times longer than female one (males: 177.75 ± 102.72 s; females: 46.57 ± 32.17 s; *M* ± *SD*). This result points to a better female ability to detour the obstacles, but it was rather unexpected. Indeed, female guppies inhabit mainly open water with no obstacles (Darden & Croft, 2008) and they are expected to be less used to navigate in complex habitats. Males instead tend to live in complex habitats close to the shoreline (Darden & Croft, 2008). I was therefore expecting selection to favour the evolution of enhanced detour abilities in males rather than in females. Interestingly, in experiment 1b, in which the barrier was not transparent, there was no evidence of sex differences. This result was confirmed also by Bayesian analysis which revealed my data are almost 20 times more likely to have occurred in the absence of sex difference. A possible reason for this difference between experiment 1a and 1b, comes from my reversal learning experiment, in which males proved to be more persistent than females in choosing the previously reward stimulus (chapter 2.4.3). To detour around the barrier, guppies had to move far from the shoal. It is possible that in experiment 1a males were more persistent that females in trying to pass through the transparent barrier. A posterior analysis of the video recordings provided support for this possibility, Indeed, I found males guppies apparently spent much more time than females in persistently trying to pass through the transparent barrier rather than in searching to detour it (*M* ± *SD*; females: 46.57 ± 81.79 s; males: 177.75 ± 251.77 s). Therefore, the result of experiment 1a is more parsimoniously explained by assuming a sex differences in persistence rather than a sex differences in detour abilities. In this species, males endlessly purchase females to obtain mating. Since more persistent males achieve greater reproductive success, male guppies might have been selected for a general cognitive function that increases behavioural persistence and affect therefore their behaviour in a large number of contexts (Rowe et al., 2005; see chapter 2.4.4 for a more detailed discussion).

In experiment 2, guppies showed no evidence of learning. Part of the problem could de due to the fact that some subjects preferentially chose the right arm of the Y maze. Similar left-right biases are often ascribed to behavioural lateralization, that causes animals to develop motorial asymmetries (Facchin et al., 1999). Very often these asymmetries are more marked in threating situation (Bisazza et al., 1997b) or in unfamiliar environments (Bisazza et al., 1997a). Since the experimental apparatus was unfamiliar, and potentially dangerous for the subjects, I could have cause guppies to show a strong lateralization bias in the task. In experiment 2, I did not detect sex differences in choice accuracy or in time to solve the task. However, it is clear that the poor performance of subjects, perhaps because of the spontaneous turning biases, made the Y maze inappropriate to study spatial learning in guppies. Moreover, since similar maze paradigms are often adopted in many other fish (Aoki et al., 2015; Takahashi et al., 2010), my result suggests that a careful validation for each species is required to avoid erroneous inferences.

In experiment 2, I found a sex difference in decision speed: females took longer than males to choose with arm of the maze to enter. Interestingly, I also found no evidence of a trade-off between decision speed and accuracy, suggesting that rapid decisions are not costly in male guppies, almost in terms of accuracy. This sex difference in decision speed is similar the one observed in visual discrimination learning with training procedures (chapter 2.3) and in the oddity concept learning task (chapter 2.4.2). It is interesting that the same effect has occurred in tasks that are very different between each other. Faster decision time appears therefore a general characteristic of males in this species rather than a specifics property of a single cognitive process. Further investigations are need to fully understand mechanisms and evolutionary explanations of this phenomenon (see chapter 2.3.5 for detailed discussion).

In experiment 3, guppies learned to find the correct route to reach the reward shoal, although this effect was apparently due to male performance only. Almost two methodological differences can explain why guppies were better in this task compared to the Y maze. Although apparently more complex than the Y maze, the maze I used in experiment 3 is possibly more similar to spatial problems guppies face in their natural environment, such as moving between rock and plants. In sharp contrast, other animals, for example rodents, perform very well in Y maze tasks. These animals dig large burrow systems and they might be therefore adapted to move in narrow corridors with left-right turns. Moreover, in my experiment 3 guppies were not forced to a distinct right-left turning decision, and therefore their choice was possibly less affected by behavioural lateralization compared to experiment 2.

From my analysis, it seems clear that only males solved successfully the task of experiment 3, whereas females chose randomly between the two available routes. The superior spatial ability of males emerged early in this task, after only a single exposure to the maze. One-trial learning is a common phenomenon in animals, including fish (Lucon-Xiccato & Dadda, 2014; Chivers & Smith, 1994). This learning process is thought to be very important in life-death situations, as when dealing with predators. Indeed, fish can show hard to believe learning and memory abilities for an object or an odour if it is associated with predation threat (Brown & Smith, 1998; Chivers & Smith, 1994). I intentionally tested guppies without precedent familiarization with apparatus to induce strong motivation to reach the social group. With this setting, males might have express such rapid one-trial learning because of the treating situation. How can we explain the absence of learning in females? Females inhabit deep open water while males prefer habitats close to shore (Darren & Croft, 2008). Therefore males, but not females, can use shelters to cope with dangerous situations. Since shelters have stable position over time, males might have been selected for rapid acquisition of spatial information to reach them. By contrasts, females might have been selected to perform better in different antipredator tasks more suitable to their habitat, such as in the discrimination of shoal size, an important antipredator defence of guppies. This interpretation is supported by results of my shoal size discrimination task in which females somewhat outperformed males (chapter 2.6.2). The clear decrease in accuracy of males can be due to motivation decrease, as they habituate to the environment faster than females do (Irving & Brown, 2013). This explanation seems supported by the trend observed in the decision time which tend to increase for males during the last trials.

As in previous experiments, it seems that sex differences in decision speed is quite common in this species, and possibly affects lots, if not all, cognitive processes. In experiment 3 of this section, the differences was not absolute, but showed a very complex pattern across the different trials that clearly needs further investigation to be understand (figure 20b).

In my three spatial learning experiments, I found support to my initial hypothesis of sex difference favouring males, but with very limited extent (experiment 3). A second important point of my research on spatial abilities is that I found several limits in the use of these tasks often adopted in fish, such as the problem of lateralization or of rapid habituation to the procedure in males. My results claimed therefore for accurate validation and improvement of spatial tasks for fish, and, eventually, the development of new paradigms.

2.6 Quantitative abilities

2.6.1 Introduction

The capacity for processing quantities is not a human prerogative. Many species of mammals, birds, fish, and even invertebrates, show quantitative abilities (Agrillo & Bisazza, 2014; Rodríguez et al., 2015). Animals can discriminate between continuous quantities, such as objects with different length (Basolo, 1990) or with different area (Bisazza et al., 1989). Animals can also discriminate numerical quantity. For example, they can estimate which set is larger between two sets made of a different number of objects

(Agrillo & Bisazza, 2014). The widespread presence of quantity estimation abilities in animal species is not surprising given the benefits an animal could gain, for example, in selecting the largest food source for feeding or the largest social group for reducing predation risk (Agrillo & Bisazza, 2014). Most of the research in animal quantitative abilities has focused on numerical abilities so far. There are many similarity between numerical abilities of nonhuman animals and those observed in infants before the acquisition of language (Starkey et al., 1990). Accordingly, some authors have suggested that all extant vertebrates share a basic system for numerical quantity processing which evolved early in this clade (Beran, 2008; Feigenson et al., 2004). The outstanding numerical performances expressed by humans in adulthood are thought to arise from the acquisition of a more refined numerical system related to language (Feigenson et al., 2004). In studies on numerical abilities of nonhuman animals researchers usually test individuals of only one sex or too few individuals to allow the investigation of sex differences (but see Ferkin et al., 2005; Uller et al., 2003).

Guppies and other fish species can perform very accurate numerical discriminations (Bisazza et al., 2014; Miletto Petrazzini et al., 2015). These abilities have usually been studied with the shoal choice task that exploits a spontaneous behaviour of social fish. Under perceived threat, such as in presence of a predator or in a novel environment without refuges, individual fish tends to select and join the larger available shoal of conspecifics (Agrillo et al., 2008; Agrillo et al., 2012b; Hager & Helfman, 1991). Shoaling with the larger group is thought to improve predator detection and to dilute the individual risk of being caught (Krause & Ruxton, 2002). In shoal choice task, the subject is moved in a novel aquarium and presented with two shoals, made of a different number of conspecifics, in two adjacent aquaria. If the subject is able to discriminate the two quantities, it is expected to preferentially shoal near the larger group (Agrillo et al., 2012b). In experiment

1 of this chapter, I aimed to investigate sex differences in guppy accuracy to discriminate shoal size. I firstly compared male and female guppies in the discrimination between shoals of 4 and 6 conspecifics (experiment 1a). This ratio between shoals was found to be the upper limit that guppies can discriminate (Agrillo et al., 2012b). If sex difference in this task exists, I would expect to find it with this ratio that is difficult to discriminate for guppies. For this experiment literature provides indications for the possible outcome. In the guppy, females suffer higher susceptibility to predation. Compared to males, female guppies usually live in deeper and more open water which are located far from shorelines and shelters (Croft et al., 2006; Darden & Croft, 2008). In these environments, there is a great predation risk (Magurran, 2005). Furthermore, predators actively prefer to attack females because females have a larger, more profitable, size (Pocklington & Dill, 1995; Magurran, 2005). Thus, I expected natural selection to favour a greater accuracy in shoal size discrimination among females to deal with their higher predation risk. In contexts other than predator avoidance, the choice of social group is affected by many factors outside size (Hoare et al., 2004) and these factors could have different relevance for male and female guppies. I therefore performed two additional experiments to control for possible confounds of this task. In experiment 1b, I looked for sex differences in the willingness to join the larger shoal by observing males and females in a very easy shoal size discrimination (4 and 10 conspecifics; Agrillo et al., 2012b). Finally, in experiment 1c I controlled whether males were attracted by social group for sexual rather than antipredator purposes.

Shoal choice tasks as experiment 1 of this chapter are very effective to study quantitative abilities of fishes. However, most of the research in animal quantitative abilities is performed with a different spontaneous choice task. The animal is presented with two sets of food items that differ for item number or item size. In this situation, most of the species tend to choose the larger food quantity. To date, there are no food choice procedure available for study quantitative abilities in fish. I therefore developed a new paradigm for studying sex differences in guppy abilities to discriminate between food quantities. In each trial of the experiment, I exposed the subject to two cards with pieces of food pasted onto them. The quantity of food was different between the two cards to observe the preference for the larger quantity. In experiment 2, I tested male and female guppies for their ability of discriminating between set of food items of different numerosity. In experiment 3, I studied sex differences in the ability to discriminate between two food items with a different size.

2.6.2 Experiment 1: Discrimination of shoal size

In this experiment, I compared males and females guppies in the discrimination between two shoals with a different number of conspecifics (4 *versus* 6 fishes, experiment 1a). Two further experiments served as controls for two possible confounds of this procedure: sex differences in the willingness to join the larger shoal (experiment 1b) and male sexual motivation (experiment 1c).

Materials and methods

Subjects: I observed 60 males and 60 females in experiment 1a; 36 males and 36 females in experiment 1b; and 8 males in experiment 1c. I used elevated sample sizes because with this procedure variance in performance is always high (e.g., Agrillo et al., 2012b). All these subjects were wild guppies that expressed greater antipredator response in novel environment compared to domestic guppies.

Experimental apparatus: The apparatus adopted in this experiment was a modification of one previously used in guppies (Agrillo et al., 2012b). It consisted of one

60 x 40 x 35 cm glass aquarium for the subject (“subject tank”) and two identical 60 x 40 x 35 cm glass aquaria for the stimuli (“stimulus tanks”; figure 21). These aquaria were filled with 18 cm of water. The bottom and the long walls of the subject tank were covered with green plastic material. The short walls of the subject tank were adjacent to the two stimulus tanks to allow the subject to see the two stimulus shoals. Two black lines delimited two choice areas (15 x 40 cm) in front of each stimulus tank. A digital video camera was placed 75 cm above the subject tank to record the experiments. Each stimulus stank, provided of gravel bottom, was divided by a green opaque barrier into two compartments (figure 21).

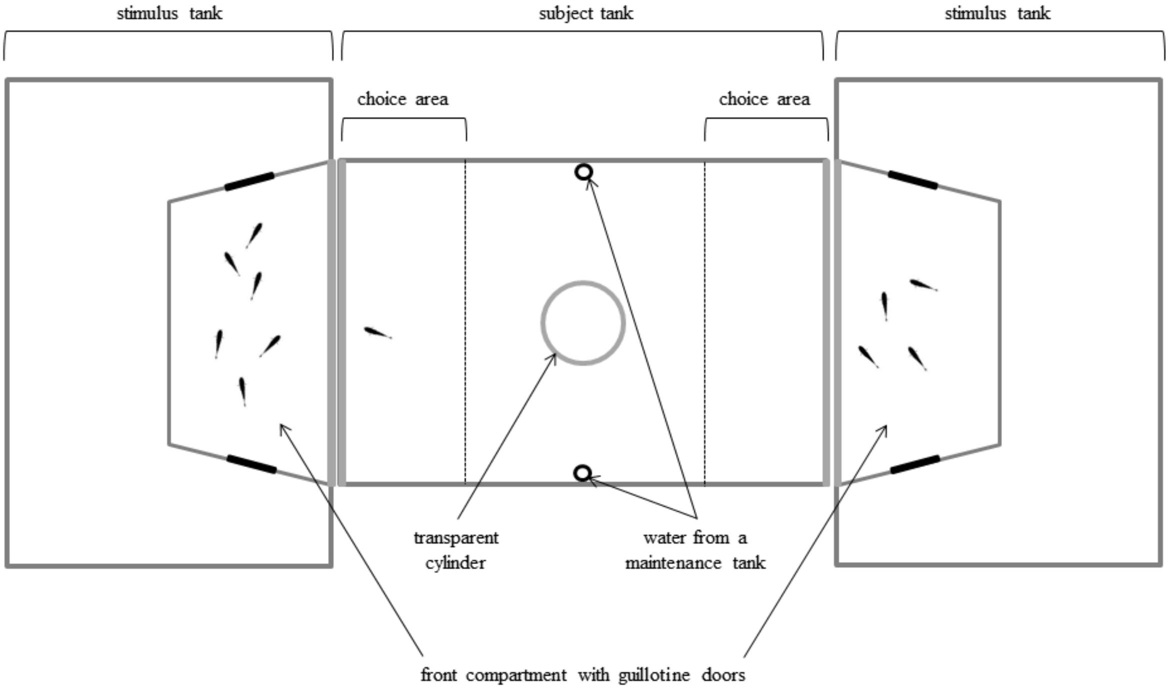

Figure 21: Aerial view of the apparatus used in experiment 1 (shoal size discrimination). After two minutes of habituation in the cylinder, the subject could freely move within the subject tank.

The back compartment contained a filter and an heater. The front compartment was empty and was the only part that could be seen by the experimental subject. The two compartments were connected by two guillotine doors. In this study, I introduced two important innovations to the experimental apparatus compared to previous works. Instead of using fresh aged water, the subject tank received a constant flow (1.5 L/min) from a 400

L tank containing a stable population of approximately 50 guppies (both sexes, all ages), as well as gravel and aquatic plants. This guarantee a more realistic situation as the subject could perceive fish odour in the subject tank. Preliminary experiments revealed with this procedure guppies behaved more naturally, reduced freezing behaviour, and achieved better discrimination performances. The second innovation concerned stimulus fish. In previous experiment stimulus fish were introduced in the stimulus tanks a few minutes before the test and therefore they were probably poorly accustomed to the new environment. Here, a group of 24 adult guppies (sex ratio 1:1) permanently inhabited each stimulus tank. These guppies could move freely between the two compartments, except during the experiments, when a predetermined number of individuals were blocked in the front compartment by mean of the guillotine doors. Females and males of the two stimulus tank were matched for body size; female stimuli were not virgin but were not used in the experiment if showing abdominal distension due to pregnancy.

Procedures: Thirty minutes before the beginning of the experiments, the guillotine doors of the stimulus tanks were closed, and the stimuli were inserted in the front compartment according each experiment’s schedule. The subject was netted from the maintenance tank and inserted in a small plastic jar filled with water. The jar was then gently emptied into a transparent plastic cylinder (diameter 8 cm, height 20 cm) that was placed in the middle of the subject tank. After 2 min of acclimatization, the transparent cylinder was lifted by mean of a nylon line, allowing the subjects to freely swim in the experimental tank for 28 min. Males and females were tested in alternation. Position of the larger shoal was balanced between the two stimulus tanks. In experiment 1a, I studied sex differences in the ability of discriminating between shoal of different numerosity. The stimuli were two shoals of 4 and 6 guppies, a numerical discrimination roughly corresponding to the threshold in fish with only some individuals being able to achieve it

(Agrillo et al., 2008; Agrillo et al., 2012b). In experiment 1b, I observed whether males and females showed equal tendency to join the larger shoal by observing guppies in a very easy shoal size discrimination, 4 and 10 conspecifics (Agrillo et al., 2008; Agrillo et al., 2012b). In the wild females form the core of guppies' shoals, but usually mixed-sex assemblages occur (Croft et al., 2003; Griffiths & Magurran, 1998). To close natural situation, half of the subjects of each sex were presented the choice between two mixed-sex shoals (2 males and 2 females *versus* 3 males and 3 females in experiment 1a and 2 males and 2 females *versus* 5 males and 5 females in experiment 1b). However, in dangerous situations females might avoid shoals with males as males' conspicuousness attracts predators which then attack females that are more profitable (Pocklington & Dill, 1995). For this reason, the other half of the subjects were required to discriminate among females-only shoals. In experiment 1c, I controlled whether males were sexual rather than social motivated when joying the shoals in our experiments. I observed 8 males using the same procedure as experiment 1a, but for a longer time (3 hrs). As stimuli, I used shoals of 4 and 6 females. To allow a lateral view of the fish, I substituted the plastic on the long walls of the subject tank with two 1 x 1 mm grids. Following a well-established procedure for behavioural observation in this species, I recorded male sexual behaviour toward the shoals by counting the number of sigmoid courtship displays and the number gonopodial swings (Houde, 1997). Males were observed uninterruptedly for 60 min from the beginning of the experiment. Thereafter, I conducted two separated 15-minute observations from minute 105 to minute 120 and from minute 165 to minute 180, respectively.

Analysis of video recordings: In experiment 1a and 1b, I collected the data from the video recordings. Time spent in the two choice areas during the 28 min of experiment was computed using a computer software (Ciclic Timer) that produces as output data divided in seven blocks of 4 min each. I then calculated for each block of minutes a preference index for each subject as time spent in the larger shoal choice area / overall time spent in the two choice areas. To evaluate the reliability of the method, a random subsample of recordings (N = 20) were double coded by a second naïve experimenter. I found clear evidence of high reliability: the bias between the two scorings (mean of the absolute difference between each pair of observations) was less than 2% of the average value (*M* ± *SD*: 1.79 ± 2.14%); there was a strong correlation between the two scorings (Spearman rank correlation: *rho* = .991, $p < .001$). I also recorded time spent before the first switch between choice areas occurred and the number of switches between the two choice areas in the first 8 min of the experiment. As a measure of coping with the stress of the experimental procedure I also recorded whether the subjects performed freezing behaviour (more than 5 seconds motionless) in the first 8 min of experiment 1a. One possible confounds in my experiments is that the subject may freeze or notice only one stimulus; to exclude this possibility, I included only subjects that moved at least twice between the two choice areas during the trial (Miletto Petrazzini & Agrillo, 2015; Dadda et al., 2015). This restricted the sample size to 53 males and 49 females in experiment 1a and 34 males and 34 females in experiment 1b. In experiment 1c, none of the subjects was discarded.

Statistical analysis: Analysis was performed using R statistical software (R Development Core Team, version 3.0.2). I performed an angular transformation (arcsine square root) on proportion data to meet normality assumptions (Sokal & Rohlf, 1995). I compared the shoaling tendency of males and females using an independent-sample *t* test on the proportion of time spent in the choice areas. To test the presence of a preference for one of the two stimuli, I compared the preference index against the mean of a random choice (50% of time spent in the larger shoal choice area) using a one-sample *t* test (Agrillo et al., 2008). Preference index was further analysed with linear mixed model (LMM) fitted with the seven blocks of four minutes each, sex (male or female) and type of stimulus (only females or mixed sex) as fixed effects, and subject ID as random effect for account the repeated measures. The ANOVAs on the preference index in the first 8 min of test was fitted with the sex and type of stimulus as factors. The number of switches between the two choice areas was modelled using quasi-GLM corrected for overdispersion (Zuur et al., 2009). GLM model was fitted with sex (male or female) and type of stimulus (only females or mixed-sex) as factors, and statistical significance was assessed using a *F*-test. The time taken to visit the second chosen shoal was analysed using Wilcoxon Rank Sum Test, assigning the highest possible value (480 s) to subjects that did not visit the second shoal within 8 min of test.

Results

Experiment 1a: Subjects spent 85.37 ± 10.45 % time in the two choice areas, with no sex difference (males: 86.76 ± 9.24 %; females: 83.87 ± 11.52 %; *t*(100) = 1.209, *p* = .229), suggesting an equal shoaling tendency in males and females. Subjects achieved the discrimination and expressed a significant preference for the larger shoal (preference index: 64.26 ± 23.6 3%; *t*(101) = 6.041, *p* < .001). This preference was significant for both males (61.99 ± 24.86 %; *t*(52) = 3.493, *p* = .001) and females (66.72 ± 22.22 % *t*(48) = 5.174, *p* < .001). LMM found no significant effect of sex (*F*(1,98) = 1.133, *p* = .290), type of the stimulus (*F*(1,98) = .560, *p* = .456) or block of time (*F*(6,588) = 0.434, *p* = .856). The only significant interaction was block of time x sex x type of stimulus interaction (*F*(6,588) = 2.893, *p* = .009); thus, I run two simpler LMMs by splitting the data on the basis of the type of stimulus. In these LMMs, there was not significant effects of sex (mixed-sex stimuli: *F*(1,50) = .164, *p* = .687; females-only stimuli: *F*(1,48) = 1.312, *p* = .258) or block of minutes (mixed-sex stimuli: *F*(6,300) = .455, *p* = .841; females only stimuli: *F*(6,288) = .915, *p* = .484). In the mixed-sex stimuli condition, a significant block of minutes x sex interaction (*F*(6,300) = 2.417, *p* = .027) revealed that at the beginning of the test females performed better than males. In the female-only stimuli condition, this effect was present but less markedly and only approach the statistically significance (*F*(6,288) = 1.853, *p* = .089).

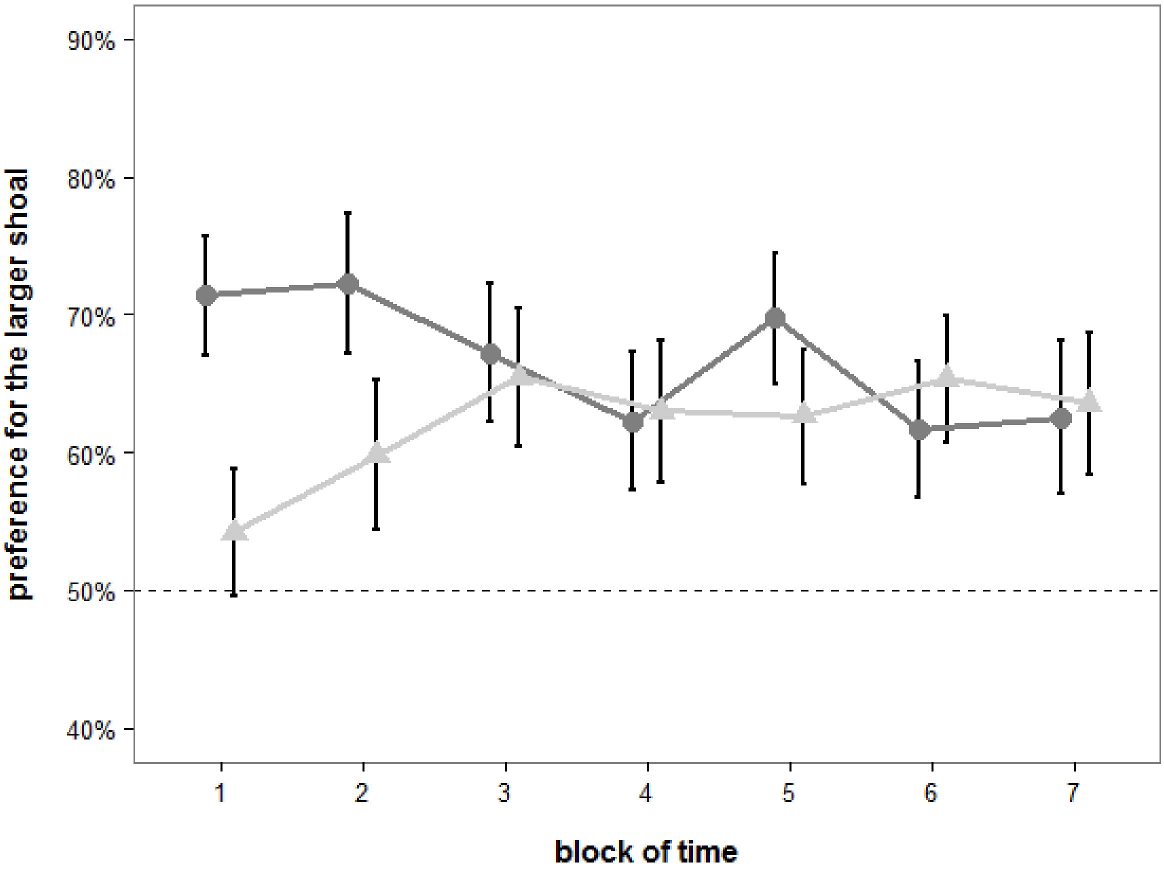

Figure 22: Preference for the larger shoal of male (grey line) and female (dark line) guppies in experiment 1 (shoal size discrimination). Data points represent *M* ± *SE* preference index in seven blocks of 4 min each.

Graphical inspection of the preference for the larger shoal across the seven blocks of time (figure 22) provided a more clear picture and suggested that the sex difference occurred in the first 8 min of the experiment (blocks 1 and 2). In this interval, females showed a significant preference for the larger shoal (71.81 ± 27.39 %; *t*(48) = 5.052, *p* < .001), whereas males did not (56.99 ± 32.70 %; *t*(52) = 1.684; *p* = .098; figure 23a). The ANOVA performed on the first 8 min of the experiment revealed a significant effect of sex

(*F*(1,98) = 4.545; *p* = .036), but no significant effect of type of stimulus (*F*(1,98) = 1.080; *p* = .301) or interaction (*F*(1,98) = .257, *p* = .613). In contrast, during the last 20 min of the experiment (blocks 3 to 7) both males and females showed a significant preference for the larger shoal (one-sample *t* test: *t*(52) = 3.813, *p* < .001 and *t*(48) = 3.775, *p* < .001, respectively). The ANOVA performed on the last 20 min of the test revealed no significant effect of sex (*F*(1,98) = .027, *p* = .869), type of stimulus (*F*(1,98) = .105, *p* = .746) or interaction (*F*(1,98) = .359, *p* = .550).

Possible explanations for the fact that males showed a performance poorer than females in the first minutes of the test are that males selected the first shoal they happened to notice or that males were more persistent with the first shoal selected, resulting in fewer chances to compare options. To test for these possibilities, I analysed the first 8 min of experiment 1a in more detail. Time taken to visit the second chosen shoal was not significantly different between males and females (mean rank: 46.65 and 56.74, respectively; *W* = 1555.5, *p* = .084), indicating no sex difference in the persistence of the first choice. The GLM revealed males and females switched between shoals at similar frequency (4.38 ± 4.66 and 4.06 ± 5.05, respectively; *F*(1,98) = .114, *p* = .737). There was no significant effect of type of stimulus (*F*(1,98) = .719, *p* = .398) or significant sex by type of stimulus interaction (*F*(1,98) = 2.614, *p* = .109). I observed freezing behaviour (indicating stress response) in equal proportion of males and females (33.96 % and 40.82 %, respectively; $\chi^2(1) = .570$, *p* = .323).

Experiment 1b: Subjects spent 84.42 ± 10.88 % time in the two choice areas with no sex difference (males: 86.05 ± 8.96 %; females: 82.79 ± 12.44 %; *t*(66) = .912, *p* = .365). Subjects expressed a significant preference for the larger shoal (preference index: 67.67 ± 27.66 %; *t*(67) = 5.395, *p* < .001). This preference was significant for both males (68.17 ± 27.20 %; *t*(33) = 3.952, *p* < .001) and females (67.18 ± 28.51 %, *t*(33) = 3.625, *p*

= .001; figure 23b). In this experiment, the preference for the larger shoal was not significantly different between males and females (ANOVA: $F(1,64) = .035$, $p = .852$; figure 23b). There was no significant effect of type of stimulus ($F(1,64) = .419$, $p = .519$) and no significant interaction ($F(1,64) = .100$, $p = .752$).

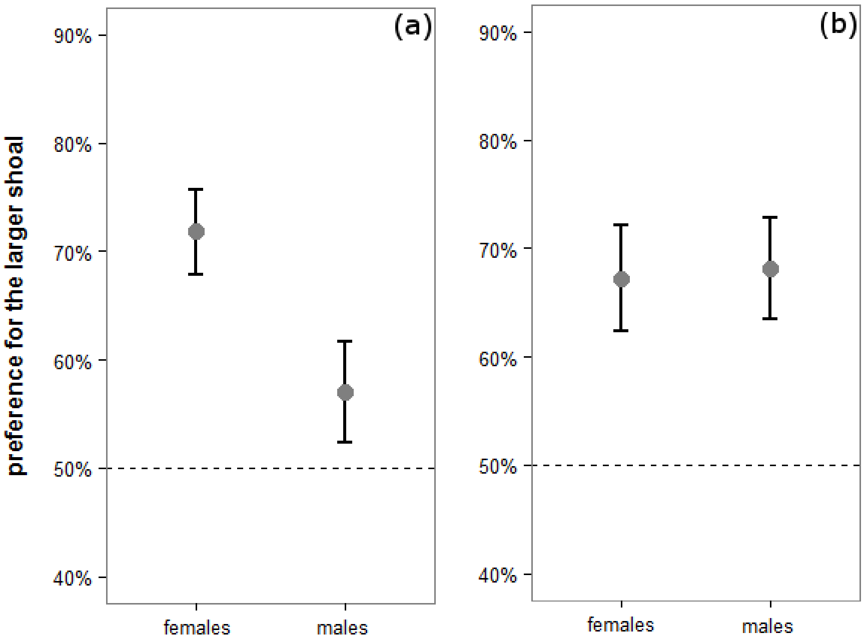

Figure 23: Preference for the larger shoal in the first 8 min of **(a)** experiment 1a and **(b)** experiment 1b. Data points represent $M \pm SE$ preference index for the larger shoal.

Experiment 1c: Two males exhibited no sexual behaviour for the whole period, five others started to exhibit sexual behaviour after the first 30 min of observation, and one male started to exhibit sexual behaviour immediately before the end of this period. The number of sexual behaviours was almost zero (0.38 ± 1.06, $M \pm SD$) for the first 30 min (the duration of the test in experiment 1) against an average of more than 20 acts per hour in the following 30 min periods of observation (min 31-60: 10.50 ± 16.80; min > 60: 13.25 ± 11.71).

2.6.3 Experiment 2: Discrimination of food item number

In this experiment, I presented male and female guppies with two sets of food that differ by item number (1 *versus* 4, 2 *versus* 4, 2 *versus* 3, and 3 *versus* 4). I aimed to study sex difference in the accuracy of discriminating food item number.

Materials and methods

Subjects: The experimental subjects were 10 male and 10 female guppies of the domestic strain.

Experimental apparatus: Each experimental apparatus was a 20 x 50 cm glass aquarium filled with gravel and 25 cm of water. The long walls of the tank were covered with green plastic. The apparatus was shaped like an hourglass (figure 24) by mean of two trapezoidal lateral compartments (10 x 5 x 25 cm) made of transparent plastic. In the lateral compartments, two aquatic plants each provided a natural and enriched environment for the subject. Two immature guppies were housed in each lateral compartment to avoid social isolation of the subject. Two 15-w fluorescent lamps were placed above the main compartments to illuminate the apparatus. Experiments were conducted in a dark room. I used six identical apparatuses at the same time.

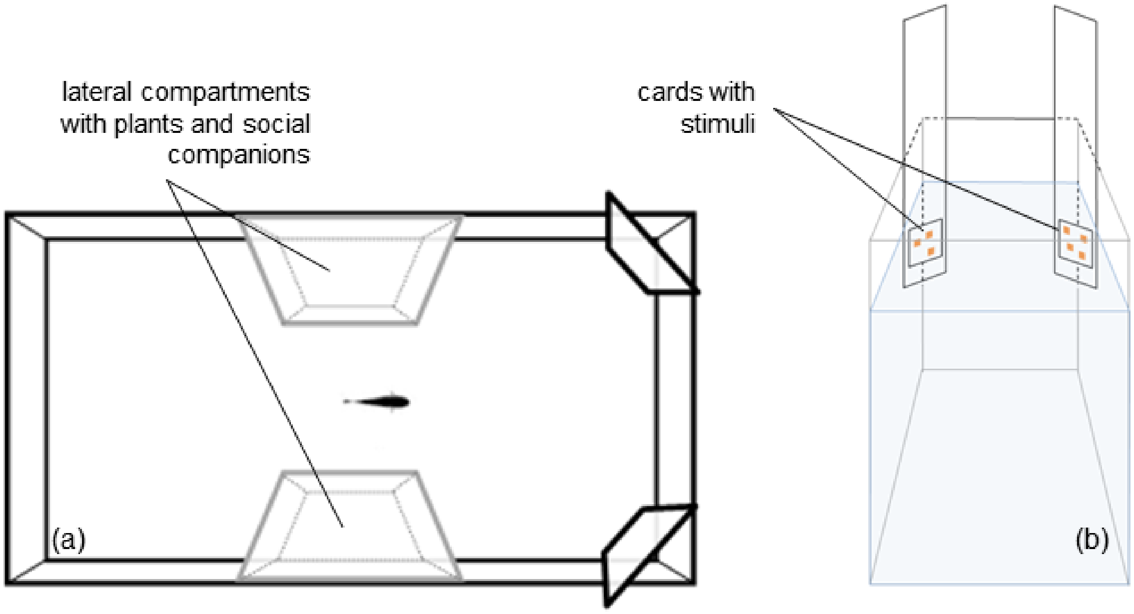

Figure 24: Aerial **(a)** and frontal **(b)** view of the apparatus of experiment 2 and 3 (food quantity discrimination). The subject was housed in the apparatus for the entire experiment. In each trial, **(b)** two cards with food items were simultaneously inserted at the corners of one of the short walls. The subject was allowed to select only one card.

Stimuli: The stimuli were small pieces of commercial food flakes with a homogeneous brown colour (GVG mix, Sera GmbH, Heinsberg, Germany) cut by using a chirurgical scalpel. The stimuli were then pasted on 3 x 3 cm white plastic cards by adding a drop of water.. This experiment investigated the ability of guppies to discriminate between discrete food quantities by presenting two sets with different numbers of food items. Therefore, the size of the stimuli was constant (1.5 x 1.5 mm), whereas the number changed according to the four discriminations administered. I administered four discriminations: 1 *versus* 4, 2 *versus* 4, 2 *versus* 3, and 3 *versus* 4 food items (numerical ratios: 0.25; 0.50; 0.67, and 0.75, respectively; figure 25). All food items were the same size (1.5 x 1.5 mm). To present the stimuli to the subjects, each card was fixed to the terminal part of a transparent panel (3.5 x 15 cm). During the experiments, I inserted the panels into the tank. Each panel was provided with a support that blocked it on the tank wall so that it could be rapidly placed in the correct position. The top end of the cards was 3 cm under water surface.

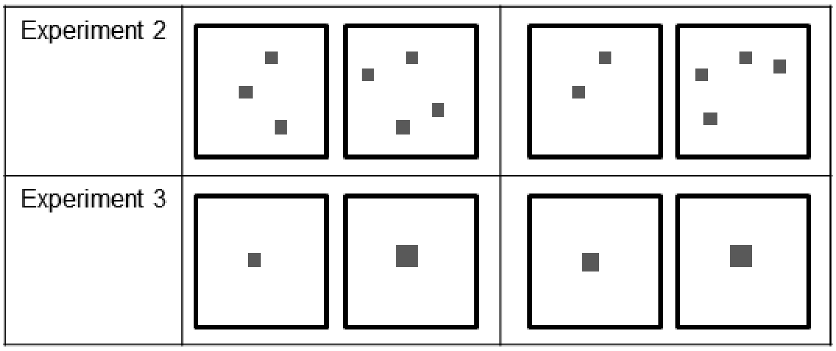

Figure 25: Examples of cards with stimuli used in the experiments 2 and 3four experiments.

Acclimation phase: Before the experiment, the subjects underwent an acclimation phase. Each subject was moved into the apparatus seven days before the start of the experiment, with the four immature companions that could freely swim in the apparatus. A preliminary experiment showed that subjects apparently familiarise faster to the apparatus when they could interact with other fish. For three days, the subject was fed three times a day by delivering food from alternate short walls of the tank. On the fourth day, the subject was fed according to the same schedule, but, a few seconds before food delivering, a single withe card without any stimulus was inserted into the tank near one of the short wall. A Pasteur pipette was used to deliver food close to the card to allow the subject to learn the association between the card and the food. To further habituate subject to the experiment, in the following two days it was fed six times per day (three in morning and three in the afternoon) by inserting in the tank a single card with some pieces of food pasted onto it. Therefore, the subject had to feed on the food pasted onto the card. Most of the subjects learned this feeding routine very fast and rapidly reached the card once inserted into the water. Other subjects did not learn, and they did not approach the card, or they approached it occasionally after longer delay. These latter subjects were not admitted to the experimental phase and were replaced with new subjects of the same sex. I discarded three males and two females. To avoid that companions might influence the choice of the subject, on the sixth day of the acclimation phase, they were removed from the main compartment of the tank and inserted into the two lateral compartments. The subject could still see them when in middle portion of the apparatus, but not while approaching the stimuli. The last day of the acclimation phase, the subject was not fed.

Experimental phase: The experiment lasted 10 days. Each day I administered eight trials, four in the morning (9:00 – 10:00) and four in the afternoon (15:00 – 16:00). The subject was therefore observed in a total of 80 trials (20 for each discrimination). In each trial, two cards that differed in number of food items were inserted simultaneously into the corners of one of the short walls of the tank (figure 24b). Before inserting the cards, I waited until the subject was in the opposite half of the tank. This way, fish could see both stimuli before choosing. After the subject chose one of the cards, the other card was gently removed from the water. The chosen card was left into the water until the subject consumed all of the food on it, which normally took around 20-30 s. The following trial began after 5 min. A preliminary experiment with this schedule showed guppies' performance was not affected by the order of the trial within each day. The side of the tank in which I inserted the cards and the relative position (right or left) of the card with the larger amount of food alternated according to a pseudo-random pre-set scheme, as did the presentation order of the different types of discriminations. The spatial configuration of the food items was varied according to a fixed sequence to prevent fish from using pattern recognition across trials. Experiments were performed 5 days per week, from Monday to Friday.

Statistical analysis: To evaluate the accuracy of the subjects, I computed the proportion of choice of the card with the larger food quantity. This variable was reported in text as percentages (*M* ± *SD*), and was always arcsine square root transformed before analysis (Sokal & Rohlf, 1995). I initially tested for a sex difference in the standard length of the subjects using independent-sample *t* test. As females were generally larger than males, I tested for sex and standard length effect on the food choice performance independently: the effect of the standard length was assessed with Pearson correlation test, whereas the effect of sex was examined with the linear mixed model (LMM). The standard length was log-transformed to achieve normality assumptions. To assess whether the proportion of choice of the larger food quantity was different from the one expected by chance (50%), I used one-sample *t* test. Pearson correlation test was used to assess the correlation between the performances of the subjects in the discriminations. A LMM fitted with the identifier name of the subject (subject ID) as random factor was used to study the possible effect of sex and discrimination in each experiment. Trend analysis was performed according to Logan (2011). Finally, I used the Bayesian information criteria of the LMM models with and without the effect of sex to approximate a Bayes factor that expressed the amount of evidence in favour of the absence of sex differences (Schwarz, 1978).

Results

Subjects measured 24 ± 3 mm. Female subjects were significantly larger than male (females: 26 ± 3 mm; males: 22 ± 1 mm; independent-sample t test: $t(18) = 3.879$, $p = .001$). There was no significant correlation between standard length of subjects and proportion of choice of the card with more food items (Pearson correlation: $r(18) = -.021$, $p = .983$), suggesting that the body size did not affect the results of the experiment.

Subjects chose the card with more food items in 55.25 ± 7.56 % of the trials, a preference significantly greater than chance (one-sample t test: $t(19) = 3.109$, $p = .006$). However, separate analysis for each discrimination found the ratio between quantities important. Subjects significantly discriminated 1 *versus* 4 (63.25 ± 14.89 %, $t(19) = 3.907$, $p < .001$) and 2 *versus* 4 food items (57.5 ± 8.51 %, $t(19) = 3.916$, $p < .001$), but not 2 *versus* 3 (53.00 ± 11.52 %, $t(19) = 1.174$, $p = .255$) or 3 *versus* 4 food items (47.25 ± 11.18 %, $t(19) = 1.104$, $p = .283$; figure 26). The proportion of choice of the card with more food items significantly correlated between the 1 *versus* 4 and 2 *versus* 3 discrimination (Pearson correlation: $r(18) = .526$, $p = .017$) and between the 2 *versus* 4 and 3 *versus* 4 discrimination ($r(18) = .472$, $p = .036$). There was no significant correlation between the remaining discriminations (all p values > .300).

The LMM on the proportion of choice of the card with more food items revealed a significant effect of discrimination ($F(3,54) = 8.721$, $p < .001$). A polynomial trend analysis suggested that the proportion of choice of more food items decreased linearly with the increase of the ratio of the discrimination ($p < .001$; figure 26). No significant effects of sex ($F(1,18) = 1.613$, $p = .220$) nor significant sex by discrimination interaction ($F(3,54) =$

1.021, *p* = .391) were found in the model. The approximate Bayes factor indicated that the LMM model without the effect of sex was 42.612 times more likely to explain the performance of the subjects than the model with the effect of sex.

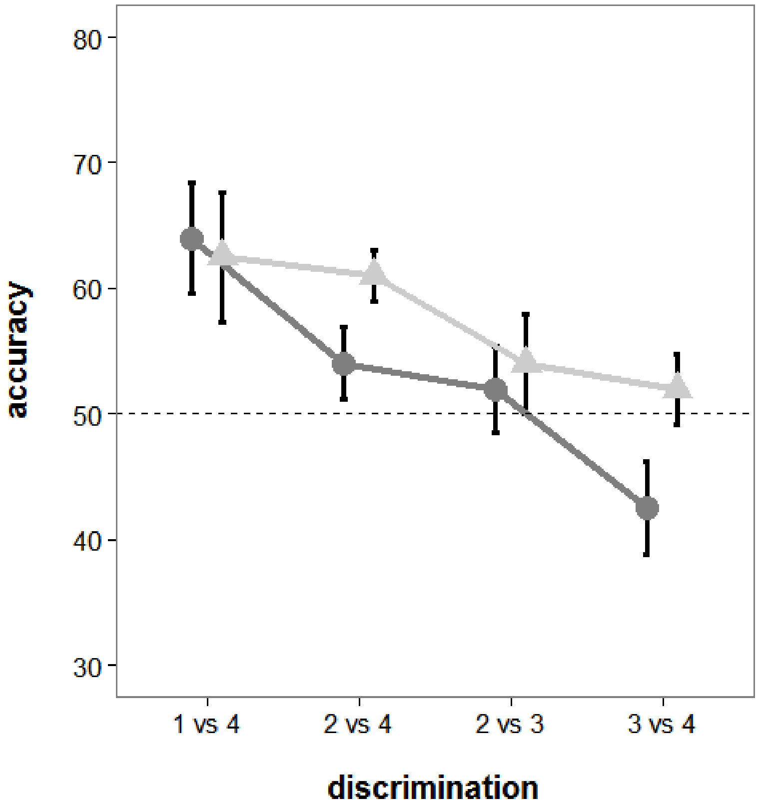

Figure 26: Accuracy of males (grey) and females (dark) in experiment 2 (discrimination of food item number). Data points represent *M* ± *SE* percentage of choice for the larger food quantity.

2.6.4 Experiment 3: Discrimination of food item size

In this experiment, I presented male and female guppies with two food items of different size (ratio between the area: 0.25, 0.50, 0.67, and 0.75, the same ratios as in experiment 2 of this section). I aimed to study sex difference in the discrimination of the larger food item.

Materials and methods

In this experiment, I used five male and five female domestic guppies. The apparatus, the procedures and the statistical analysis are the ones adopted for experiment 2 (described in details in chapter 2.6.3). The only differences compared to experiment 2 is that the stimuli were cards with a single piece of food each. The two pieces of food differed in size following the ratios of experiment 2 (ratio between the area: 0.25, 0.50, 0.67, and 0.75; figure 25). The item sizes adopted in this experiment were 1.5 x 1.5 mm, 2.1 x 2.1 mm, 2.6 x 2.6 mm, 3 x 3 mm. One male was discarded and replaced as it did not learn to feed from the cards in the acclimation phase.

Results

Subjects measured 23 ± 2 mm. Females were significantly larger than males (females: 25 ± 2 mm; males: 21 ± 1 mm; independent-sample *t* test: *t*(8) = 3.860, *p* = .005). There was no significant correlation between standard length and proportion of choice of the card with the larger food item (Pearson correlation: *r*(8) = -.107, *p* = .768), suggesting that body size did not affect the results of the experiment.

Subjects chose the card with the larger food item in 74.38 ± 5.72 % of the trials, a preference significantly greater than chance (one-sample *t* test: *t*(9) = 11.316, *p* < .001). A separate analysis for each discrimination revealed the proportion of choice of the larger food item was significant in all quantity ratios (0.25 ratio: 88.50 ± 5.80 %, *t*(9) = 15.306, *p* < .001; 0.50 ratio: 79.00 ± 4.59 %, *t*(9) = 16.157, *p* < .001; 0.67 ratio: 65.00 ± 11.3 %, *t*(9) = 3.851, *p* = .004; 0.75 ratio: 65.00 ± 12.69 %, *t*(9) = 3.660, *p* = .005; figure 27). The proportion of choice of the larger food item was significantly correlated between the 0.50 and 0.67 discrimination (Pearson correlation: *r*(8) = .705, *p* = .023). There was no significant correlation between all the remaining discriminations (all *p* values > .100).

The LMM on the proportion of choice of the larger food item revealed a significant effect of discrimination (*F*(3,24) = 18.911, *p* < .001). A polynomial trend analysis suggested that the proportion of choice of the larger food item decreased linearly with the increase of the ratio (*p* < .001; figure 27). No significant effect of sex (*F*(1,8) = .011, *p* =

.920) or significant sex by discrimination interaction (*F*(3,24) = .441, *p* = .726) were found. The approximate BF_{01} indicated that the model without the effect of sex was 48.565 times more likely to explain the performance of the subjects than the model with that effect.

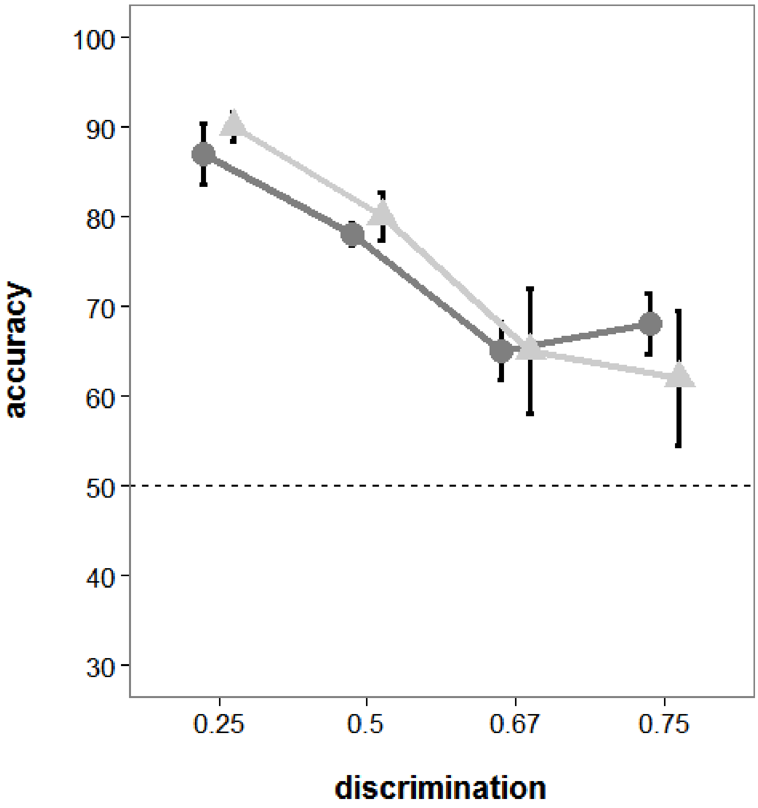

Figure 27: Accuracy of males (grey) and females (dark) in experiment 3 (discrimination of food item size). Data points represent *M* ± *SE* percentage of choice for the larger food quantity.

2.6.5 Discussion

The aim of the experiments reported in this chapter was to study sex differences in quantitative abilities in guppies. I found evidence of sex differences in only one experiment among the three experiments conducted. However, this difference in performance is not likely to be related to quantitative abilities and it is possibly due to difference the speed of information processing.

In experiment 1a, I compared male and female guppies in the discrimination of two shoals of 4 and 6 conspecifics. Because of sex differences in morphology and habitat utilization, and active preference of predators, female guppies suffer higher predation risk compared to males (Croft et al., 2006; Darden & Croft, 2008; Magurran, 2005;

Pocklington & Dill, 1995). I was therefore expecting females to perform better than males in this task. Experiment 1 found no support for this prediction. Male and female guppies did not differ in their overall discrimination accuracy.

One difference between the two sexes emerged when I looked at the temporal pattern of shoal choice. Females showed a significant preference for the larger group from the beginning of the test. In sharp contrast, the position occupied by males was random in the first 8 min. Males apparently achieved the same performance as females only in the following minutes. This could be interpreted as an indication that female guppies are faster than males in estimating which shoal is larger. However, before accepting this hypothesis, I examined alternative explanations. The first alternative explanation is that males were more sensitive than females to experimental manipulations such as netting and transportation, resulting in a negative effect on male performance due to stress (Beiko et al., 2004). My analysis tend to exclude this possibility. Indeed, freezing behaviour, that indicates stress response, have occurred with the same frequency in males and females. The two sexes might also have different willingness to reach the two shoals. This possibility is unlikely for two reasons. The first is that in experiment 1a males and females spent an equal amount of time near the two shoals (time spent close to the larger shoal plus time spent close to the smaller shoal). The second is that in experiment 1b, where I compared males and females in a cognitively easy task (discriminating between 4 and 10 fish; Agrillo et al., 2008; Agrillo et al., 2012b) both sexes rapidly choose the larger shoal and showed similar performances from the beginning of the experiment. Another potential issue is that males might join the stimulus shoals for sexual purposes. This could have affected the cognitive processes and the neural circuits used by males in the choice of the shoal and therefore the comparison between male and female performance. When sexually motivated, male guppies perform high rates of courtship display and sneak mating attempts (approximatively one per minute; Magurran & Seghers, 1994). These behaviours start the instant males encounter a female (Griffiths, 1996). In experiment 1c, I showed that, in a situation identical to that of experiment 1a, most males exhibited sexual behaviour, but only after the first 30 min of the test. Previous observations found also that male poeciliids from wild populations need a long period of time to resume sexual motivation after being captured and transferred in a new place (Bisazza et al., 1997b). Thus, it is extremely unlikely that in experiment 1a males were sexually motivated, especially at the beginning of the test when sexual difference has been observed. Lastly, in guppies there is suspect of selection for a greater behavioural persistence in males, a characteristic that helps to overcome females resistance to mating (Rowe et al., 2005). In my experiment, male guppies might have remained for longer periods in the same choice area and switched less frequently between shoals. This results in fewer chances to compare the two options. A detailed analysis of experiment 1a tends to rule out this hypothesis. Indeed, the frequency of switching did not differ between the two sexes.

Taken together, these results suggest that female guppies are faster than males at discriminating the two shoals with subtle differences in numerosity. My initial prediction - females should have greater accuracy in the shoal size discrimination task- has not been confirmed. However, my result suggests that selection has been acting in the predicted direction, but on a cognitive process diverse than numerical accuracy. Interestingly my result is similar to the one reported in a study by Krause and colleagues (1998). They compared shoal discrimination performance in two fish species with different susceptibility to predators. The species suffering higher predation risk was faster at identifying the larger shoal, but there was no differences in the overall accuracy (Krause et al., 1998). Therefore, it seems that numerical acuity in discrimination of shoal choice has limited or absent response to selective pressures. However, selection may act on other cognitive mechanisms involved in shoal size discrimination, by allowing a more rapid information acquisition and processing. An interesting possibility for future investigations is that male and female guppies exploited different cues to estimate shoal size, such area of fish, quantity of movement, number of fish. The exploitation of different cues could cause differences in processing speed. Yet, the investigation of this hypothesis require to develop and to adopt training procedures that allow a greater control of stimuli characteristics.

In experiment 2, guppies were administered four discriminations between discrete food quantities, from 1 *versus* 4 up to 3 *versus* 4. Overall, subjects selected the larger food quantity, but their performance was affected by the numerical ratio. Subjects had a significant preference in the two easier discriminations (1 *versus* 4 and 2 *versus* 4 food items), but showed a chance performance in the two difficult discriminations (2 versus 3 and 3 versus 4 food items). The ratio dependence of the accuracy aligns with previous literature in mammals (Ward & Smuts, 2007) and birds (Al Aïn et al., 2009). To explain this phenomenon, several authors have hypothesised the existence of an approximate number system for discrimination whose accuracy is set by Weber’s law (Cantlon & Brannon, 2007). However, the performance of guppies in food choice appears to be lower than that found in studies of other species (chimpanzee: Beran, 2006; orangutan: Call, 2000; macaque: Hauser et al., 2000; New Zealand robin: Hunt et al., 2008; salamander: Uller et al., 2003) and similar only to dogs (Ward & Smuts, 2007). Interestingly, the upper limit of the discrimination ability I observed in food choice test was also lower than the one observed in shoal choice experiments, where guppies discriminated up to 3 *versus* 4 conspecifics (Agrillo et al., 2012b) and 4 *versus* 6 conspecifics (chapter 2.6.2), or in experiments based on training procedures where some guppies achieved a 4 *versus* 5 items discrimination (Bisazza et al., 2014). It is possible that the choice of the larger food quantity is not fundamental for guppies life in the wild and therefore this species lack of refined cognitive abilities that control decision making in this context. Male and female guppies showed equal discrimination performance, suggesting they own the same ability to estimate food item number. The evidence for the absence of sex differences in experiment 2 was “very strong” according to Bayesian analysis (Jeffreys, 1998).

In experiment 3, I investigated guppies’ ability to discriminate between two food items differing in size. The ratios between the surface area of the stimuli followed the same quantity ratios of experiment 2 (0.25, 0.50, 0.67, and 0.75). Here, the guppies performed much better than in experiment 2. The preference for the larger food quantities was significant even in the 0.75 ratio. Again, I found a ratio-depend decrease of the accuracy, suggesting Weber’s law sets the accuracy of this discrimination as well. Also in this experiment, the accuracy of male and females guppies was essentially the same. Again, Bayesian analysis revealed my data provides “strong” evidence in favour of the absence of sex differences in this task (Jeffreys, 1998).

The overall result of my three experiments on quantitative abilities is that the accuracy in performing quantity discrimination is not different between male and female guppies. Surprisingly, I found absence of sex difference in accuracy also in task of experiment 1 in which literature strongly suggested females should perform better. Indeed, the sex differences that I found in experiment 1 seems to be due to processing speed rather than accuracy. How can we explain the general absence of sex differences in quantity discrimination ability in guppies, especially in experiment 1? Some authors have proposed the existence of a single unified system for numerical processing across contexts, such as foraging, discrimination of social groups, mate choice (Agrillo & Miletto Petrazzini, 2013; Walsh, 2003). In this scenery it is reasonable to assume that this system has low evolvability because of constraints arising from concurring selective pressures. However, a comparative approach suggests the lack of sex differences in numerical acuity in female guppies could be explained also from a different perspective. Outside primates and few exceptional species (e.g., Hanus & Call, 2007), all vertebrates show similarity in numerical acuity (reviewed in Agrillo & Bisazza, 2014). Some authors believe all extant species share a system for quantity processing which has appeared early in the evolution of this clade (Beran, 2008; Feigenson et al., 2004). This conservation can be explained by the possibility that the improvement in numerical computation is constrained by the increase in the complexity of underlying neural circuits. Therefore, only few species with outstanding nervous system development such as primates can evolve a superior ability to discriminate quantity.

3. GENERAL DISCUSSION

3.1 Similarities in cognitive performance of male and female guppies

In the research for this thesis, I compared male and female guppies' performance on 11 tasks that arguably required different cognitive skills to solve. Overall, I found limited evidence of sex differences (table 2). On the visual discrimination learning tasks (chapter 2.3), males and females expressed similar discrimination ability, reaching comparable accuracy in choosing the correct stimulus both in experiments with training procedure and in an experiment exploiting spontaneous one-trial learning. There was also an absence of sex differences in visual discrimination learning in the first phase of the reversal learning experiment (chapter 2.4), in which both males and females easily learned a red *versus* yellow discrimination. Males and females obtained comparable scores on the oddity discrimination learning task (chapter 2.4), and I found no evidence of sex differences in performance or in the strategy adopted to solve the task. Regarding spatial learning tasks (chapter 2.5), both males and females rapidly learned to navigate around an obstacle (if the obstacle was not transparent). Moreover, the two sexes showed a similar reduced performance in the Y maze task, possibly because of behavioural lateralization. In the last field investigated, quantitative abilities (chapter 2.6), I found no evidence of sex differences in the accuracy of discrimination between shoals of different numbers, different numbers of food items, and food items of different sizes. Only two clear sex differences in performance emerged from my experiments, in the second phase of the reversal learning and in the speed of recognizing the larger shoal in the discrimination of shoal size. I also

Table 2: Summary of the main results of experiments in guppies. Presence and absence of sex differences are indicated with + and -, respectively. Marginal sex differences are indicated with *****. Most of the sex differences and marginal sex differences (grey highlighted) regarded either cognitive style or behaviour; for two of them (dark highlighted) it is not clear.

Experiment	**Task**	**Procedure**	**Variable**	**Type**	**Sex Diff**
Visual discrimination	S vs bar	Training	Accuracy	Ability	-
	S vs bar	Training	Decision Speed	Cognitive style	+*
	triangle vs square	Training	Accuracy	Ability	-
	triangle vs square	Training	Decision Speed	Cognitive style	+
	Image vs mirror-image	Training	Accuracy	Ability	-
	Image vs mirror-image	Training	Decision Speed	Cognitive style	+
	Novel object recognition	Spontaneous	Accuracy	Ability	-
	Novel object recognition	Spontaneous	Exploration	Behaviour	+
	Novel object recognition	Spontaneous	Timing	Cognitive style	+
Concept learning	Oddity discrimination	Training	Accuracy	Ability	-
	Oddity discrimination	Training	Decision Speed	Cognitive style	+
Reversal learning	Red vs yellow	Training	Errors to learning criterion	Ability	-
	Reversal learning	Training	Errors to learning criterion	Ability / Cognitive style	+
Spatial learning	Detour – transparent barrier	Training	Time to reward	Cognitive style	+
	Detour – opaque barrier	Training	Time to reward	Ability	-
	Y maze	Training	Accuracy	Ability	-
	Y maze	Training	Time to reward	Ability	-
	Y maze	Training	Decision Speed	Cognitive style	+
	Complex maze	Training	Accuracy	Ability	+
	Complex maze	Training	Time to reward	Ability	-
	Complex maze	Training	Decision Speed	Cognitive style	-
Quantity discrimination	Shoal size	Spontaneous	Accuracy	Ability	-
	Shoal size	Spontaneous	Timing	Ability / Cognitive style	+
	Food item number	Spontaneous	Accuracy	Ability	-
	Food item size	Spontaneous	Accuracy	Ability	-

found a third sex difference in performance on a complex spatial learning task, but its nature was less clear.

Taken together, my results show that sex differences in cognition exist in guppies but are limited to very few contexts. This result is interestingly similar to the one found by reviewing researches in other species, such as humans and rats, in which sex differences exist only in a limited number of tasks (conclusion *i*, chapter 1.3.2). In guppies, as in other vertebrates, there are more similarities than differences between male and female cognition.

In the discussion of the single sections I provided some possible explanations for this absence of sex differences in guppies. Some of these explanations are specific to the cognitive task or the species investigated. Yet, some other explanations are more general and could be adapted to many contexts and, potentially, many species. The first of these general explanations is that, despite differences in behaviour and ecology, males and females of many vertebrate species face cognitive challenges that are roughly similar. As a consequence, selective pressures on cognitive performance are similar in males and females, and the two sexes evolve similar cognitive performances. The second general explanation is that cognitive challenges that differ greatly for males and for females exist, but these challenges are limited in number. In such a scenario, selection might act differentially on male and female cognition, but only in a limited number of contexts. If the same cognitive functions are involved in the solution of many different tasks, functional pleiotropy might occur. Selective pressures imposed in situations in which males and females experience similar challenges might constrain the evolution of sex differences in those situations in which the challenges are sex-specific. Lastly, it is also possible that selective pressures are different for males and females, but that the cognitive functions involve lack of evolvability for sex differences because of genetic constraints (i.e., there is no genetic variation for the trait and for sex-specific expression) or developmental ones. The lack-of-evolvability hypothesis is supported by the growing evidence that many basic cognitive functions are conserved across vertebrates. For example, the mechanisms for learning (Papini, 2002), visual perception and discrimination (Gori et al., 2014; Sovrano & Bisazza, 2008), numerical processing (Agrillo & Bisazza, 2014) are substantially the same from fish to humans. The stability of these functions across vertebrate species with large ecological differentiation suggests the existence of robust constraints that prevent differentiation of the cognitive systems. These constraints are likely to be very strong in prevent the evolution of sex differences because males and females share most of the genome and ecological niche.

It should be said that the experiments in this thesis also have a limit. Guppies observed in the experiments were bred in the laboratory in standard conditions. Although these conditions resembled natural ones as much as possible, they were equal for males and females. This approach was chosen because it could disclose eventual cognitive differences that have occurred through evolution and that would therefore be interesting for comparative psychology. However, it is possible that sex differences in cognitive tasks arise only if males and females experience different environments during development (e.g., Ebbesson & Braithwaite, 2012), as in the case of gender differences that occur in humans (Halpern, 2013). Therefore, an interesting direction for future investigations is the use of wild-caught guppies or, alternatively, the study of sex by environment interaction in guppies reared in the laboratory.

All these general explanations for the absence of sex differences in cognitive tasks in guppies and other vertebrates deserve attention in future research. The fact that cognitive abilities are similar across all vertebrates, rather than different between the two sexes, suggests that in future investigations more attention should be devoted to understanding why sex differences have not occurred rather than whether they have occurred.

3.2 Sex differences in cognitive performance of guppies

The three tasks in which male and female guppies obtained differential performances were very different in nature. In the second phase of the reversal learning task (chapter 2.3), females switched faster than males to the new rewarding stimulus after reversal of contingency, whereas males persisted longer in choosing the previously rewarding stimulus. This difference is not likely to be due to general learning or discrimination ability, because in the first part of the experiment males and females discriminated equally well between the two stimuli. In the shoal size discrimination, females appeared to be faster than males at recognizing and choosing the larger shoal. It is worth noting that the overall accuracy of the two sexes was similar. Therefore, this sex difference is not likely to be related to quantity discrimination abilities. Rather, the observed sex difference is more likely due to a difference in speed of information processing or a difference in cognitive style, such as in the attention devoted to the environment and the stimuli.

The last task in which a sex difference in performance emerged was a complex maze adopted to study spatial learning (chapter 2.5). In this task, guppies had to choose the correct routes among four possibilities to reach a social reward. Here, only males achieved the task, suggesting that males possess enhanced spatial learning abilities compared to females, but this difference is subtle and therefore visible only in complex tasks. However, there were a number of issues with the experiment (discussed in chapter 2.5.5) that indicate caution in interpreting this result. For example, this was the only sex difference across three spatial learning experiments in which males and females performed similarly; also, there appeared to be a rapid decrease in male motivation to participate in the task. Therefore, these results are promising in suggesting a sex difference in spatial learning tasks in guppies, but future investigations with other paradigms are needed for confirmation.

Similarly to the results obtained in other vertebrates (conclusion *ii*, chapter 1.3.2), the nature of the sex differences observed in the performance of guppies is not clear. It is not possible to affirm whether sex differences in guppies' performances arise from sex differences in cognitive abilities or are due to other factors. Observed sex differences in performance could be related to sex differences in cognitive abilities, such as learning flexibility, processing speed, and spatial learning. On the other hand, sex differences in cognitive style could also explain these results. For example, poor male performance in reversal learning could be due to greater male persistence. Faster female discrimination in shoal size discrimination could be due to greater attention to the environment. Enhanced male learning in spatial tasks could be due to the use of different navigation strategies. My results are similar to those of research on other vertebrates regarding another aspect (conclusion *iii*, chapter 1.3.2). Sex differences in cognitive abilities appear to be scarce or, in most cases, non existent.

Another similarity between the results obtained in guppies and the general conclusion from the review of sex differences in vertebrates is that cognitive sex differences appear to be related to sex differences in ecology and behaviour (conclusion *iv*, chapter 1.3.2). Therefore, also in guppies sex differences are likely to be adaptive and to have evolved because of the different challenges faced by the two sexes. Indeed, male guppies, which showed more persistence in the reversal learning task, showed persistent behaviour during mating. Female guppies, which were faster in recognizing the larger shoal in an antipredator task, suffer a greater predation risk than males (Magurran, 2005; Pocklington & Dill, 1995). The male advantage in the complex maze, if confirmed by further studies, could be explained by the fact that male guppies were reported to move more, and in a more complex environment, compared to females (Croft et al., 2003; Darden & Croft, 2008). In guppies, as in other vertebrates, sex differences in cognitive performance are therefore likely to derive from sex-specific selective pressures imposed by the environment (Sherry et al., 2006).

It is worth noting that two out of three of the differences in performance identified in guppies, reversal learning and spatial learning, are also present in mammals and birds (conclusion *vii*, chapter 1.3.2). This similarity suggests that when sex-specific selective pressures on cognition occur, they are substantially the same regardless of the species.

3.3 Sex differences in behaviour and cognitive style in guppies

In many of my experiments, male and female guppies behaved quite differently, despite a large similarity of the accuracy and learning scores. In two visual discrimination tasks and in two spatial learning tasks based on different training procedures, males showed shorter decision times compared to females and were faster at deciding which option to choose. This suggests increased impulsivity in males. In a spatial navigation task, males persisted longer than females in trying to reach a target behind a transparent barrier. In the novel object recognition task, males and females expressed equal ability to discriminate the two objects. However, while males explored the novel object at the beginning of the test, females did so some time afterwards. This difference could be explained by a sex difference in the tendency to explore novel objects. In addition to these three points, the sex differences observed in the reversal learning task could also be explained by a sex difference in cognitive flexibility and persistence.

Altogether, this evidence suggests that the sex differences in cognitive style and behaviour are expressed frequently during cognitive tasks in guppies, as they are in other vertebrates (conclusion *iv*, chapter 1.3.2). Surely, these differences are more frequent than sex differences in performance, which are likely to derive from sex differences in cognitive abilities.

Why are these differences in behaviour and cognitive style so common? Some of these differences could be explained in the light of the different ecological requirement of males and females, as suggested for sex differences in cognitive performance. For example, the sex difference in decision speed might have evolved because of the limited foraging time budgeted for males or because females have been selected to make careful decisions in the mating context. Increased persistence in males might have been selected to overcome female resistance to mating.

However, the growing literature on individual differences in animal behaviour suggests another evolutionary origin. The sex differences in cognitive style in guppies and in other vertebrates are extremely similar to the differences reported between individuals who differ in personality traits (Sih & Del Giudice, 2012). In many species, males and females often differ for personality traits (Schuett et al., 2010), perhaps because of the differences in life history traits such as growing rate or investment in reproduction (Biro & Stamps, 2008; Wolf et al., 2007). Therefore, this sex difference in personality traits might be an explanation of sex differences in cognitive style. If this hypothesis is correct, it provides a possible alternative interpretation of the widespread evidence of sex differences in cognitive style in vertebrates (conclusion *iv*, chapter 1.3.2) and also of the fact that differences such as in flexibility and persistence are present in different phylogenetically distant species (conclusion *vii*, chapter 1.3.2). This new hypothesis deserves attention in future investigations.

3.4 Conclusions and future directions

The results of this thesis provide the first extended data on sex differences in a species outside mammals. However, these results are far from being conclusive, and cognitive sex differences in fish deserve many more investigations. For example, a greater characterization of cognitive sex differences in these and other tasks is needed for biomedical purposes. This first requires the development of many more procedures to study cognition in fish. In any case, the partial results of this thesis have clearly proven that in terms of cognitive research, male fish are not equal to female fish. Therefore, future investigations should at least carefully consider the sex of the subjects.

One issue that this thesis did not address is the study of proximate mechanisms for sex differences. The broad literature in model species such as laboratory rodents and chickens allows understanding of several key mechanisms of hormonal regulation controlling sex differences in cognition as well as their possible neural basis. This thesis, as the first deep investigation on fish, has focused on behavioural and cognitive aspects, but the investigation of proximate mechanisms could be an interesting topic for future studies.

Although this research substantially expands the knowledge on cognitive sex differences in lower vertebrates, there is still a need to investigate many more species. For example, it was impossible to study and consider one of the conclusions that emerged from the review across all vertebrates in the introduction of this thesis: that differences in cognition appear larger in polygamous species (conclusion *vi*, chapter 1.3.2). This conclusion can be investigated only by adopting a study of many more fish species.

On the other hand, the results of this thesis and previous work in vertebrates have delineated a very interesting scenario. Cognitive sex differences in fish species appear to follow the same general rules observed for other vertebrates. Almost all of the conclusions that emerged from the review of the literature across vertebrates are also valid for fish (chapter 1.3.2). The causes and mechanisms underlying the evolution of sex differences in cognition therefore appear to be equal across all vertebrates. This evidence is in line with the growing literature suggesting that basic characteristics of cognitive systems are shared among all vertebrates. As a consequence, this work raises the interesting possibility that lower vertebrates, such as fish, can be used as a simpler model for research on sex differences in cognition.

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