Early environment and the development of social behaviours in the Trinidadian guppy, *Poecilia reticulata*

Ioannis Leris

Leris, Ioannis
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Early environment and the development of social behaviours in the Trinidadian guppy, *Poecilia reticulata*

Vroege omgeving en de ontwikkeling van sociaal gedrag van de Trinidadiaanse guppy, *Poecilia reticulata*

(met een samenvatting in het Nederlands)

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Ioannis Leris

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te Agrinio, Griekenland

Promotor: Prof. dr. J. J. Bolhuis
Copromotor: Dr. S. M. Reader

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Chapter 1

General Introduction

It has been more than half a century since Tinbergen (1963) published his influential article 'On aims and methods of ethology', in which he elegantly describes the four main problems that behavioural biologists tackle in their research. This thesis examines what I find the most fascinating of these questions, that is, how do specific characters or traits develop during an individual's lifetime and how does the environment define and shape animals' phenotypes later in life. We have slowly but steadily shifted from a 'genocentric' evolutionary theory, towards a more integrated approach that addresses the nature versus nurture controversy by essentially merging them under the concept of phenotypic plasticity (Whitman & Agrawal, 2009). Phenotypic plasticity is no longer neglected as 'noise' but considered adaptive under a wide range of circumstances, and genes have been argued to more likely be followers rather than leaders in evolutionary processes (West-Eberhard, 2003 – but see Luttikhuizen & Drent, 2004). Social learning – learning from others – represents a particular type of plasticity allowing animals to detect and respond to environmental change quickly and inexpensively by observing the behaviour of other individuals (Heyes, 1994; Hoppitt & Laland, 2013). Furthermore, the use of social learning is itself plastic, since an individual's reliance on personal or social information can be dependent on the relative costs and benefits of each option in different circumstances and environments (Boyd & Richerson, 1985; Rendell et al., 2011; Webster & Laland, 2008).

Aims of the Thesis

The present thesis aims to extend current knowledge on the developmental plasticity of behaviour and specifically of social behaviour and social learning, by utilising a series of developmental experiments on a fish system. I examine whether elements of social behaviour such as grouping tendencies and social learning propensities are consistent and heritable traits (Chapter 2), and whether they are developmentally plastic i.e. influenced by external factors and experiences during early life such as social cues (Chapter 3), rearing density and predation risk (Chapters 4-5). For these purposes I used the guppy, *Poecilia reticulata*, a popular study system with well-studied social behaviour. My first set of experiments involved repeated testing of individuals and their offspring, to assess individual consistency and heritability (Chapter 2). I then followed a developmental approach, where I manipulated the subjects' rearing environment to reveal early life influences on their behavioural phenotype later on, either directly varying the informational value of social cues (Chapter 3) or varying traits such as density and predation risk that are hypothesised to impact the payoffs of social learning as well as other social behaviours (Chapters 4-5). In this introductory chapter, I discuss the basic theoretical concepts behind these experiments and present previous relevant work on the main topics that will be analysed in the following chapters.

Social Behaviour and Social Learning

Living in groups brings numerous benefits to group members, including decreased vigilance costs and reduced predation risk, as well as advantages in finding food and mates (reviewed by Krause & Ruxton, 2002). However, animals may also face increased competition for resources and higher risk of parasite and disease transmission when forming social groups (Altizer et al., 2003). Hence, there is a trade-off between the costs and benefits of social (group) living that, when animals are free to move, will impact decisions about joining or leaving groups. Whether social or solitary, animals often interact with each other in various ways and contexts, engaging in so-called social behaviour. These social interactions may potentially result in the acquisition of new behavioural patterns and learned responses by social learning. This thesis examines social behaviour in general and social learning in particular. I thus introduce social learning here, and its relationship to learning.

Animals change their behaviour through experience, a process we call learning. Basic requirements of learning are the capacity to detect and process external sensory inputs and a memory system to store and retrieve the relevant information when necessary (Krause & Ruxton, 2002). Animals can learn about the environment individually (or asocially), from their own experiences, or socially from other individuals. Social learning refers to situations where the observation of or interaction with other individuals (or their products) facilitates the learning of a new behaviour (Heyes, 1994; Hoppitt & Laland, 2008; Hoppitt & Laland, 2013), and has become an increasingly popular topic of study in behavioural and evolutionary biology as it affects a wide range of processes such as foraging, predator avoidance, mating choices, and communication (reviewed by Hoppitt & Laland, 2013). The knowledgeable individual that provides the social cues is usually termed a 'demonstrator' and the naïve individual learning from these cues is termed an 'observer' (Hoppitt & Laland, 2008). Although social learning typically includes any learning from others, some argue that it should be distinguished from cases of directed information transfer from the demonstrator to the observer via active signalling, teaching or other forms of directed communication (Heyes, 2012). Moreover, Heyes (1994) restricts the category of social learning to social influences that directly result in learned responses. However, recent classification schemes include social learning processes that can lead to learning indirectly. For example, an individual's tendency to follow others might not directly result in learning, but if following an individual with knowledge of a food patch allowed the naïve individual to discover this food patch, then learning this foraging location would be considered a social learning process (Hoppitt & Laland, 2008). Social learning is often contrasted with other forms of learning about the environment such as asocial or individual learning, learning by trial and error and innovation, which all typically happen without any influences from other individuals (Heyes, 2012). However, it is argued that social and asocial learning potentially rely on the same underlying processes (Heyes, 1994), but this remains an open question (Hoppitt & Laland, 2013). In relatively stable or slowly changing environments, social learning is expected to be favoured over individual learning but the pattern is reversed in rapidly changing environments where information needs to be constantly updated (Boyd & Richerson, 1985).

Social learning is widespread in the animal kingdom and has been demonstrated both in the wild and in the laboratory (Hoppitt & Laland, 2013; Reader & Biro, 2010). Two classic studies proposing social learning in natural populations are the social transmission of the sweet potato washing behaviour in Japanese macaques, *Macaca fuscata* (Kawai, 1965) and the spread of milk bottle opening behaviour in British tits, *Parus* spp. (Hinde & Fisher, 1951). Although the role of social learning (by imitation) in these studies is controversial (e.g. see Galef, 1992,1998) they are interesting historical observations which have promoted interest in the field and have served as useful guides for subsequent investigations. A well-studied example of social learning in the laboratory is the acquisition of food preferences from conspecifics in Norway rats, *Rattus norvegicus* (Galef & Wigmore, 1983). Galef and his colleagues have shown that when rats interact with conspecifics that have previously eaten a particular food, they develop a preference for this food. The information (food odour) is transferred to the ‘observer’ through the breath of the ‘demonstrator’, coupled with contextual cues like metabolic products. As a result, the ‘observer’ develops a long-lasting increased preference for the food items the demonstrator had eaten (Galef et al., 1985). Numerous other examples exist from insects, cephalopods, fish, reptiles, amphibians, birds and mammals, learning about predators, mates, nesting sites, foraging techniques, food preferences and locations, grouping and travel routes, and communicative signals (Corten, 2002; Danchin et al., 2004; Heyes & Galef, 1996; Hoppitt & Laland, 2013; Mathis et al., 1996; Reader & Biro, 2010; Whiten et al., 2016). In addition, there are several different psychological processes that have been proposed to underlie these social learning observations (social learning mechanisms), ranging from fairly simple such as local or stimulus enhancement, to more complex like imitation and emulation (reviewed by Hoppitt & Laland, 2013).

Gathering information personally can be costly in terms of effort and risk, therefore animals often rely on inexpensive information provided by others (Boyd & Richerson, 1985; Rendell et al., 2010; Webster & Laland, 2008). However, socially acquired information can be unreliable or outdated depending on the spatial and temporal variability of the environment (Rendell et al., 2011; Webster & Laland, 2008). This trade-off between the costs and benefits of social information use and social learning might differ between individuals and between species, which could explain the observed variation in their reliance on social information (Lefebvre & Palameta, 1988; McCabe et al., 2015; Reader et al., 2011). Due to this trade-off, animals would benefit if they were selective regarding their reliance on social information. Several so called 'social learning strategies' have been proposed about 'when' and 'who' individuals should copy (Hoppitt & Laland, 2013; Laland, 2004; Rendell et al., 2011). For example, individuals may choose their sources of information based on the level of familiarity and affiliation between them (Galef & Whiskin, 2008; Kavaliers et al., 2005; Scheid et al., 2007; Swaney et al., 2001), based on the size and age of the demonstrators (Biondi et al., 2010; Duffy et al., 2009; Dugatkin & Godin, 1993; Vukomanovic & Rodd, 2007), demonstrator dominance status (Horner et al., 2010), kinship (Kavaliers et al., 2005) and sex (Scheid et al., 2007). In addition animals are more likely to socially learn when the number of demonstrators increases (Boyd & Richerson, 1985; Lachlan et al., 1998; Laland & Williams, 1997; Sugita, 1980) and there is evidence for conformity, i.e. a general tendency to copy the majority (Day et al., 2001; Kendal et al., 2004; Pike & Laland, 2010). Regarding 'when' to copy, there are a lot of examples where animals 'copy when asocial learning is costly'. Usually the cost refers to increased predation risk for the acquisition of an anti-predator defence (Chivers & Smith, 1995; Kelley et al., 2003) or during foraging (Kendal et al., 2004; Webster & Laland, 2008). Furthermore, animals may choose to 'copy when uncertain' (in the broad sense; see Toelch et al., 2014), for example naïve individuals may copy food preferences or follow chemical trails of others, while experienced individuals rely on personal information (Galef, 2009; Grüter et al., 2011). Apart from 'who' and 'when' to copy, strategies about 'what' to copy have also recently been proposed, suggesting that individuals socially learn disproportionally according to the content of the copied information (Hoppitt & Laland, 2013). However, there are situations where unbiased social learning (random copying of others) might be more advantageous than following a strategy or using personal information (Rendell et al., 2010).

Social learning has been a popular research topic for several decades in a broad range of scientific fields including behavioural biology and ecology, cognitive neuroscience, psychology, evolutionary biology, anthropology, and economic science (reviewed by Hoppitt & Laland, 2013). Social learning has important ecological and evolutionary consequences as it can shape the structure of social groups (Cantor et al., 2015), influence evolutionary dynamics and equilibria (McElreath et al., 2005), affect processes like speciation (Lachlan & Servedio, 2004), and it is the key for understanding human cumulative culture (Hill, 2010). However, the importance of early environment on the development of social learning propensities of individuals has not received much empirical attention, as most studies in the literature either concern new observations of social learning, or they focus on strategies, mechanisms and underlying psychological processes. There are only a handful of studies that have tried to address developmental influences on social information use and social learning. Chapman et al. (2008b) reared guppies in different housing densities and found that fish reared at low densities exhibited a higher tendency to follow conspecifics to a food location and were consequently better at socially learning this route, compared to high-density-reared fish. Katsnelson et al. (2008) exposed house sparrows, *Passer domesticus*, to artificial parents that either reliably indicated food locations or did not. Sparrows that were previously exposed to reliable social cues were more likely to use social information later in life compared to others that were exposed to unreliable cues. However, both of these studies maintained the developmental manipulations and exposures until just before testing, making it hard to distinguish between the effects of early and recent experiences (Kendal et al., 2005; Leadbeater & Chittka, 2009). To my knowledge, there are only three cases where manipulations specifically confined in early life have been demonstrated to influence the reliance of social information later on. Japanese quails, *Coturnix japonica*, exposed to an early life stressor like unpredictable food availability, were less likely to copy the foraging choices of videoed demonstrators later on (Boogert et al., 2013). Similarly, zebra finches *Taeniopygia guttata*, developmentally exposed to high levels of stress hormones, followed a different social learning strategy and learned a novel foraging skill from unrelated birds, compared to control individuals which socially learned from their parents (Farine et al., 2015). Finally, in Norway rats maternal care during early life has been found to influence social learning propensities later in life (Levy et al., 2003; Lindeyer et al., 2013; Melo et al., 2006). These previous studies have provided novel evidence on how early environment can affect an individual's reliance on social information, and have stimulated new investigations on the developmental plasticity of social behaviour. However they have only focused on a narrow range of developmental factors (mainly developmental stressors and maternal care), while some have demonstrated influences on social information use rather than social learning. Thus, we lack a general picture of how social learning propensities develop and several important questions remain unanswered. Are these propensities heritable and to what extent are they fixed or flexible? How and to what degree can they be influenced by conditions and experiences confined in early life or by the individual's knowledge of the value of social information? Chapters 2, 3 and 5 of this thesis aim to extend our understanding on these interesting topics.

Early Environment and Phenotypic Plasticity

The phenotype of an individual is shaped by complex interactions between its genes and the environment it encounters. The organism's genotype provides it with a certain degree of plasticity allowing adjustment to a variety of environmental regimes. Phenotypic plasticity refers to this ability of organisms with specific genotypes to produce multiple different phenotypes in response to different environments (Bradshaw, 1965; Callahan et al., 1997; Pigliucci et al., 2006; Schlichting & Levin, 1986; Schlichting, 1986; Via et al., 1995; West-Eberhard, 1989,2003).

Although phenotypic plasticity is generally acknowledged as an important characteristic of organisms, its evolutionary role has been controversial. It has been previously proposed that phenotypic plasticity is in direct competition with adaptive trait evolution (Fitzpatrick, 2012); i.e. highly plastic organisms do not 'need' to evolve new characters to survive in changing environments, whereas organisms with narrow phenotypic plasticity can only cope with environmental change by evolving new traits through selection (Schlichting, 1986). Recent theory suggests that phenotypic plasticity plays an important role in various evolutionary processes and can promote speciation, divergence and adaptive radiation under certain circumstances (reviewed by Pfennig et al., 2010). When faced with environments that change in a predictable way, organisms with appropriate genetic variability can evolve adaptive plastic adjustments (Via et al., 1995). For example, the micro-crustacean, *Daphnia melanica*, rapidly reduces its expression of melatonin, when faced with a recently introduced fish predator (Scoville & Pfrender, 2010). Thus, phenotypic plasticity is often adaptive, i.e. it increases the organism's chances of survival and reproduction in different environments (Fitzpatrick, 2012). There are also cases where induced plasticity results in non-adaptive (and even maladaptive) plastic responses which do not enhance fitness (Schlichting, 1986). These are often induced by poor environmental conditions and stress (Handelsman et al., 2013). However, even non-adaptive phenotypic plasticity has been proposed to significantly influence evolutionary change (Fitzpatrick, 2012). Overall, plasticity can either stimulate or dampen adaptive evolution, divergence and speciation, depending on the combined effects of the genetic architecture of the focal trait, the degree of plasticity exhibited by the population and its effects on gene flow (Fitzpatrick, 2012; Pfennig et al., 2010).

Behavioural plasticity, a type of phenotypic plasticity, refers to the capacity of an individual to change its behaviour as a response to varying environments (Bateson 1983). Behavioural plasticity can be further classified in two categories, activational and developmental (Snell-Rood, 2013). Activational behavioural plasticity occurs when an organism exhibits different behaviours in different environmental conditions or as a response to changes in the environment (e.g. Rendell et al., 2011; Webster & Laland, 2008). Developmental behavioural plasticity refers to cases where different environments can lead a particular genotype to follow different developmental trajectories which result in different behavioural phenotypes (e.g. Adkins-Regan & Krakauer, 2000; D'Andrea et al., 2007; Sundström et al., 2003). For example, an individual that increases its cryptic behaviour and reduces foraging activity in the presence of predators would be exhibiting activational behavioural plasticity, while individuals that develop bolder or shyer phenotypes as a result of being raised in high-predation environments would be exhibiting developmental behavioural plasticity (Snell-Rood, 2013). Developmental plasticity of behaviour and social learning in particular, will be explored further in Chapters 3-5. Of course, there are limits to the behavioural plasticity of an organism, in the sense that not all individuals can express the optimum behaviour across all contexts. For instance, individuals that are generally bolder or more aggressive may benefit in intraspecific competitive situations but might be at a disadvantage in other contexts (e.g. anti-predator contexts) where shyness or caution is favoured. The existence of behavioural syndromes and personalities in animals (Réale et al., 2007; Sih et al., 2004), which are often genetically or epigenetically heritable (Dingemanse et al., 2002; Drent et al., 2003; van Oers et al., 2004), further supports the notion that animals are limited in the degree of behavioural plasticity they can exhibit, and this limited plasticity could explain situations where individuals appear to behave in a suboptimal or maladaptive way (Sih et al., 2004). The elements of consistency and heritability of social behaviour will be further analysed in Chapter 2.

The early life environment in particular plays a very important role, as it can potentially program the organism's phenotype early on, to better match the conditions it will encounter later in life. In Norway rats for example, the level of maternal care during early life provides cues for local environment in a proposed form of 'adaptive programming' (Diorio & Meaney, 2007), and it can directly influence offspring's behavioural phenotype in terms of responsiveness to stress and reliance to social information later in life (Levy et al., 2003; Lindeyer et al., 2013). In a different example, in Japanese quails, pre- and post-natal exposure to stressors (cortisol and unpredictable food availability respectively) had combined effects on their behaviour. The developmental stressors increased the quails' propensities to explore and take risks, which would potentially be beneficial in novel/changing and potentially stressful environments (Zimmer et al., 2013). Moreover, there is increasing evidence that developmental effects can potentially overshadow genotypic variation (Jonsson & Jonsson, 2014). For example, wild- and hatchery-reared populations of coho salmons, *Oncorhynchus kisutch*, have been repeatedly demonstrated to differ in terms of physiology, morphology and behaviour, and the observed differences are attributed to the rearing environment rather than genetic variation (Chittenden et al., 2010; Swain et al., 1991). There are several other studies focusing on differences between wild- and hatchery-reared fish that illustrate the importance of early environment effects on fish brain development and plasticity (see Ebbesson & Braithwaite, 2012 for review).

Predation is a strong selective force for organisms that has well-established long-term effects on populations in evolutionary time, while also influencing animal morphology, physiology and behaviour in ecological time (Lima & Dill, 1990). Three-spined sticklebacks, *Gasterosteus aculeatus*, for example, increase their growth when exposed to cues of rainbow trout, *Oncorhynchus mykiss*, a size-selective predator (Bell et al., 2011) and gastropods have been found to change the timing of certain developmental events in the presence of predator cues (Rundle et al., 2011). Early environment can provide the necessary stimuli to induce the development of anti-predator defences in animals. Developing these defences (being behavioural, physiological or morphological) early in life in anticipation of high predation risk, can potentially incur less costs than having to develop them later in life (Hoverman & Relyea, 2007). Moreover, for several species, early life experience with predators is important for building potent anti-predator skills (e.g. Bass & Gerlai, 2008) and learning about potential threats during early life – mostly through exposure to chemical cues – can assist with fine tuning predator recognition and avoidance (Ferrari et al., 2007). The effects of early exposure to predator cues on several behavioural traits will be addressed in Chapters 4 and 5. Social environment during early life can also affect several aspects of an animal's behavioural phenotype. In mice, *Mus musculus*, social enrichment such as communal nesting has been demonstrated to increase social competences (D'Andrea et al., 2007). Zebra finches raised in colonies without adult males, were less adept at choosing a suitable mating partner (Adkins-Regan & Krakauer, 2000), and trout, *Salmo trutta*, reared in hatcheries under high stocking densities were more territorial and conducted more contests compared to wild conspecifics (Sundström et al., 2003). The effects of early social environment (social cues and rearing density) on behaviour will be further discussed in Chapters 3-5.

Plasticity has received great empirical attention in the past century, as it can potentially provide the means for animals to withstand environmental change (e.g. the appearance of a new predator – Agrawal et al., 1999; Scoville & Pfrender, 2010) and adjust to novel environments after dispersal (Handelsman et al., 2013; Thibert-Plante & Hendry, 2011). Phenotypic plasticity has important ecological consequences, ranging from simple susceptibilities to environmental stressors and influencing interactions between species, to impacting the structure of animal communities (reviewed by Whitman & Agrawal, 2009). Consequently, these ecological effects influence evolutionary processes like adaptation and speciation, and by generating novelty plasticity can facilitate evolutionary change (West-Eberhard, 2003). However, environment-induced developmental effects are still being neglected or misinterpreted for genetic variation, when studying morphological, physiological or behavioural variation (Jonsson & Jonsson, 2014), and there is a significant lack of empirical evidence for the developmental plasticity of social behaviour. Therefore, a developmental perspective can improve our understanding of the ecology and evolution of social behaviour. We live in a constantly changing world and within the next 100 years, climate change is expected to severely impact populations and ecosystems across the globe (Williams et al., 2008). Such rapid environmental changes may not allow the development of adaptive responses through evolution, particularly in animals with long lifespan like us.

Phenotypic plasticity, acting in ecological time and within an organism's lifetime, can provide the means to cope with a rapidly changing world.

Study System

Studying social learning is of paramount importance for understanding the evolution of human culture, and experiments with animal systems in the wild and in the laboratory allow us to examine simpler social learning mechanisms which might have contributed to the evolution of this more complex biological adaptation that is human cumulative culture (Hill, 2010). More specifically, to address questions on the developmental plasticity of social learning and social behaviour in general, one needs a study system with particular characteristics. First of all, the system must exhibit a rich repertoire of social behaviours which can be investigated under a range of experimental conditions. Moreover, the system should show a certain degree of phenotypic plasticity, i.e. there should be an established relationship between its early environment and its phenotype (physiology, morphology or behaviour) later in life, from which we can hypothesise similar patterns of plasticity in social behaviour. In addition, the experimental species should exhibit a natural variation in their sociality and prior knowledge on aspects of its ecology, evolutionary history and behaviour are useful tools for more accurate hypotheses formation and more efficient experimental design. For practical and logistical matters, the system should be easy to maintain and breed in laboratory conditions, with short generation times (but long enough to allow the study of sensitive developmental windows), and its rearing environment should be relatively easy to manipulate. The ability to conduct tests both in the laboratory and in the wild would also constitute a big advantage for a more complete experimental approach (Reader & Biro, 2010). In the next paragraphs, I describe the key features that make a small tropical fish, the Trinidadian guppy, an ideal study system for examining the developmental plasticity of social behaviour.

Apart from being highly diverse in terms of their morphology, physiology and ecology, fishes are extremely social animals. Approximately 50% of all teleost species spend their early life in social groups and about 25% shoal throughout their life (Shaw, 1978). One of the advantages of group living is that information can be rapidly transmitted among shoal members both through directed communication and active signalling and by observing the behaviour of their conspecifics in response to external stimuli (Hoare & Krause, 2003). Consequently, fishes often display complex social behaviours and commonly rely on social cues to detect and respond to changes in their environment. Sticklebacks, for example, are known to use social information to evaluate a food patch and while three-spined sticklebacks rely on simpler cues like number of demonstrators near a foraging location (Webster & Hart, 2006), nine-spined sticklebacks, *Pungitius pungitius*, can make decisions following more subtle cues like the feeding rate of a group of conspecifics (Coolen et al., 2005b). Nine-spined sticklebacks are also more selective in their social learning strategy regarding whom to copy compared to three-spined sticklebacks, as they preferentially use social information from larger/older individuals (Duffy et al., 2009). Social guidance may not only be advantageous to the receivers, but essential for the survival of the entire population. For example, herring, *Clupea harengus*, form stable migrating traditions which are socially transferred from one generation to the next, but in the absence of older knowledgeable individuals (e.g. due to overfishing) these traditions can go extinct (Corten, 2002).

Early environment is known to play a significant role in the development of fish phenotypes (see Jonsson & Jonsson, 2014 for a review). Early social environment and predation risk in particular, which are targeted by this thesis in Chapters 3-5, have been repeatedly demonstrated to influence life history, morphological and behavioural traits of fish. For example, three-spined sticklebacks exposed to predator cues during early life grew faster but reached a smaller size when adult, compared to unexposed fish (Bell et al., 2011). In addition, early experiences with predators facilitates predator recognition by prey fish later in life, thus increasing survival chances (Lönnstedt et al., 2012). In *Neolamprologus pulcher*, a cooperatively breeding cichlid, early social environment has been found to directly affect gene expression of the stress axis and consequently the adult individuals' social behaviour (Taborsky et al., 2013). There are even cases where early social environment programs the juveniles for the predation regime they will encounter later on, as social interactions with conspecific adults in early life have been found to induce the development of anti-predator responses in guppies and sticklebacks (Chapman et al., 2008a; Jonsson & Jonsson, 2014). However, the effects of early environment on social learning propensities in fish have remained largely unexplored.

The Trinidadian guppy is a small tropical fish inhabiting streams and rivers in the Northern Mountain Range of Trinidad, but it is also commonly found in other Central and South American regions. Nowadays it has spread to most parts of the world as an introduced species for mosquito control or after release in the wild by aquarium owners. In the wild, guppies encounter a wide spectrum of predation threat. In low-predation habitats, they co-exist with the killifish Hart's Rivulus, *Rivulus hartii*, a gape-limited predator that occasionally preys on guppy juveniles, whereas in high-predation areas, they live among several predatory species including pike cichlids, *Crenicichla* spp., and wolf fish, *Hoplias malabaricus*, which cause high mortalities in adult guppies (Magurran, 2005; Seghers, 1974). These populations also differ in various demographic characteristics, such as sex ratio, age ratio and density (Rodd & Reznick, 1997). Because of these particular characteristics, guppy has been established study system in behavioural and evolutionary ecology for several decades.

Guppies are known to form social groups in the wild and more importantly guppy populations exhibit remarkable variation in their sociality. Seghers (1974) analysed the shoaling behaviour in different guppy populations, and linked the observed differences to differences between the populations' predation regimes. Guppies from high-predation sites typically show stronger grouping tendencies than guppies from low-predation sites (Magurran, 2005; Magurran & Seghers, 1991; Seghers, 1974) and this pattern is observable even in laboratory-reared fish (Magurran & Seghers, 1994a). These populations also differ in terms of productivity (Magurran, 2005) and resource availability may in turn affect sociality (Edenbrow et al., 2011), since grouping can increase foraging success (Krause & Ruxton, 2002). There are several factors that influence shoaling decisions and the formation of social networks in guppies, such as body size, sex ratio and the level of familiarity between individuals (Croft et al., 2003,2004; Magurran & Seghers, 1994c).

Being highly social animals, guppies frequently rely on social information to learn about their environment. Numerous empirical studies have demonstrated social learning in guppies in various contexts, such as social learning of foraging sites in the wild (Reader, Kendal, & Laland, 2003) and in the laboratory (Chapman et al., 2008b; Lachlan et al., 1998; Laland & Williams, 1997; Reader & Laland, 2000; Swaney et al., 2001), mate choice (Dugatkin & Godin, 1992,1993; Vukomanovic & Rodd, 2007), avoidance tasks and escape routes (Brown & Laland, 2002; Reader et al., 2003; Sugita, 1980), and anti-predator behaviour (Kelley et al., 2003). There are even cases where maladaptive information is socially transmitted between conspecifics (Laland & Williams, 1998). These well-established elements of social behaviour combined with the extensive background knowledge of its ecology and evolution, and the ease of breeding and raising large populations under specific rearing conditions in the laboratory, made the guppy an ideal study system for the present project.

Thesis Outline

In Chapter 2, I explore the consistency and heritability of shoaling and social learning in guppies by testing male and female guppies in similar but distinct shoaling and social learning tests and then testing their offspring after breeding randomly formed pairs. I provide evidence that shoaling tendency is consistent in males while it also appears to have a genetic component, exhibited as differences between sibling groups. In Chapter 3, by manipulating the reliability and utility of social information in the rearing environment and testing both immediately after the manipulation and after a period of common-garden rearing conditions, I demonstrate that guppy social learning propensities develop over time and are linked to exposure to reliable social cues early in life. In Chapter 4, by rearing subjects in standard or high stocking densities while exposing them to either predator or control cues, I show how early predation risk and density can influence the guppy behavioural phenotype using a series of standard tests to assess boldness and risk avoidance, exploration, grouping tendency and propensity to leave a shoal to forage. In Chapter 5, I examine how these developmental manipulations (predation risk and density) affect social information use and social learning, using a dichotomous shoal choice test with shoals providing differing cues regarding local predation danger. Finally, in Chapter 6, I summarise the most important results of the thesis and discuss the implications of my findings in relation to previous literature.

Chapter 2

Individual consistency and heritability of guppy social behaviour

Ioannis Leris and Simon M. Reader

(to be submitted to 'Journal of Fish Biology')

ABSTRACT

Social learning, learning from others, provides an efficient and relatively risk-free way to acquire information about the local environment. Social learning has been repeatedly demonstrated in several animal taxa, and social learning propensities have been found to correlate with grouping tendencies. However, although there is compelling evidence that grouping tendencies consistently differ between individuals and are heritable, relatively little evidence supports individual consistency and heritability in social learning propensities. In this study, we examined individual consistency and heritability of these two aspects of social behaviour using the Trinidadian guppy, *Poecilia reticulata*, a well-studied tropical fish easily bred and maintained in the laboratory, which has become an established study system in behavioural and evolutionary ecology. Fish were presented with two grouping tests and two social learning tests to investigate whether individual differences in grouping tendencies and social learning propensities were consistent across tasks. After testing, male and female fish were placed in breeding pairs at random. The newborn fry were collected, divided into small groups of siblings and reared in common-garden conditions until adulthood. Subsequently, they were individually tested with a shoaling and a novel social learning task. We estimated heritabilities by conducting regressions of offspring to parent performance and by examining sibling group effects. We found evidence for consistency in shoaling tendencies but not in social learning propensities. We also found evidence for a genetic component underlying shoaling differences, but the possibility of maternal effects cannot be ruled out. We discuss the implications of our results for the development and evolution of grouping and social learning tendencies.

INTRODUCTION

Social living conveys significant benefits for group members, particularly by means of enhanced defences against predators (such as the many-eyes effect, dilution of risk, predator confusion etc.), but also in the form of increased foraging efficiency and mating opportunities (reviewed by Krause & Ruxton, 2002). Another important advantage of group living is the swift transfer of information between individuals, both through active signals and publicly available information, with animals adjusting their behaviour after observing the behaviour of other individuals (Hoare & Krause, 2003). Minnows, *Phoxinus phoxinus*, for example change their foraging behaviour and initiate anti-predator responses after observing the behaviour of a conspecific exposed to a sham predator (Magurran & Higham, 1988). Social learning, learning facilitated by observing or interacting with other individuals (Heyes, 1994; Hoppitt & Laland, 2013), is widely employed by animals, allowing them to acquire information about their environment rapidly and economically, without having to pay the personal costs associated with individual learning. Social learning is often directly linked to social living in the literature and it has been proposed that social learning is an adaptive specialization to group living (e.g. Templeton et al., 1999), but there is limited empirical support for this hypothesis (Reader & Lefebvre, 2001). Moreover, there is increasing evidence that propensities to use social information and socially learn can emerge through simple associative learning during an individual's lifetime, rather than develop as adaptive specializations (Leadbeater, 2015).

Although the evolutionary link between social learning and group living is obscure, there are several examples, particularly in fish, where increased grouping tendencies were found to be correlated with increased reliance on social information. For example, guppies exhibiting high grouping tendencies were better at using demonstrator cues to locate food in a maze, which resulted in more efficient learning of the task (Chapman et al., 2008b), and Laland & Williams (1997), suggested that a general tendency to follow others rendered demonstrated foraging routes more preferable for guppy subjects and resulted in learning those routes, through a simple form of guided social learning. However, the social learning tasks in these studies involved some type of shoaling (the subject following the demonstrators to learn), thus an interesting question is whether grouping tendency can predict social learning that does not directly involve following others. There is also evidence that social learning propensities develop alongside grouping tendencies during an organism's lifetime. Takahashi et al. (2014) for example, demonstrated that the development of social learning propensities coincides with the appearance of schooling behaviour, in jack mackerel, *Trachurus japonicus*, juveniles.

Personality traits were first proposed to account for human behavioural variation. However, animals also often exhibit consistent individual differences in a variety of behavioural traits, and there is a growing body of evidence on the existence of animal temperaments or personalities, i.e. behavioural phenotypes that are consistent over time and across contexts and situations (Budaev, 1997; Réale et al., 2007). Individual variation in exploratory behaviour, for example, has been often shown to be consistent in several species (Dingemanse et al., 2002; Nomakuchi et al., 2009; Stöwe et al., 2006). Some of these behavioural traits appear to correlate with each other and manifest alongside in a stable and consistent way across situations, a concept described as 'behavioural syndromes' (Sih et al., 2004). Marchetti & Drent (2000) demonstrated that in great tits, *Parus major*, stable individual differences in exploratory behaviour can also predict their copying behaviour. In this paradigm, fast explorers significantly increased their visits to feeders demonstrated by tutors, while slow explorers did not alter their foraging behaviour. In three-spined sticklebacks, *Gasterosteus aculeatus*, increased exploratory behaviour in a novel environment was correlated with increased reliance on social information to solve a maze (Nomakuchi et al., 2009). There is limited evidence for individual consistency of social learning propensities and such examples typically arise from human studies. Toelch et al. (2014), for instance, tested a group of students in decision-making computer games, and demonstrated that although participants were flexible regarding when to use social information and the amount they invested in their strategy, they exhibited consistent individual differences in the way they valued and used social information. If grouping tendency is a stable behavioural trait of an organism's phenotype, we expect that social learning, a behaviour commonly employed by grouping individuals, will follow a similar pattern where individual variation in the propensity to use social information will remain consistent over time and across contexts, and increased grouping tendencies are predicted to correlate with increased social learning propensities.

There is also evidence that cross-context consistency in individual differences is genetically and epigenetically heritable (Dingemanse et al., 2002; Drent et al., 2003; van Oers et al., 2004). There are several examples of heritable traits in fish, including anti-predator responses in minnows (Magurran, 1990b), aggressive behaviour in sticklebacks (Bell, 2004), and foraging preferences (Karino et al., 2005) and aggression in guppies (Magurran & Seghers, 1991). Previous empirical work has shown that population variation in guppy shoaling behaviour has a heritable genetic component (Breden et al., 1986; Paxton, 1996; Seghers, 1974), but it is argued that these propensities, which have originally developed as a potent anti-predator defence, should fade over time in the absence of predation threat (Kelley & Magurran, 2003). Here we examine the heritability of shoaling behaviour disentangled from predation risk, by testing domesticated guppies born and raised in captivity for numerous generations. Although the heritability of fish grouping behaviour has been extensively studied, the potential transfer of social learning propensities across generations through genetic inheritance has not received similar attention. One of the few existing examples demonstrating potential heritability of social information use is the discovery of family-related differences in social foraging tactics in zebra finches, *Taeniopygia guttata* (Mathot & Giraldeau, 2010). In this study, the authors conducted full sibling comparisons regarding their propensity to join an occupied feeding well ('scrounger' tactic) instead of personally exploring novel feeders ('producer' tactic) and found that sibling groups differed significantly in their preferred foraging tactic. Since reliance on social cues can be consistent and is often correlated with other behavioural traits such as exploratory activity and boldness (Nomakuchi et al., 2009), which have previously been found to be heritable (e.g. Brown et al., 2007), we can hypothesise that an individual's propensity to socially acquire information will also have a genetic component.

In this study we tested domestic guppies in a series of shoaling and social learning tasks to establish whether any observed individual differences are stable across time and contexts, thus confirming the hypothesised existence of personality elements in these behavioural traits. We also bred these individuals, reared their offspring and then compared offspring performance with the average performance of their parents. Evidence for heritable components in shoaling behaviour would confirm previous findings in the literature, while demonstration of inherited social learning propensities would present novel evidence that social learning has inherent adaptive benefits by itself and is not simply a by-product of grouping tendency.

METHODS

Overview

We investigated whether individual differences in shoaling and social learning are consistent across time and contexts by testing male and female domestic guppies in similar but not identical shoaling and social learning tests, allowing an interval of at least 5 days between tests. We first conducted a social learning test, followed by a shoaling test, then a second social learning test and a second shoaling test. We looked for evidence of individual consistency by examining correlations of the various behavioural measures within and between tests. We randomly paired males and females, allowed them to mate and collected the offspring. We raised the fry in common-garden conditions for 5-6 months and then tested them with a new social learning test and a shoaling test. Finally, we compared the performance of the offspring with the performance of their parents and examined sibling group effects in several behavioural measures to examine if these behaviours have a genetic component.

Individual Consistency Study

Subjects and Standard Housing Conditions

We used 40 female and 20 male domestic guppies as subjects. Eleven female and one male fish were removed during the experiment due to poor health, leaving a final sample of 29 females and 19 males. These subjects originated from a mixed strain population of approximately 1400 fish, first established in 2003 within the Utrecht University Biology aquarium and based on a founder population of approximately 480 guppies purchased from two commercial suppliers (Ruinemans, Montfoort and Ruisbroek, Maassluis, both The Netherlands). The subjects were kept in groups of 5 or 6 fish in separate compartments (in order to be able to discriminate them by size and colour patterns without invasive marking procedures) in three 70-l rectangular glass tanks (90 cm x 40 cm x 25 cm, water depth: 20 cm). The tanks were equipped with thermostat heaters (Jaeger, Germany), external canister filters (Ecco Pro 200, Eheim, Germany), airline and air-stones for aeration, plastic plants and pieces of ceramic pots for shelter and gravel as substrate. Temperature was regulated at 26.5 ± 0.5 °C, while the photoperiod was set at 12 h light/12 h dark, with lights on at 07:00. We fed the fish in the morning between 09:00 and 10:00 and in the afternoon between 16:30 and 17:30 with commercial flake food (TetraMin and TetraPhyll, Tetra, Germany). To ensure optimal water conditions, we measured nitrites, nitrates, ammonia and pH weekly, and conductivity and oxygen concentration biweekly. Every 15 days we replaced 30% of the tank water with fresh warmed dechlorinated copper-free water and tanks were cleaned more thoroughly if necessary. On training or testing days fish were not fed until 30 minutes after all training and testing was completed for that day.

Demonstrators

We used 42 female guppies as demonstrators for the social learning tests and 27 female guppies as stimulus fish for the shoaling tests. We used large rectangular glass tank (160 cm x 40 cm x 20 cm, water depth: 18 cm) that was divided in half (crosswise) using an opaque PVC partition. Demonstrators were housed in standard conditions in one of these two compartments while the first social learning test was conducted in the other compartment, thus minimising demonstrators' stress from transferring between tanks. Stimulus shoal fish were housed in standard conditions in a 70-l tank (90 cm x 40 cm, water depth: 20 cm). Demonstrators were trained one or two times daily for up to two months before the social learning tests, in order to consistently perform the required tasks successfully. Stimulus shoal fish were also habituated to being caught by a net and placed inside transparent containers twice daily, for at least 3 days prior to the shoaling tests.

Social Learning Test 1 (SL1)

Social learning test 1 was conducted in an 80 cm x 40 cm compartment separated from a larger tank (160 cm x 40 cm x 20 cm, water depth: 18 cm) with an opaque PVC divider (Fig. 1A). The subject was introduced inside a transparent plastic cylinder that was attached to a string. Two transparent plastic containers (12.3 cm x 8.5 cm x 20 cm) at one end of the tank and were separated by a transparent divider, thus forming two choice areas. One of the containers held a demonstrator and the other a sham demonstrator, which had been selected at random from their housing tanks. A feeding tube was attached on one of the walls of each container, designed to allow the demonstrator to see any food item dropping through it, but not the subject. The rear side of the two demonstrator containers was covered with green or blue plastic sheets to aid visual discrimination by the subject.

The lower front side was also covered with a piece of identically-coloured plastic sheet, to hide the food item at the end of each demonstration bout, so that only the demonstrators feeding behaviour was visible. Our intention was that subject's choice would be based only on the demonstrator's feeding behaviour and not on viewing the reward. This approach is commonly found in previous studies measuring public information use, where the focal fish is restricted from individually sampling the feeders and the food items and is thus expected to make a choice based on its conspecifics' feeding rate (e.g. Webster & Laland, 2011). After a short habituation period of 2 min, we added a 2- to 3-mm piece of frozen bloodworm that had been thawed in tank water in the demonstrator's feeding tube using a plastic pipette. For a successful demonstration, the demonstrator was expected to peck at the tube as the bloodworm piece slowly sunk to the bottom end of the tube where it could be eaten. At the same time, water was added to the feeding tube of the other container, which did not elicit any observable response from the sham demonstrator. After 1 min, another bloodworm piece and water were added in the feeding tubes, and the procedure was repeated for a total of 5 bouts. Immediately after the 5 demonstrations, the subject's view was blocked by an opaque PVC partition for a 5-min time interval. During this period we returned the demonstrators to their housing tanks, emptied and replaced the containers in their original locations and released the subject in its holding compartment by pulling the string and lifting the cylinder. After the end of the time interval, the opaque partition was removed and the subject was released and its behaviour was scored for 5 min. Demonstrated location (left or right) and feeder colour were alternated in a pseudorandom way. Between trials we replaced the container water and mixed the water in the tank.

Shoaling Test 1 (S1)

The first shoaling test was a modified version of previously established paradigms for measuring sociability and grouping tendency (e.g. Chapman et al., 2010; Paxton, 1996). It was conducted in a rectangular glass tank (90 cm x 40 cm x 25 cm, water depth: 10 cm) with a line drawn on the bottom dividing it in two compartments, left and right (Fig. 1B). The subject was placed in the centre of the tank, between two transparent PVC partitions attached to strings to minimise disturbance during release. Two transparent plastic containers were present at each end of the tank, one holding a shoal of 4 stimulus fish and the other one being empty. The shoal was introduced in the tank 2 min before the subject. A 6-cm shoaling zone (equivalent to approximately 2 guppy body lengths – see Chapman et al., 2008b) was drawn around each container for scoring purposes. After a time interval of 3 min that allowed the subject to habituate while maintaining visual contact with the shoal, the two transparent partitions were lifted by pulling the strings and the subject was allowed to choose between the two containers. The test lasted 5 min. Shoal position (left or right) was counterbalanced between trials and the water in the shoal containers was changed before every trial. Every morning we replaced approximately 10 litres of water with fresh copper- and chlorine-free water of the same temperature and the tank water was mixed between trials to avoid any accumulation of olfactory cues.

Social Learning Test 2 (SL2)

For the second social learning test, we used a small rectangular glass tank (40 cm x 25 cm x 25 cm, water depth: 10 cm) that was divided in 4 compartments with transparent PVC partitions (Fig. 1C). The tank was filled every morning before the first trial using water from the demonstrator tank to equalise any possible olfactory cues that were present. The subject was transferred to the tank inside a transparent plastic cup and placed inside an observation compartment behind a removable transparent divider attached to a string. From there, the subject had visual contact with the other 3 compartments. After 2 min of habituation, a group of 4 demonstrators (caught at random from their housing tank by a net) was placed in the other central compartment of the tank (henceforth 'demonstrator compartment') inside a transparent plastic cup, and the subject was released in its holding compartment by slowly tilting the cup. At this point, crushed flake food (TetraMin, Tetra, Germany) was added to two floating feeders in the two choice compartments. The feeders were floating rings (5.5 cm in diameter) of plastic airline tubing, which confined the flake food in a small area without it spreading across the water surface. A minute later, we released the demonstrators by tilting their cup and the demonstration lasted another 2 min (3 min in total). From the demonstrator compartment the fish had access to the outer choice compartments by swimming under clear PVC transparent partitions. These partitions were covered with pieces of coloured plastic tape (left: green stripes, right: blue squares) to make them visually distinctive and were suspended 2 cm above the bottom of the tank, allowing fish to swim under them. The demonstrators were trained to enter either the left or the right compartment within 1 min from their release, and feed from floating feeders. The demonstration ended with the placement of 3 opaque partitions around the observation compartment, thus blocking the view of the subject, for a period of 5 min. During this period, we removed the demonstrators and returned them to their housing tank and added fresh food to both feeders ensuring they have approximately the same amount. The subject was then released by lifting the transparent barrier and its behaviour was scored for 5 minutes. After each trial the tank was cleaned, any uneaten food was removed and a portion of the water was replaced with water from the demonstrators' housing tank. This test is identical to the social learning test described in Chapter 3.

Shoaling Test 2 (S2)

The second shoaling test was also conducted in a rectangular glass tank (90 cm x 40 cm x 25 cm, water depth: 10 cm) with two main compartments, left and right, defined by a line on the bottom (Fig. 1D). The subject was introduced in the tank inside a transparent cylinder attached to a string. The subject's cylinder was located near the wall closest to the observer and equidistant from two transparent cylindrical plastic containers, one of which held a group of 6 shoal fish and the other one being empty. A circular 6-cm shoaling zone was drawn around the cylindrical containers to assist with the scoring of shoaling behaviour. At the beginning of each trial, the cylindrical containers were placed in the tank and the fish allowed to acclimatise for 2 min. We then introduced the subject in its holding cylinder and let it habituate for 2 min. The cylinder was lifted by pulling the string and the behaviour of the subject was scored for the next 5 min (part A). Afterwards, we placed the subject in the transparent cylinder and returned it to its original position where it remained for 2 min. During this period, we simultaneously swapped the transparent containers so that the shoal was now at the previous location of the empty container and vice versa. The shoal container was always moved closest to the subject (while the empty one was moved further back), thus allowing the subject to maintain visual contact with the shoal. We then released the subject again by lifting the transparent container and scored its behaviour for another 5 min (part B). Every morning we replaced approximately 10 litres of tank water with fresh copper- and chlorine-free water of the same temperature. Between trials we mixed the tank water and replaced portion of the water inside the cylindrical containers to avoid the accumulation of olfactory cues.

Heritability Study

Pairing, Fry Collection and Rearing

After all testing for individual consistency ended, we randomly selected 20 female subjects and paired them at random with the 20 male subjects. Female guppies are known to store sperm for long periods and can produce fry in the absence of males (e.g. López-Sepulcre et al., 2013; Winge, 1937), but because of deterioration of stored sperm's quality they will preferentially use the fresh sperm of a new mate (Winge, 1937). In our study, the female fish were not virgins, but had been housed in single-sex groups for a period of at least 9 months, and had not given birth to fry for at least 3 months before being paired, to ensure that any sperm reserves were previously depleted. The mating pairs were placed in commercial breeding nets (Breeding Container, Sera GmbH, Germany) inside two

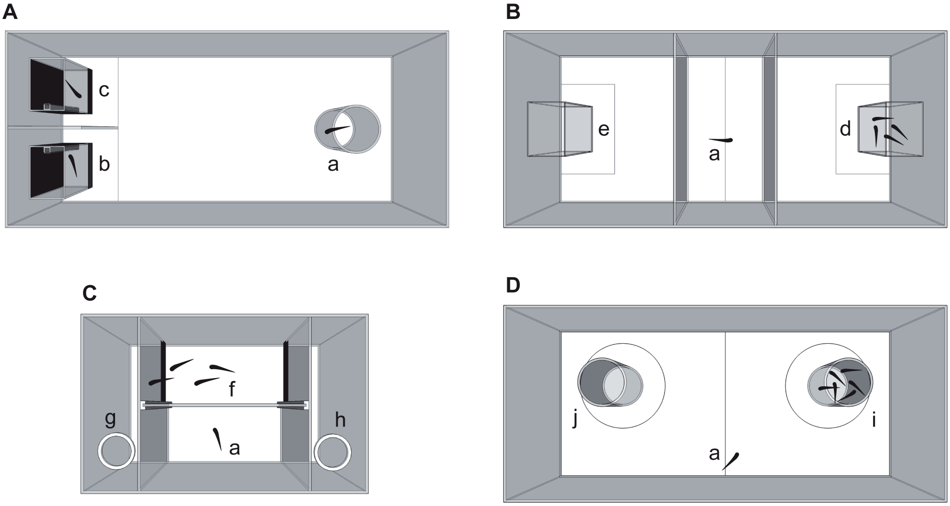


Figure 1. Top views of the behavioural tests presented to the parental generation. A: Social learning test 1 (SL1); B: Shoaling test 1 (S1); C: Social learning test 2 (SL2); D: Shoaling test 2 (S2). Labels: a: Subject; b and c: containers with a feeding tube, holding a demonstrator and a sham demonstrator; d: container holding a group of 4 stimulus shoal fish; e: empty container; f: group of 4 demonstrators in the demonstrator compartment; g and h: floating feeder rings in the outer choice compartments; i: container holding a group of 6 stimulus shoal fish; j: empty container.

large rectangular glass tanks (120 cm x 40 cm x 40 cm) equipped with thermostat heaters (Jaeger, Germany) and external canister filters (Professionel 3 250T, Eheim, Germany) (10 breeding nets per tank). Plastic plants, pieces of ceramic pots and gravel were used as enrichment in each tank. Immediately after their transfer to the breeding nets, the males started courting and performing mating attempts. We allowed the pair to mate for 48 hours and then removed the males and returned them to their original housing tank. The females remained in the breeding nets until they gave birth (approximately 1-1.5 months later). A small piece of filter floss (Biofibres, Sera GmbH, Germany) attached to a pebble was placed in the bottom of each breeding net to provide shelter for newborn fry.

We examined the breeding nets daily for newborn fry and transferred any to pre-established rearing tanks. Once they had given birth, the females were returned to their original housing tanks. The rearing tanks were rectangular glass tanks (90 cm x 40 cm x 25 cm) divided in 5 or 6 compartments depending on the number of fry they would hold. The fry batches ranged from 2 to 25, thus sibling groups larger than 6 individuals were split into smaller groups (henceforth termed 'rearing groups') of 5-6 individuals in size. Each compartment was equipped with a thermostat heater and an air-driven sponge filter to maintain water quality, and plastic plants, ceramic pot pieces and gravel for enrichment. The fish were reared under standard conditions for at least 5 months, before being tested in a social learning and a shoaling task as described below. This ensured that the offspring were tested well after they had reached maturity, allowing comparison of their performance with the performance of their parents.

Social Learning Test 3 (SL3)

A preliminary analysis of the data from SL1 and SL2 revealed that our subjects did not show a significant preference the demonstrated feeding compartments as we had expected, thus we used new test to measure the social learning propensities of the offspring. This test was a modified version of SL2 that allowed the subject to feed from a feeder alongside the demonstrators during the demonstration phase. It was conducted in a rectangular glass tank (90 cm x 40 cm x 25 cm, water depth: 15 cm) divided longitudinally in half with a fixed transparent PVC partition, forming a 'subject area' and a 'demonstrator area' (Fig. 2). The subject was introduced in the central compartment of the 'subject area' ('observation compartment'), which was separated from the two outer compartments with removable transparent PVC partitions. The two outer compartments of that area were the choice compartments of the test with coloured floating feeders (green on the left and blue on the right side) attached to both sides of the central partition. The 'demonstrator area' was divided in two compartments (left and right) with a transparent PVC barrier. At the beginning of each trial, the subject was introduced to the tank by tilting a transparent plastic cup in the 'observation compartment' and allowed to habituate for 2 min. Subsequently, we placed a group of 5 demonstrators inside a transparent plastic container and in one of the two demonstration compartments (left with green feeders or right with blue feeders) depending on the trial order, and let them acclimatise for 1 min. The demonstration location was counterbalanced in a pseudo-random way. At this point, crushed flake food (TetraMin, Tetra, Germany) was added in all feeders. We then released the demonstrators by tilting their container and they were free approach and feed from the feeder in their compartment. Ten seconds later, we lifted the removable partitions in the subject area and allowed the subject to approach the demonstrated feeder and feed near the demonstrators. The demonstration lasted 3 min in total. After this period, the subject was shepherded back into the observation compartment using the back of a net and opaque barriers were lowered to block its view for a 6-min time interval. During this period the demonstrators were caught with a net and returned to their housing tanks, leftover food was removed with a fine siphon, water was mixed between the two choice compartments to avoid olfactory

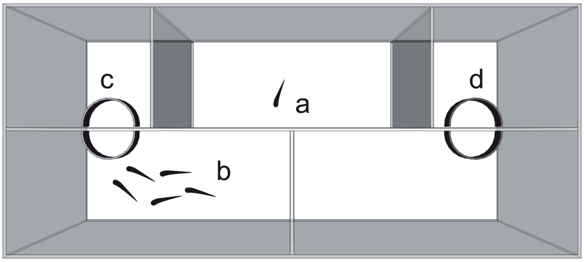


Figure 2. Social learning test 3 (SL3), top-view of the testing tank. Labels: a: subject; b: group of demonstrators; c and d: feeders.

cue accumulation and new food was added to both feeders in the 'subject area'. Finally, we released the subject by lifting all partitions, thus allowing it to choose compartments and feed from the feeders. The test lasted 5 min. Between trials we removed any uneaten food and mixed the water in the tank. Every morning before the first test, the tank was filled with water from the demonstrators' tank to control for olfactory cues present in the tank.

Shoaling Test 3 (S3)

This test was identical to the two-part shoaling test 2 (S2) described in the previous section (Fig. 1D).

Behavioural Measurements

In all tests we measured time spent moving and motionless, time spent in each tank compartment i.e. left, right and middle (if present), dashing events (rapid darting movements) and events of 'repetitive' behaviour (swimming back and forth against the tank wall). In the social learning tests we measured latency to each choice compartment, time spent in each choice compartment and time interacting with each feeder container (SL1) or time feeding from each feeder (SL2 and SL3). In the shoaling tests we measured latency to enter each shoaling zone, time spent shoaling or near the empty container (i.e. swimming within the 6-cm shoaling zone) and time interacting with each container (touching the container walls).

Data Collection and Analysis

All trials were recorded using a high resolution camera (QuickCam Pro 9000, Logitech, USA) suspended above the testing tanks. We scored behaviour live or by video (in cases where live scoring was not possible), using JWatcher (v1.0, Blumstein et al., 2006). We used relative measures to establish subject preferences. For S1 and both parts of S2 (A and B), we analysed 'time shoaling', defined as time spent near the shoal minus time spent near the empty container, 'time interacting', defined as time spent interacting with (touching the walls of) the shoal container minus time spent interacting with the empty container, and 'latency to shoal', defined as latency to the empty container minus latency to the shoal container. We also analysed the subject's 'shoaling preference', defined as time spent within the shoaling zone of the actual shoal divided by the total time spent in both shoaling zones. For SL1 and 2, we analysed 'time in the demonstrated area', defined as time spent in the demonstrated compartment minus the time spent in the opposite compartment, and 'latency to the demonstrated area', defined as latency to the non-demonstrated compartment minus the latency to the demonstrated compartment. For SL1 we also analysed 'time interacting with feeder', defined as time interacting with the feeder container previously holding a demonstrator minus the time interacting with the feeder container previously holding the sham demonstrator. In SL2 few fish fed from the feeders, therefore measures involving 'time spent feeding' were not further analysed. We compared the mean values with theoretical values predicted by chance using Student's *t*-tests or Wilcoxon signed ranks tests (on medians) if needed, to assess the validity of our tests. We then examined individual consistency in social learning by conducting non-parametric correlations (Spearman's ρ) between behavioural measures both within and between tests and compiling them into correlation matrices. For logistical reasons, male and female subjects were tested at different time points and thus they were analysed separately.

We estimated heritabilities from the regression of offspring performance on the average performance of the parents. Additionally we examined sibling group effects with factorial ANOVAs on the main behavioural measures, 'time shoaling' and 'time in the demonstrated area' with 'sex', 'sibling group', 'rearing group' and 'shoal location' as fixed factors. 'Shoal location' and 'rearing group' were removed from the analysis, if they had no statistically significant effect ($P > 0.1$), to increase power. Normality was assessed with Shapiro-Wilk's test and homoscedasticity with Levene's test. If these failed, data were square root transformed. All statistical analyses were performed in SPSS (v20, IBM, USA).

Ethical Note

All experimental procedures were approved by the Utrecht Ethics and Animal Care and Use Committee (DEC) under protocol number 2011.I.05.050, and conform to ASAB guidelines and Dutch law. At the end of the study all fish were returned to breeding populations at Utrecht University.

RESULTS

Individual Consistency Study

Shoaling Tests

Both male and female subjects showed a clear preference for the shoal over the empty container in both tests and in both parts of the S2 shoaling test (Table I).

Table I. Shoaling tests' results for males and females.

Sex	Test	Behavioural measure	Statistic	Comparator	*P*
Male	S1	Time shoaling	$t_{19} = 14.37$	0	< 0.001
		Time interacting	$t_{19} = 13.08$	0	< 0.001
		Latency to shoal	$W_{n=20} = 153$	0	0.073
		Shoaling preference	$W_{n=20} = 210$	0.5	< 0.001
	S2A	Time shoaling	$t_{18} = 8.52$	0	< 0.001
		Time interacting	$t_{18} = 6.55$	0	< 0.001
		Latency to shoal	$W_{n=19} = 165$	0	0.005
		Shoaling preference	$W_{n=19} = 187$	0.5	< 0.001
	S2B	Time shoaling	$t_{18} = 7.92$	0	< 0.001
		Time interacting	$t_{18} = 6.21$	0	< 0.001
		Latency to shoal	$W_{n=19} = 178$	0	0.001
		Shoaling preference	$W_{n=19} = 189$	0.5	< 0.001
Female	S1	Time shoaling	$W_{n=37} = 703$	0	< 0.001
		Time interacting	$t_{36} = 14.1$	0	< 0.001
		Latency to shoal	$W_{n=37} = 675$	0	< 0.001
		Shoaling preference	$W_{n=37} = 703$	0.5	< 0.001
	S2A	Time shoaling	$W_{n=29} = 415$	0	< 0.001
		Time interacting	$t_{28} = 5.98$	0	< 0.001
		Latency to shoal	$W_{n=29} = 336$	0	0.01
		Shoaling preference	$W_{n=29} = 384$	0.5	< 0.001
	S2B	Time shoaling	$t_{28} = 10$	0	< 0.001
		Time interacting	$t_{28} = 7.37$	0	< 0.001
		Latency to shoal	$W_{n=29} = 380$	0	< 0.001
		Shoaling preference	$W_{n=28} = 406$	0.5	< 0.001

Social Learning Tests

We found no group-level evidence for social learning in either males or females. That is, no significant preference for the demonstrated locations was observed, since the means of all measured variables did not significantly differ from the values predicted by chance in both SL1 and SL2 (Fig.3; Table II). However, test performance was quite variable, and thus we analysed correlations between per-

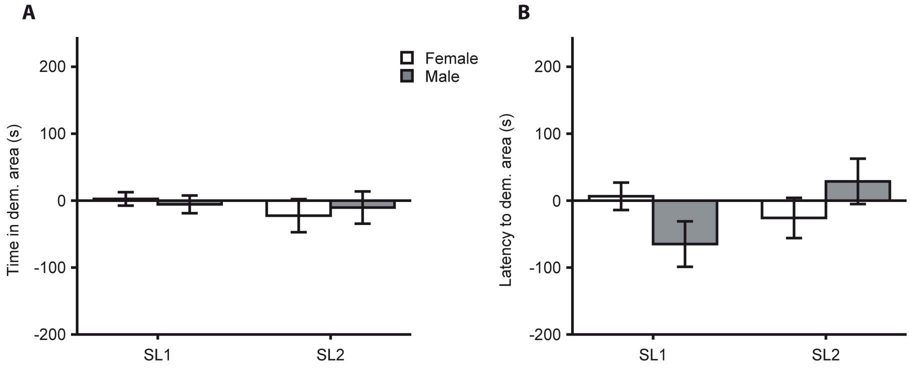
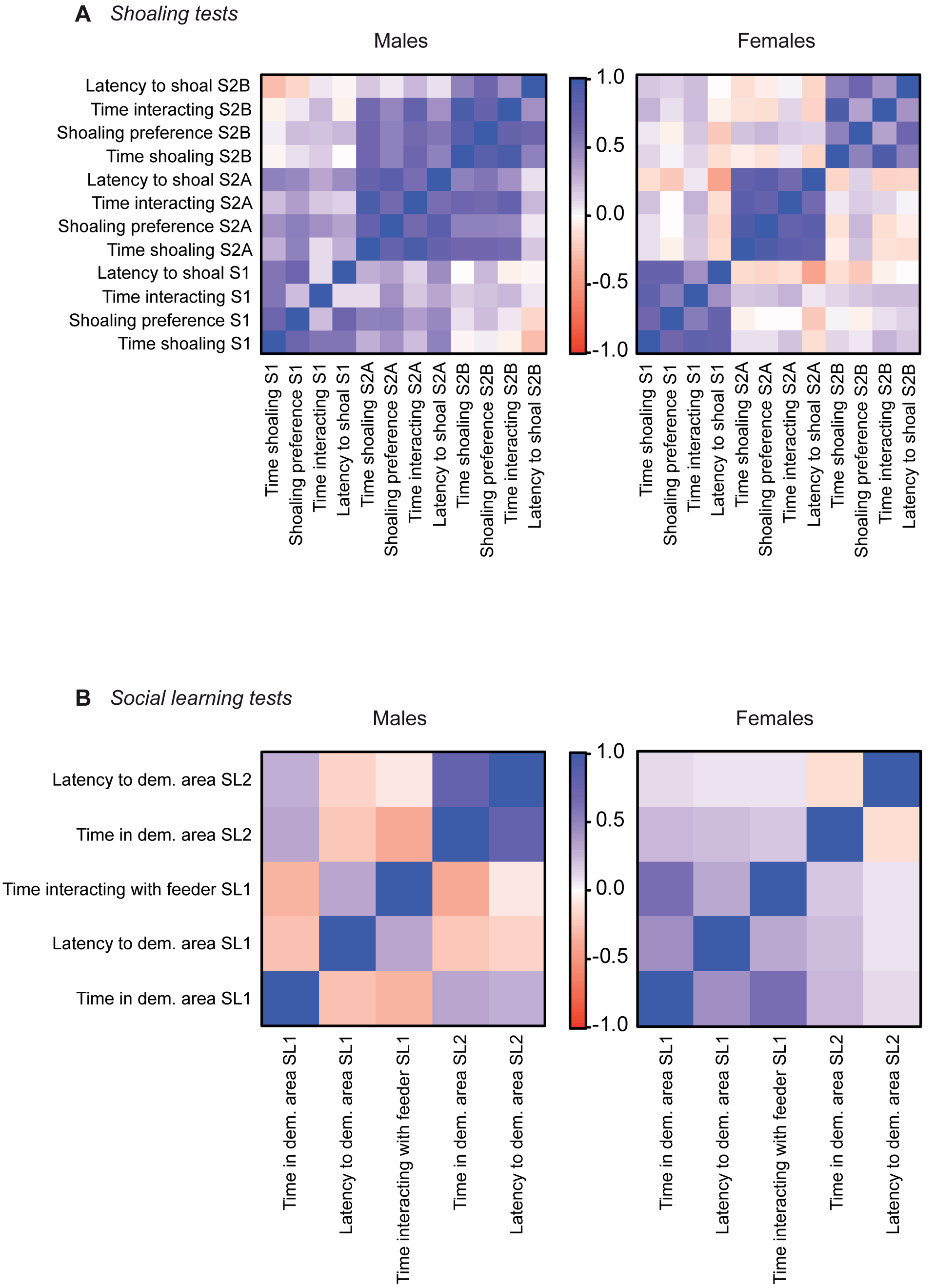


Figure 3. Social learning tests' results for males and females in two social learning tests (SL1 and SL2). A: Time in the demonstrated area; B: Latency to the demonstrated area. Bars represent the standard error of the mean.

Table II. Social learning tests' results for males and females.

Sex	Test	Behavioural measure	Statistic	Comparator	*P*
Male	SL1	Time in dem. area	$W_{n=20} = 55$	0	0.18
		Time interacting	$W_{n=20} = 33$	0	0.64
		Latency to dem. area	$t_{19} = 1.91$	0	0.07
	SL2	Time in dem. area	$t_{18} = 0.43$	0	0.67
		Latency to dem. area	$t_{18} = 0.85$	0	0.41
Female	SL1	Time in dem. area	$t_{39} = 0.25$	0	0.80
		Time interacting	$W_{n=40} = 226$	0	0.85
		Latency to dem. area	$t_{39} = 0.31$	0	0.755
	SL2	Time in dem. area	$t_{31} = 0.91$	0	0.37
		Latency to dem. area	$t_{31} = 0.86$	0	0.40

formance on the social learning tests and between the social learning and shoaling tests (see below).


A Shoaling tests
Males
Females
Latency to shoal S2B
Time interacting S2B
Shoaling preference S2B
Time shoaling S2B
Latency to shoal S2A
Time interacting S2A
Shoaling preference S2A
Time shoaling S2A
Latency to shoal S1
Time interacting S1
Shoaling preference S1
Time shoaling S1
1.0
0.5
0.0
-0.5
-1.0
Time shoaling S1
Shoaling preference S1
Time interacting S1
Latency to shoal S1
Time shoaling S2A
Shoaling preference S2A
Time interacting S2A
Latency to shoal S2A
Time shoaling S2B
Shoaling preference S2B
Time interacting S2B
Latency to shoal S2B
B Social learning tests
Males
Females
Latency to dem. area SL2
Time in dem. area SL2
Time interacting with feeder SL1
Latency to dem. area SL1
Time in dem. area SL1
1.0
0.5
0.0
-0.5
-1.0
Time in dem. area SL1
Latency to dem. area SL1
Time interacting with feeder SL1
Time in dem. area SL2
Latency to dem. area SL2

Behavioural Correlations

In males there were several significant correlations both within and between the shoaling tests (Fig. 4A). Most of the behavioural measures within each test were significantly correlated, as predicted given that these variables were measured simultaneously and thus are somewhat dependent on one another, and are not presented here for brevity. There were several significant correlations between the two parts of S2 (S2A and S2B), providing evidence for short-term consistency in shoaling behaviour. 'Time shoaling S2A' was significantly correlated with 'time shoaling S2B' (Spearman's $\rho_{n=19} = 0.69$, $P < 0.01$), 'time interacting S2B' (Spearman's $\rho_{n=19} = 0.68$, $P < 0.01$), and 'shoaling preference S2B' (Spearman's $\rho_{n=19} = 0.70$, $P < 0.01$). 'Time interacting S2A' was significantly correlated with 'time shoaling S2B' (Spearman's $\rho_{n=19} = 0.72$, $P < 0.01$) and 'shoaling preference S2B' (Spearman's $\rho_{n=19} = 0.67$, $P < 0.01$). 'Shoaling preference S2A' was significantly correlated with 'time shoaling S2B' (Spearman's $\rho_{n=19} = 0.50$, $P < 0.05$), 'time interacting S2B' (Spearman's $\rho_{n=19} = 0.475$, $P < 0.05$), and 'shoaling preference S2B' (Spearman's $\rho_{n=19} = 0.515$, $P < 0.05$). Finally 'latency S2A' was significantly correlated with 'time shoaling S2B' (Spearman's $\rho_{n=19} = 0.50$, $P < 0.05$) and 'shoaling preference S2B' (Spearman's $\rho_{n=19} = 0.59$, $P < 0.01$). More importantly, we found evidence for long-term consistency in shoaling behaviour with correlations between S1 and S2A. 'Shoaling preference S1' was significantly correlated with 'shoaling preference S2A' (Spearman's $\rho_{n=19} = 0.53$, $P < 0.05$), 'latency S2A' (Spearman's $\rho_{n=19} = 0.47$, $P < 0.05$) and 'time shoaling S2A' (Spearman's $\rho_{n=19} = 0.50$, $P < 0.05$). In addition 'time shoaling S1' was significantly correlated with 'latency S2A' (Spearman's $\rho_{n=19} = 0.51$, $P < 0.05$). All other correlations were not statistically significant and for brevity are not presented here.

In contrast to males, females showed no evidence for short-term individual consistency between the two parts of S2 test, or for long-term consistency between S1 and S2 tests (Fig. 4A). Although the majority of variables within each test were correlated as expected, there was only one significant correlation between tests, between 'latency S1' and 'latency S2A', and the relation is negative (Spearman's $\rho_{n=29} = -0.42$, $P < 0.05$). Thus, this relationship does not provide any evidence for consistent shoaling behaviour in the females. All other correlations were not statistically significant and for brevity are not presented.

We found no evidence for consistency in the social learning tests of male

Figure 4. Correlation matrices (Spearman's ρ) of the main behavioural measures taken within and between the two shoaling tests (A) and the two social learning tests (B) for male and female subjects. The calculated Spearman's ρ value for each correlation is illustrated as a colour in a blue-white-red spectrum (see figure insert), with blue representing positive values close to 1, white values near 0 and red negative values near -1.

individuals, as there was only one significant correlation within SL2 (Fig. 4B). In the case of females, we found one significant correlation within SL1 but no significant correlations between tests that would indicate consistency in social learning propensities (Fig. 4B).

Regarding correlations between shoaling and social learning tests, there was only one significant correlation between 'interaction time S1' and 'latency to dem. area SL2' (Spearman's $\rho_{n=32} = 0.37, P < 0.05$) in the case of females. In males, 'interaction time S1' was significantly correlated with both 'time in dem. area SL1' (Spearman's $\rho_{n=20} = 0.55$, $P < 0.05$) and 'time in dem. area SL2' (Spearman's $\rho_{n=19} = 0.58$, $P < 0.01$).

Heritability Study

A total of 76 fry were born to 6 of the 20 mothers, with brood sizes of between 2 and 25 fry (mean ± SE = 12.67 ± 4.51). The small number of mothers breeding compromised our power to be able to examine parent-offspring correlations.

The subjects exhibited a significant preference for the shoal in both parts of the shoaling test (S3A and S3B) since mean 'time shoaling S3' differed significantly from 0 (*t*-tests: A, $t_{75} = 16.56$, $P < 0.001$; B, $t_{75} = 13.07$, $P < 0.001$). This measure was significantly correlated between the two parts of the test (Spearman's $\rho_{n=76} = 0.58$, $P < 0.001$) providing further evidence for short-term consistency in grouping tendency. When the sexes were analysed separately, this correlation was significant only in the case of males (Spearman's $\rho_{n=47} = 0.69$, $P < 0.001$; females: Spearman's $\rho_{n=29} = 0.35$, $P = 0.063$). The subjects showed no preference for the demonstrated area in the social learning test (SL3), neither in terms of 'time in the demonstrated area SL3' (*t*-test: $t_{68} = 0.88$, $P > 0.1$)' nor in 'latency SL3' (*t*-test: $t_{68} = 1.7$, $P > 0.1$). Again, there were considerable individual differences in performance on the social learning test.

We estimated heritabilities for shoaling tendency and social learning propensities by conducting linear regressions of the offspring performance on the average performance of their parents for our two main behavioural variables of our shoaling and social learning tests, i.e. 'time shoaling' (Fig. 5A) and 'time in the demonstrated area' (Fig. 4B). We calculated offspring scores by taking each sibling group's average 'time shoaling S3' (average of 'time shoaling S3A' and 'time shoaling S3B') and 'time in the demonstrated area SL3'. We calculated midparent scores (average scores of the two parents) by taking the average 'time shoaling' from S1 and S2, and 'time in the demonstrated area' from SL1 and SL2. We found no significant linear relationship in either case (linear regressions: $r^2 = 0.05$, $P > 0.1$ and $r^2 = 0.22$, $P > 0.1$ respectively).

In 'time shoaling S3' there was a significant effect of 'sex', with females spending more time shoaling than males (ANOVA: $F_{1,65} = 4.58$, $P < 0.05$). Moreover, there

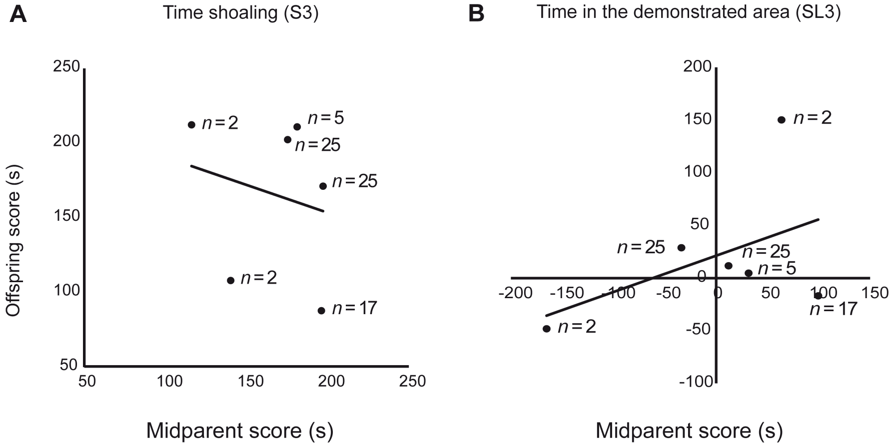


Figure 5. Heritability regressions of offspring-midparent performance for 'time spent shoaling' in S3 (A) and 'time in the demonstrated area' in SL3 (B). Relationships were not significant but trend lines are provided for illustration.

was a significant effect of 'sibling group' (ANOVA: $F_{5,65} = 4.02$, $P < 0.01$). Thus families of siblings differed significantly from each other in their shoaling tendency. We found no significant effects of 'sex' (ANOVA: $F_{1,58} < 0.001$, $P > 0.1$) or 'sibling group' (ANOVA: $F_{5,58} = 0.75$, $P > 0.1$) on 'time in the demonstrated area SL3'. Similarly, in 'latency SL3' we found no significant effects of 'sex' (ANOVA: $F_{1,58} = 0.43$, $P > 0.1$) or 'sibling group' (ANOVA: $F_{5,58} = 0.42$, $P > 0.1$).

DISCUSSION

Subjects showed high grouping tendencies in all shoaling tests, as expected, with males and females in both the F_0 and F_1 fish significantly preferring a group of female guppies over an empty container, in a novel test environment. Males, but not females, showed evidence for individual consistency in shoaling tendencies. We also found evidence that social learning performance was correlated with grouping tendency. Moreover, we found evidence, by comparing F_1 sibling groups, for heritability in shoaling tendencies. However, we found no group-level evidence for social learning in any of our three tests, although individual performance was quite variable. Social learning performance was not correlated across social learning tests, and there was no evidence for heritable differences in social learning.

The clear shoaling preference remained consistent regardless of the size of the shoal (4 or 6 individuals) and despite changing the shoal location during the test. This observation is in line with results from previous empirical studies which have demonstrated high grouping tendencies in guppies both in the field and in the laboratory (Chapman et al., 2008b; Day et al., 2001; Jones et al., 2010; Lachlan et al., 1998; Magurran & Seghers, 1990a; Seghers, 1974; Song et al., 2011). We found evidence for consistency in male guppy shoaling behaviour. Consistent grouping tendency was found both at longer time scales, after an approximately 1-month interval between S1 and S2, and shorter time scales, after a 2-min interval between S2A and S2B (as well as S3A and S3B). Individual consistency in guppies, and particularly male individuals, has been previously reported in exploratory and mating behaviour (Harris et al., 2010; Magellan & Magurran, 2007). Our results are in line with previous findings demonstrating consistent shoaling tendency in male guppies (Budaev, 1997) and further support the existing idea that propensity to join groups can be an individual characteristic. We however present evidence for the existence of sex differences in this consistency. Females have been previously reported to be more consistent than males in a boldness-related task (Harris et al., 2010) and they have also been found to make mate choices with high repeatability (Kodric-Brown & Nicoletto, 1997) but to our knowledge, no previous study has examined female guppies' shoaling consistency. In our case, males appeared more consistent in their shoaling tendency than females, which showed no evidence for consistency. Males and females did not differ in absolute time spent near the shoals but males were faster to approach the shoal in SL1. We cannot exclude the possibility that the observed shoaling tendency of males was due to increased motivation to mate, since the stimulus shoals consisted of only female individuals. We intentionally used all-female shoals, since they are considered more appealing to both sexes regarding group joining decisions. Previous experimental work on poeciliid fishes revealed that males prefer to shoal with female or female-biased shoals to increase their mating opportunities (Agrillo et al., 2008) and females prefer the company of same-sex conspecifics to dilute the persistent mating efforts exerted by males (Agrillo et al., 2006). In guppies, similar patterns of harassment towards females are reported (Magurran & Seghers, 1994c). Shoals with female-biased sex ratios are more common in wild populations (Seghers, 1973) and male individuals have been reported to actively search for shoals with higher numbers of receptive females (Croft et al., 2003; Lindström & Ranta, 1993). In the present study, male subjects were housed separately from females for several weeks prior to the shoaling tests, which could further increase their mating motivation, and during the tests, we frequently observed courtship behaviour (sigmoid displays) towards the shoal members. Therefore the observed consistency in grouping behaviour in male fish could reflect consistent individual differences in the motivation to seek possible mates.

Social learning regarding foraging decisions is a well-described phenomenon in the guppy study system. For example Reader et al. (2003) found that guppies significantly preferred demonstrated feeders over non-demonstrated ones in the field, and Laland & Williams (1997) reported that guppies learned novel foraging routes by following informed demonstrators. However, our tests may have suffered from a floor effect. To reveal individual differences, it is necessary to have social learning tests sufficiently difficult to tease apart individual performance (compared to the aforementioned tests that had the aim of establishing whether social learning occurred). It is possible that our tests were too complicated or insufficiently motivating for the subjects. Fish are known to follow certain strategies about when to socially learn and who to learn from (Boyd & Richerson, 1985; Laland, 2004; Rendell et al., 2011), and there are several factors that could influence their social learning propensities in our tests. For example the number of demonstrators, their size relative to the subjects' size and the level of familiarity between them, could all contribute to the observed pattern (Lachlan et al., 1998; Lindeyer & Reader, 2010; Swaney et al., 2001). It is possible that part of our sample did socially learn from the demonstrators, since there were several cases where the choice of the subject matched the choice of its demonstrators. However, since we only used dichotomous choice tests that did not involve repeated trials of the same individual, we cannot distinguish whether their choice was informed (influenced by the demonstration) or at random. Hence, since our tests did not provide compelling evidence for social learning, it is not possible to draw conclusions on consistency and heritability of social learning. A less challenging social learning task or a task that would allow us to convincingly distinguish between 'good' and 'bad learners', would be required for this purpose.

We found evidence that grouping tendencies were correlated with social learning performance, as 'time interacting' with the shoal (time that the subject was in contact with the walls of the shoal container) in S1 was significantly correlated with 'latency to the demonstrated area' in SL2 in the case of females and with 'time in the demonstrated area' in both social learning tests in the case of males. This measure can be perceived as a type of very tight shoaling (tighter than common definitions of shoaling/schooling within 3-5 body lengths – e.g. Kelley & Magurran, 2003; Magurran & Seghers, 1994a; Weetman et al., 1999) or as active attempts to join the shoal and be in close contact with the group members. This tendency to interact with conspecifics could be linked to the 'tendency to follow others' to which previous studies have attributed the observed increased social learning propensities measured in their tests (Chapman et al., 2008b; Laland & Williams, 1997). However, since we only detected very few significant correlations between our social learning and shoaling tasks, which did not include our main shoaling measure (time spent near the shoal), we cannot draw confident conclusions on whether grouping tendencies can directly predict social learning propensities.

We were unable to find evidence for heritability in shoaling tendency or social learning propensity by comparing parent-offspring performance. Unfortunately, only 6 pairs mated successfully and gave offspring for the heritability tests, compromising our comparison between the performance of parents and offspring. We did, however, find evidence for heritability in shoaling tendency, in terms of sibling groups differing significantly in the time they spent shoaling in S3, even when reared in different rearing groups. This result is consistent with previous findings demonstrating that shoaling or schooling tendency has a strong heritable component in guppies (Breden et al., 1986; Paxton, 1996; Seghers, 1974). Mathot & Giraldeau (2010) also reported significant sibling group differences regarding zebra finch social information use in a 'produced-scrounger' paradigm. They found that families differed in the propensity to join feeders being used by others (following the 'scrounger' tactic), but they could not distinguish whether the observed pattern was due to genetic or shared parental and environmental effects in early life. Similarly, we cannot determine whether the observed similarities within sibling groups are due to genetic inheritance or due to maternal effects from a shared maternal environment. Maternal effects are present in guppies, for example females that experience low food availability have been shown to produce smaller broods but of larger offspring (Reznick et al., 1996), and such maternal effects are known to inflate estimates of genetic heritability (Kruuk et al., 2000). For these reasons, the evidence for heritability of shoaling tendency we present here is relatively weak and further studies in a larger scale, with multiple matings between individual females and males would be needed to make a valid distinction.

In conclusion, we found evidence that shoaling tendency is individually consistent, but only in male guppies, and that it potentially has a heritable genetic component. We also found limited evidence that grouping tendencies were correlated with social learning propensities. We did not find any evidence for consistency or heritability regarding our subjects' social learning propensities. This was possibly due to the poor performance in most of our social learning tests by male and female individuals alike. However, combined with the lack of evidence from the literature on individual consistency and heritability of social learning, this leaves open the possibility that social learning propensity is not a consistent behavioural trait, and that it can be employed differentially, under specific circumstances or environmental conditions (e.g. when predation risk rises; Webster & Laland, 2008). Moreover, even if social learning propensities are transferred from the parents as inherited predispositions like shoaling tendency (Magurran & Seghers, 1990a; Magurran, 1990b), they potentially need to be fine-tuned to match the early environment conditions encountered by the offspring. The degree of plasticity of social learning propensities, and to what extent these can be shaped by prior experiences, remains an open objective for future work.

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Chapter 3

Age and early social environment influence guppy social learning propensities

Ioannis Leris and Simon M. Reader

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ABSTRACT

Social learning, learning from others, allows animals to quickly and adaptively adjust to changing environments, but only if social learning provides reliable, useful information in that environment. Early life conditions provide a potential cue to the reliability of social information later in life. Here, we address whether direct early life experience of the utility of social learning influences later social learning propensities. We reared guppy *Poecilia reticulata* fry for 45 days in three different social conditions which involved the presence of adult demonstrators providing cues about feeding locations in the tanks (‘follow adults’ and ‘avoid adults’ treatments), or their absence (‘no adults’ treatment). In the ‘follow adults’ treatment, juveniles that swam in the same direction as the adult demonstrators found food, whereas in the ‘avoid adults’ treatment, subjects that swam in the opposite direction to the demonstrators found food. We then tested the fish with a social learning task, to examine whether prior experience had influenced the social learning tendencies of the juveniles. After another 45 days of rearing under common-garden conditions with no adult fish present in the tanks, subjects were re-tested with the same social learning task, to investigate whether early experiences had effects persisting into adulthood. After 45 days of rearing we found no evidence for social learning in any of the experimental groups. However, after 90 days of rearing, we found evidence of social learning, but only in the ‘follow adults’ treatment. These results suggest that social learning propensities may develop over life, and that prior exposure to conspecifics providing useful foraging information during early life can shape the degree of reliance on social learning in adulthood.

INTRODUCTION

Social learning, learning facilitated by observation of or interaction with other individuals or their products (Heyes, 1994; Hoppitt & Laland, 2013), is widespread across the animal kingdom, with examples from insects, cephalopods, fish, reptiles, amphibians, mammals and birds in numerous contexts, such as learning about predators, mates, nesting sites, foraging techniques, food preferences and locations, grouping and travel routes, and communicative signals (Danchin et al., 2004; Heyes & Galef, 1996; Hoppitt & Laland, 2013; Reader & Biro, 2010; Whiten et al., 2016). While social learning has intuitive benefits, such as rapid learning about a changing environment with minimal personal risk, there is a growing realisation that its costs and benefits will vary between individuals and circumstances, leading to the prediction that animals will employ social learning discriminatorily, following so-called ‘social learning strategies’ to maximise net benefits (Boyd &

Richerson, 1985; Rendell et al., 2011). Variation in such costs and benefits could potentially explain the observed diversity within and between species in their apparent reliance on social information and social learning (Efferson et al., 2008; Lefebvre & Palameta, 1988; McCabe et al., 2015; Reader et al., 2011; Toelch et al., 2014; Webster & Laland, 2011). However, as several researchers have noted, an important question is whether an individual's tendency to seek out and rely on social information is plastic, and to what degree it can be shaped by past experience (Heyes, 2012; Leadbeater, 2015; Mesoudi et al., 2016; Reader, in press).

Behavioural plasticity, a type of phenotypic plasticity, is the capacity of an individual to change its behaviour as a response to varying environments (Bateson, 1983; Pigliucci et al., 2006). Social learning can thus be considered as a process underlying behavioural plasticity, and may itself be plastic. Behavioural plasticity can be further classified in two categories, 'activational', where the organism exhibits different behaviours in different environmental conditions or as a response to changes in the environment, and 'developmental', where different prenatal or early environments lead to different developmental trajectories and different behavioural phenotypes (Snell-Rood, 2013). While multiple examples of activational plasticity in social learning propensities exist (see e.g. Rendell et al., 2011), as do examples of developmental plasticity in social behaviour (e.g. Adkins-Regan & Krakauer, 2000; D'Andrea et al., 2007; Sundström et al., 2003), there are relatively few investigations of the developmental plasticity of social learning or social information use.

Such investigations of developmental plasticity and social information use typically manipulate or measure conditions confined specifically to early life. For example, developmental stressors shape the use of social information in Japanese quail, *Coturnix japonica*, and zebra finches, *Taeniopygia guttata* (Boogert et al., 2013; Farine et al., 2015); while in rats, *Rattus norvegicus*, maternal care influences social learning propensities later in life (Levy et al., 2003; Lindeyer et al., 2013; Melo et al., 2006). To our knowledge, however, only one study has directly manipulated the value of social information early in life and investigated how this affects the development of social information use. Katsnelson et al. (2008) hand-reared house sparrows, *Passer domesticus*, in the presence of an artificial parent that either reliably indicated food locations or did not. Later in life, the sparrows previously exposed to the 'reliable' parent were more likely to use social information by joining others at a food patch than sparrows previously exposed to an 'unreliable' parent. However, the sparrows were exposed to the artificial parents until immediately prior to test, making it different to ascertain whether the findings were the result of early or recent experience. Here, we use guppies, to investigate whether experimental manipulations of the value of social information restricted to early life can shape adult social learning propensities. We focus specifically on social learning, a subcategory of social information use where social information is acquired and has a subsequent influence on behaviour (Reader & Biro, 2010).

The Trinidadian guppy in particular and poeciliid fish in general provide useful study systems for studies of developmental influences on social behaviour and social learning, because of the large background knowledge on their evolutionary and behavioural ecology (Brown et al., 2011; Evans et al., 2011; Magurran, 2005), the ease of experimentally manipulating rearing conditions in the laboratory, and evidence for social learning in both the wild and captivity. For example, guppies have been demonstrated to learn foraging and anti-predator behaviour in the laboratory (Brown & Laland, 2002; Lachlan et al., 1998) and in the wild (Reader et al., 2003). Different aspects of developmental phenotypic plasticity have been examined in a variety of different contexts using the guppy. For instance, early social environment specifically, and interactions with adults in particular, has been shown to inhibit sexual maturity (Magellan & Magurran, 2009), shape sexual behaviour (Guevara-Fiore, 2012) and promote the development of anti-predator defences (Chapman et al., 2008a). Particularly relevant to our current study, Chapman et al., (2008b) manipulated rearing density until giving tests of grouping and social learning propensities. Guppies reared in low densities were more likely to group with others and to socially learn in a task involving following others through a maze, compared to fish reared at high densities.

In the present study, we constrain our experimental manipulations to early life, to examine the effects of early social environment. We reared juvenile guppies in the presence or absence of adult demonstrator fish that provided differing information about feeding locations, subsequently testing them at two different time points (once immediately after the manipulation and once after a period of time in common-garden conditions) to assess their propensity to socially learn a foraging task. We manipulated the value of social information such that the adult demonstrators either swim towards or away from a feeding location. In our study, food was only provided at one of two locations, and thus both demonstrator groups provided reliable cues, but in the former group subjects must swim in the demonstrated direction to locate food, while in the latter group they must swim in the opposite direction. This latter treatment could be considered akin to a situation where competitors exploit and deplete a food source, and thus provide a reliable cue to food absence. We predicted that differing early experience with how social cues indicate food would lead to fish utilising these social cues differently, and for these differences to weaken but persist to adulthood.

METHODS

Overview

We raised guppy fry for 45 days, delivering food in two locations for 96 feeding bouts. The fry were exposed to three different social conditions: (1) to adult 'demonstrators' that reliably swam to the same location that food would be delivered to the fry, (2) to adult demonstrators that reliably swam to the opposite location of food delivery to the fry, or (3) to no adults. After these 45 days, we measured subjects' social learning propensities. We then reared subjects in common-garden conditions without any demonstrators for another 45 days, and then re-tested subjects with an identical social learning test. This procedure allowed us to establish whether experiences confined to early life influenced social learning propensities when adult.

Subjects and Housing Conditions

Subjects were fry born to domestic guppies that had been reared in 150-l glass aquaria (120 cm x 40 cm, water depth: 30 cm) in mixed-sex and mixed-age conditions. These domestic guppies were a mixed strain population of approximately 1400 fish, first established in 2003 within the Utrecht University Biology aquarium and based on a founder population of approximately 480 guppies purchased from two commercial suppliers (Ruinemans, Montfoort and Ruisbroek, Maassluis, both The Netherlands). Demonstrators for the social learning test came from the same population, and were housed separately in a 70-l glass tank (90 cm x 40 cm, water depth: 20 cm) divided in half with a transparent PVC barrier to form two demonstrator groups. All housing tanks were equipped with external canister filters (Eheim, Germany) and thermostat-controlled heaters and were enriched with gravel, artificial plants and ceramic pots. Water quality was closely monitored (nitrates, nitrites, hardness and pH were measured weekly, dissolved oxygen and conductivity biweekly). Water temperature was maintained at 26.5 ± 0.5 °C. Every 15 days 30% of the water was replaced with fresh de-chlorinated and copper-free 26.5 °C water.

Rearing Tanks

Newborn guppy fry (< 10 mm total length [TL]) were taken from four 150-l tanks with dip nets and were placed together in a transparent plastic container. Body size was visually assessed by a 10-mm grid under the container. Fry larger or considerably smaller than 10 mm were returned to the housing tanks. The remaining fry were randomly allocated to groups of 14 fry and transferred to the experimental rearing tanks. We thus randomised any housing tank effects. A total of 168 fry were subjects.

Fry were reared in six 70-l glass tanks (90 cm x 40 cm, water depth: 20 cm). Each tank was divided into three compartments with two clear PVC partitions (Fig. 1), with one group of subjects in each of the two outer compartments. The 20-cm-wide central compartment held six male adult guppies (Fig. 1b), except in the 'no adults' condition (Fig. 1c). Male demonstrators were used to avoid births during the study. The compartments were not sealed, thus allowing olfactory cues to pass between them. All tanks were equipped with thermostat-controlled heaters and air-driven sponge filters in the fry compartments.

Feeding Regime and Experimental Treatments

Crushed flake food (TetraPhyll, Tetra, Germany) was delivered to floating feeders (Fig. 1) by automatic feeders (fry: Eheim 3581; adult demonstrators: Eheim 3582; Eheim, Germany). Fry were fed 60 s after the demonstrators. For the first 3 days of rearing, demonstrators and fry were fed four times per day, twice at a random time between 08:00 and 11:00 h ('morning') and twice between 15:00 and 18:00 h ('afternoon'). Random feeding times were produced using a pseudorandom number generator function in Matlab (function 'randi', Matlab, v. R2011b). After this period of habituation to the feeders, feeding was conducted twice a day at a random time in the morning and in the afternoon. Every 5 days, new random feeding times were generated, to avoid any habituation to specific feeding times.

We reared fry in three experimental treatments. In the 'follow adults' treatment, six adult males were present in the central compartment of the tank and were fed on the same side of the tank that food would be delivered to the juveniles. Thus demonstrator movement predicted where the food would be administered, and subjects that utilised this cue would be able to locate the food more rapidly than other fish. The second 'avoid adults' treatment was identical to 'follow adults', except that fry were fed in the opposite location to the adults. Thus subjects that swam in the opposite direction to the adults would be able to locate the food more rapidly than other fish. We set up four replicate tanks, and within each tank paired subjects in the 'follow' and 'avoid adults' treatments: one group of fry per tank were in the 'follow adults' treatment, the other group in the 'avoid adults' treatment. Thus subjects were exposed to matched demonstrators and feeding conditions, apart from the fact that demonstrator behaviour predicted either the presence or absence of food. In the third 'no adults' treatment, there were two replicate tanks each with two groups of subjects, and no adults present. In this treatment, demonstrator feeding rings were replaced with perforated opaque plastic cups suspended from the tank walls, where food was delivered as in the

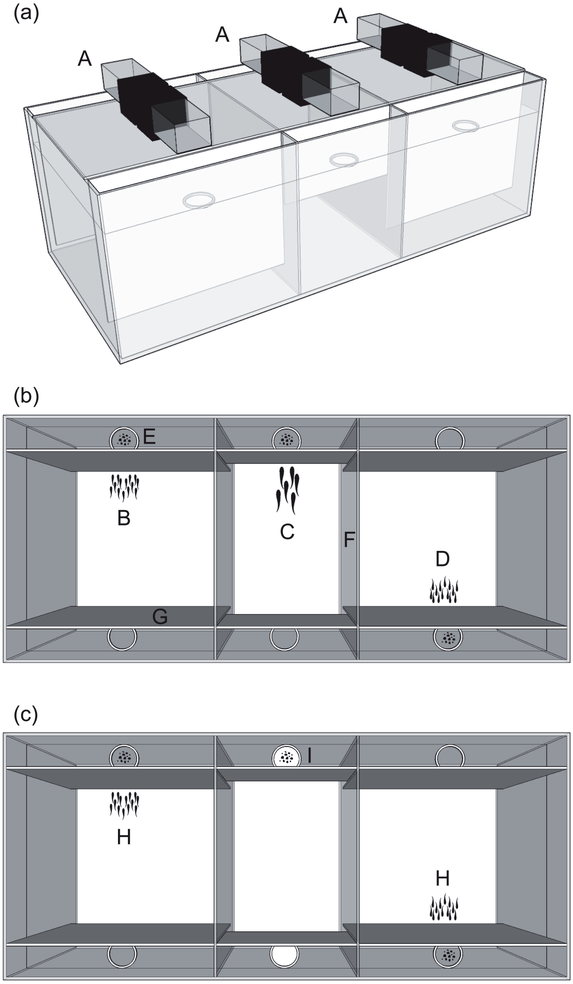

Figure 1. Rearing tanks: three-dimensional side view (a) and top view of the 'follow adults'/'avoid adults' tanks (b) and the 'no adults' tanks (c). The tanks were split by transparent dividers into three compartments, each with two automatic feeders (A). Each compartment contained two floating ring feeders made of 5-mm air tubing. Two longitudinal opaque PVC partitions with a 5-cm gap prevented subjects from seeing the feeders but allowed them to swim underneath to access them. Labels: B: 'follow adults' fry in their compartment, fed in the same location as the adult demonstrators; C: adult male demonstrators located in the central compartment of the tank; D: 'avoid adults' fry in the second outer compartment, fed in the opposite location to the adult demonstrators; E: feeder; F: transparent PVC partition separating the compartments; G: opaque partition that allowed feeder access; H: 'no adults' fry in both outer compartments of the tank; I: perforated opaque plastic cup used to collect the food delivered in the empty demonstrator compartment and thus ensure food odour cues were present in all experimental conditions.

other two treatments. This ensured that food odour was present in the central compartment during feeding time as in the other two treatments. The apparatus was designed so that subjects did not see the food itself being administered in any of the experimental treatments (Fig 1b). Ramshorn snails, *Planorbis* spp., were present in all tanks to consume any leftover food, and in the 'no adult' treatment they were also placed in the cups since uneaten food would result in deterioration of water quality.

After 45 days of rearing under the aforementioned developmental conditions, the juvenile fish were presented with a social learning test (described below). At the end of this test, fish were returned to their housing tanks (now divided in two compartments with a single clear PVC partition) and were reared for another period of 45 days in common-garden conditions, with no other partitions and no adult demonstrators present. During this second rearing period, the floating feeders were removed and crushed flake food was delivered by automatic feeders as before in the centre of each compartment twice per day. This ensured that no further learning regarding foraging locations could occur during this period. At day 90 the fish were retested with the same social learning test.

Social Learning Test

Tests were conducted in a 25-l glass tank (40 cm x 25 cm, water depth: 10 cm) divided into four compartments with clear PVC partitions (Fig. 2). A subject was placed in an observation compartment, where, during a demonstration phase, it could observe demonstrators choose between one of two feeders in two feeding compartments. After demonstration, subjects could choose a feeding compartment in a similar manner to the rearing period by swimming through a 2-cm gap under clear PVC transparent partitions. Coloured plastic tape (left: green stripes, right: blue squares) was used to make the partitions visually distinctive. A heater in the observation compartment maintained water temperature. No gravel was present to aid visibility from above.

Demonstrators (mean body mass ± standard error: 0.21 ± 0.05 g) were selected each day from a pool of 16 adult male guppies trained to the 'left/green' side and a pool of 13 trained to the 'right/blue' side. Demonstrators had been trained for two weeks prior to the experiment to enter their respective feeding area and feed from a floating ring feeder within 60 s. They were also habituated to being caught by a net and being transferred to the experimental tank in a transparent plastic cup. Each group was used maximally twice per day to ensure that demonstrators were not satiated and reliably fed from the feeder.

Every day before testing, the experimental tank was filled with water from the demonstrator housing tank to standardise olfactory cues present in the water. A subject fish was selected at random, caught by net and transferred in a transpar-

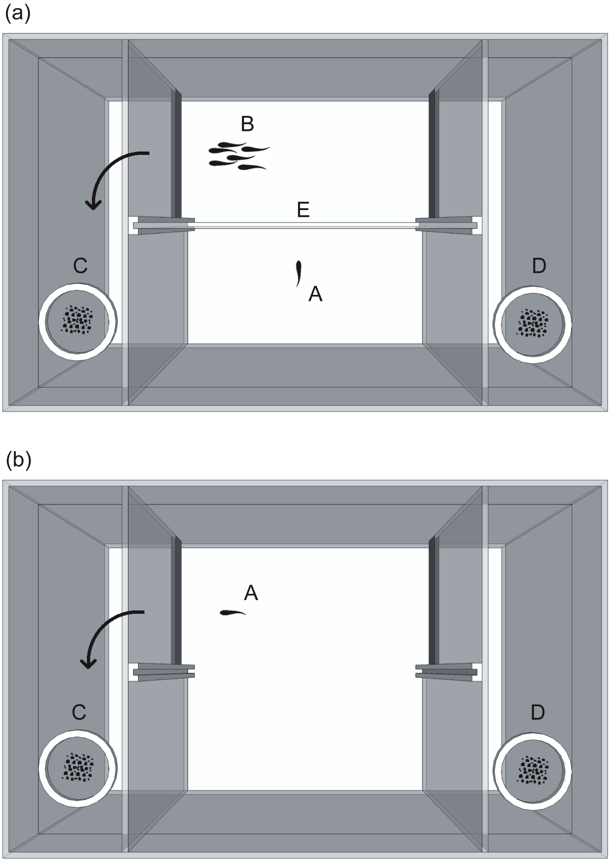

Figure 2. Social learning task: demonstration phase (a) and testing phase (b). Labels: A: subject held in the observation compartment; B: group of demonstrators in the demonstrator compartment, in this example trained to swim under the left partition to the left compartment; C and D: floating feeder ring with food in the left and right choice compartments; E: removable transparent PVC partition. In the testing phase the PVC partition is lifted by a string and the subject (A) can make a choice by swimming under the left or right partition.

ent plastic cup to the observation compartment of the experimental tank. Crushed flake food was added to the two floating feeders in the choice compartments and a group of six demonstrators transferred to the demonstrator compartment. After allowing 2 min for acclimation, the subject's cup was slowly tilted until it swam out into the observation compartment. Sixty seconds later, the demonstrators' cup was tilted and they were released. In all trials the demonstrators reliably swam to the feeding compartment they had been trained to and fed. The demonstration lasted 3 min, after which we placed three opaque partitions around the observation compartment, blocking the subject's view for 5 min. The demonstrators were shepherded back to their start compartment and removed, all food was removed with a siphon, then new food was added to both feeders. After the 5 min, the opaque partitions were removed and the transparent divider was slowly lifted using a string/pulley system, allowing the subject to swim freely and choose a feeder compartment. Behaviour was recorded for 5 min. One fish did not leave the observation compartment within 5 min after the divider was lifted and thus no measurements were taken. Four additional trials were compromised by apparatus failure and thus were also removed from the dataset. Between trials, uneaten food was removed, a portion of water was replaced with fresh water, and the water was mixed to mix any remaining olfactory cues. Identical social learning tests were run at days 46-48 and days 91-93. On days 46-48 (henceforth 'Day 45') 96 subjects were tested, and at days 91-93 (henceforth 'Day 90') 87 subjects were tested. All fish that left the observation compartment entered a feeder compartment. Few fish had reached sexual maturity at day 45, and thus subject sex was not analysed at Day 45. Given the small size of the fish at day 45 it was not possible to use elastomer tags or other methods to identify individuals, and thus we did not track individual identity across time points.

Behavioural Measures and Data Analysis

Fish movement and behaviour was recorded during the trial using JWatcher (v1.0, Blumstein et al., 2006). Trials were also recorded with a high resolution webcam (QuickCam Pro 9000, Logitech, USA) suspended above the experimental tank. For the social learning test we measured the time spent in the demonstrated and non-demonstrated areas and the latency to enter the demonstrated and non-demonstrated compartments. We recorded the time spent motionless and the total number of dashing events (i.e. rapid darting movements) as measures of stress (Budaev, 1997; Elvidge & Brown, 2015), but such events were infrequent and were thus not analysed further. Data are archived online in the Dryad repository (https://datadryad.org/resource/doi:10.5061/dryad.8538d).

If fish socially learned in the social learning test, demonstrators would influence the subsequent choices of subjects, with subjects either copying or avoiding demonstrated locations. We conducted analyses in two steps, first examining whether there was evidence for social learning in any experimental treatment, and second examining whether the evidence for social learning differed between treatments. We subtracted the time spent in the non-demonstrated compartment from the time spent in the demonstrated compartment (henceforth 'time-in-compartment difference'), and the latency to enter the demonstrated compartment from that to enter the non-demonstrated compartment (henceforth 'latency-to-enter difference'), to examine and illustrate any demonstrator influences. Positive values indicate a preference for the demonstrated location in both measures. We predicted that fish from the 'follow adults' treatment would copy the demonstrators' choice, while fish from the 'avoid adults' treatment would choose the opposite location to that demonstrated, and thus in the two treatments demonstrated location would have a significant but opposite effect. We predicted that fish from the 'no adults' treatment would be minimally influenced by the demonstrators' behaviour.

Data were tested for normality with Shapiro-Wilk's *W* test and for homoscedasticity with Levene's test. If these assumptions were not met, the data were square root or root 10 log transformed as appropriate so that normality and homoscedasticity were achieved. We used factorial ANOVA, with 'treatment', 'sex' (at day 90 only), and 'demonstrated location' being fixed factors, while 'tank' was treated as a random blocking factor and 'body mass' was assessed as a covariate. Analyses were conducted with SPSS Statistics v20 (IBM, USA). Sex was removed from analyses if it had no significant effect ($P > 0.1$).

We also compared behaviour at Day 45 and 90, with sexes and tanks pooled together. These data represent repeated measures on the same individuals, but as we were unable to mark individuals we could not include individual identity in the analyses. For these analyses, we examined 'time-in-compartment difference' and 'latency-to-enter difference', as well as the subjects' latency to enter any compartment ('latency-to-choose') using factorial ANOVAs, with 'testing age' (45 or 90 days from the beginning of rearing) and 'treatment' as fixed factors. To partially account for non-independent data, we calculated the *F*- and *t*-values as if data were independent and then compared these values to the critical *F*- and *t*-value for reduced degrees of freedom represented by our sample size.

Ethical Note

All experimental procedures were approved by the Utrecht Ethics and Animal Care and Use Committee (DEC) under protocol number 2011.I.05.050, and conform to ASAB guidelines and Dutch law. At the end of the study all fish were returned to breeding populations at Utrecht University.

RESULTS

Day 45

At Day 45, we found no evidence for social learning in any of the three experimental treatments, since the mean 'time-in-compartment difference' did not significantly differ from 0, the value predicted by chance (one-sample *t*-tests: 'follow adults', $t_{37} = 0.71$, $P = 0.48$; 'avoid adults', $t_{27} = 0.21$, $P = 0.83$; 'no adults', $t_{29} = 0.19$, $P = 0.85$; Fig. 3a). Similarly, the mean 'latency-to-enter difference' did not differ from 0 in any of the experimental treatments (one-sample *t*-tests: 'follow adults', $t_{37} = 0.37$, $P = 0.71$; 'avoid adults', $t_{27} = 0.19$, $P = 0.85$; 'no adults', $t_{29} = 0.91$, $P = 0.37$; Fig. 3c). Looking across treatments, the three experimental treatments did not significantly differ on either the 'time-in-compartment' measure (ANOVA: $F_{1,75} = 0.18$, $P = 0.69$) or the 'latency-to-enter' measure (ANOVA: $F_{1,75} = 0.10$, $P = 0.77$). There was no evidence for a significant side bias in either measure (ANOVAs examining the effect of demonstrated location: 'time-in-compartment difference': $F_{1,75} = 4.32$, $P = 0.11$; 'latency-to-enter difference': $F_{1,75} = 3.30$, $P = 0.14$; a side bias would be represented by a significant effect of demonstrated location on these measures). 'Tank' had no significant effect (ANOVA: 'time-in-compartment difference', $F_{4,75} = 0.076$, $P = 0.98$; 'latency-to-enter difference', $F_{4,75} = 0.09$, $P = 0.98$) and body mass had no significant effect as a covariate (ANOVA: 'time-in-compartment difference', $F_{1,75} = 2.74$, $P = 0.10$; 'latency-to-enter difference', $F_{1,75} = 2.19$, $P = 0.14$).

Day 90

At Day 90, we found evidence for social learning in the 'follow adults' treatment, with subjects faster to enter and spending more time in the demonstrated compartment compared to the non-demonstrated compartment (Fig. 3b, 3d; one-sample *t*-tests: 'time-in-compartment difference': $t_{31} = 2.42$, $P = 0.022$; 'latency-to-enter difference': $t_{31} = 2.27$, $P = 0.03$). However, we found no evidence for social learning in the 'avoid adults' (one-sample *t*-tests: 'time-in-compartment difference', $t_{27} = 0.25$, $P = 0.81$; 'latency-to-enter difference', $t_{27} = 0.93$, $P = 0.36$) and 'no adults' treatments (one-sample *t*-tests: 'time-in-compartment difference', $t_{26} = 0.99$, $P = 0.33$; 'latency-to-enter difference', $t26 = 0.78$, $P = 0.44$). Looking across treatments, sex did not have a significant effect on the 'time-in-compartment difference' or 'latency-to-enter difference' (ANOVAs: $F_{1,44} = 3.05$, $P = 0.14$; $F_{1,44} = 0.08$, $P = 0.79$, respectively) and was thus removed from subsequent analysis. The experimental treatments did not significantly differ on either the 'time-in-compartment difference' or 'latency-to-enter difference' measures (ANOVAs: $F_{1,66} = 3.07$, $P = 0.17$; $F_{1,66} = 4.29$, $P = 0.12$, respectively). There was no evidence for a side bias (ANOVAs: no significant effect of demonstrated location on 'time-in-compartment difference': $F_{1,66} = 0.10$, $P = 0.77$ or 'latency-to-enter difference': $F_{1,66} = 0.13$, $P = 0.74$). 'Tank' had no significant effect (ANOVAs: 'time-in-compartment difference', $F_{4,66} = 0.79$, $P = 0.60$; 'latency-to-enter difference', $F_{4,66} = 0.61$, $P = 0.68$). Body mass was a significant covariate in the case of 'latency-to-enter difference' (ANOVA: $F_{1,66} = 4.99$, $P = 0.029$) but not in the case of 'time-in-compartment difference' (ANOVA: $F_{1,66} = 1.01$, $P = 0.32$).

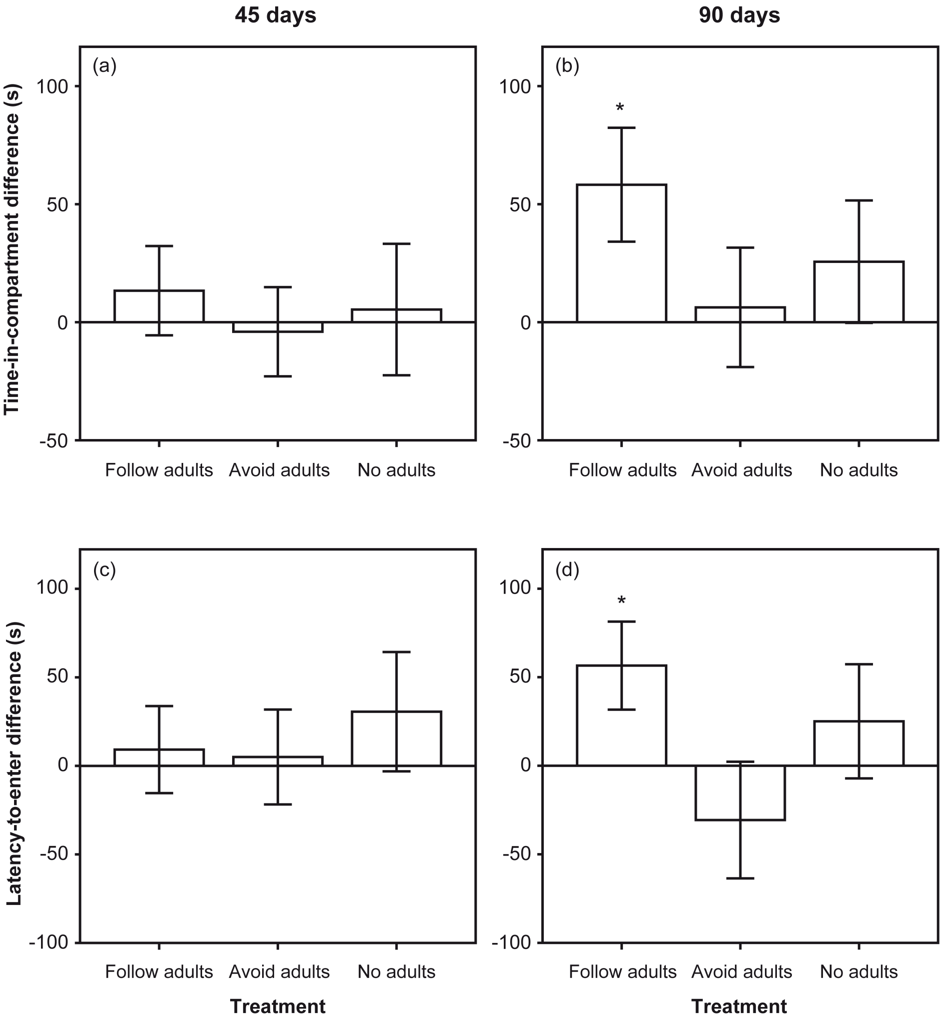

Figure 3. Mean time-in-compartment difference (time spent in the non-demonstrated compartment subtracted from the time spent in the demonstrated compartment) in the social learning test at 45 (a) and 90 days (b) and mean latency-to-enter difference (latency to enter the demonstrated compartment subtracted from the latency to enter the non-demonstrated compartment) at 45 (c) and 90 days (d). Positive values of both measures indicate a preference for the demonstrated location. *: $P < 0.05$. Error bars represent the standard error of the mean.

Comparing Day 45 and 90

Testing age had no significant effect on 'time-in-compartment difference' (ANOVA: $F_{1,177} = 1.70$, P based on 81 degrees of freedom [henceforth 'P_{81}'] > 0.1) or 'latency-to-enter difference' (ANOVA: $F_{1,177} = 0.01$, $P_{81} > 0.1$). However, fish at Day 90 were significantly faster to enter any of the two compartments than they were on Day 45 (ANOVA: $F_{1,177} = 8.34$, $P_{81} < 0.01$). This difference is mainly driven by the subjects in the 'follow adults' treatment, which entered a compartment significantly faster on Day 90 than Day 45 (t-test: $t_{67} = 2.91$, $P_{30} < 0.01$; Fig. 4a), while this effect was not significant in the 'avoid adults' or 'no adults' treatments (t-tests: $t_{54} = 1.63$, $P_{26} > 0.1$; $t_{55} = 0.87$, $P_{25} > 0.1$, respectively; Fig. 4a). Overall, however, latency to enter any compartment did not differ between treatments at Day 45 (ANOVA: $F_{2,93} = 0.23$, $P = 0.80$) or Day 90 (ANOVA: $F_{2,84} = 0.35$, $P = 0.71$). Body mass significantly increased from Day 45 to Day 90 (ANOVA: $F_{1,177} = 164.25$, $P_{81} < 0.001$; Fig. 4b), in all three treatments (t-tests: 'follow adults', $t_{68} = 8.70$, $P_{30} < 0.001$; 'avoid adults', $t_{54} = 7.20$, $P_{26} < 0.001$; 'no adults', $t_{55} = 6.46$, $P_{25} < 0.001$). Mass did not significantly differ across treatments at Day 45 (ANOVA: $F_{2,93} = 0.62$, $P = 0.54$). At Day 90, 'follow adults' subjects were 0.03 g or 30% heavier than subjects from 'avoid adults' treatment and 0.04 g or 36% heavier than subjects from the 'no adults' treatment (principally due to heavier females), but this difference between treatments was not statistically significant (ANOVA: $F_{2,84} = 2.59$, $P = 0.08$).

DISCUSSION

We found no evidence for social learning after 45 days of experimental rearing. After 45 additional days of rearing in standard conditions, we found evidence for social learning on two behavioural measures, but only in those fish that had been exposed to demonstrators that reliably indicated food locations for the first 45 days of life. Our results are consistent with the ideas that performance on this social learning test is dependent upon age, and that experience confined to early life affects social learning propensities in later life.

The most striking aspect of our results is that early life experience had an effect 45 days later, after rearing in common-garden conditions. This suggests that early life had long-lasting effects that were not expressed until adulthood. We predicted, in contrast, that the strongest effects on social learning would be observed at 45 days and these effects would be potentially weakened after 45 days in common-garden conditions. Our results therefore suggest that social learning propensities may change over the lifetime of an individual. Similarly, a comparison of different ages of jack mackerel, *Trachurus japonicus*, revealed that social learning tendencies develop in older individuals, together with enhanced schooling behaviour (Takahashi et al., 2014). A number of explanations could account for

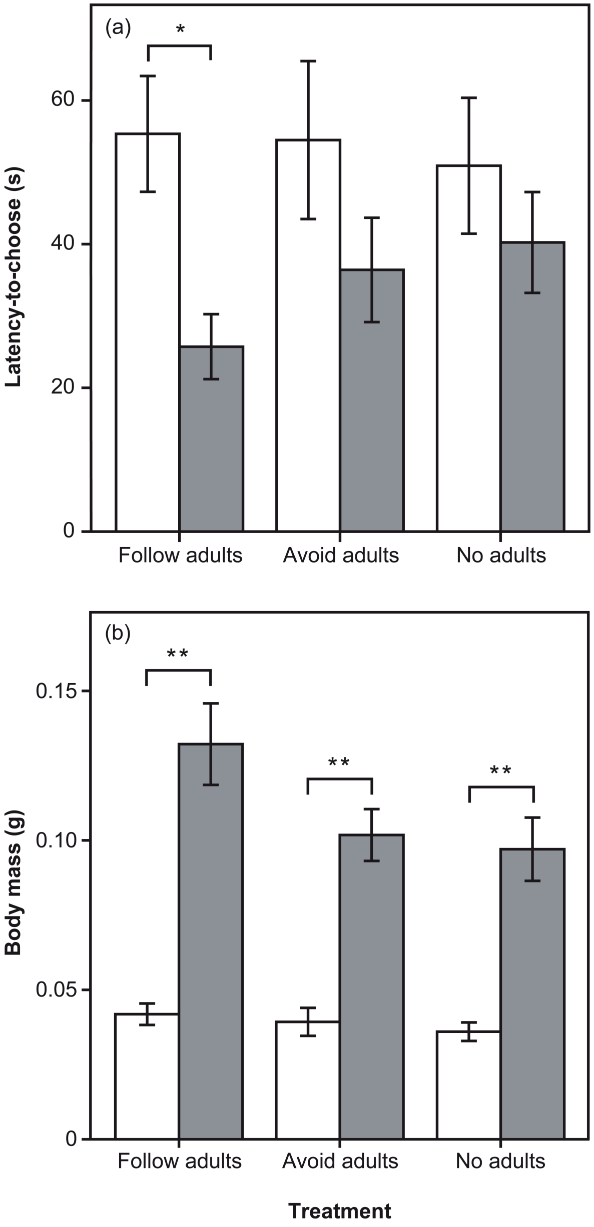

Figure 4. Mean latency to choose any feeding compartment (maximum trial length was 300 s) in the social learning test (a) and mean body mass (b) at 45 (□) and 90 (■) days. *: $P < 0.01$; **: $P < 0.001$. Error bars represent the standard error of the mean.

the difference in social learning propensities with age that we observed. The social learning task may simply be too challenging or insufficiently motivating for younger fish. For example, younger fish, which are also smaller in body size, may be less motivated to visit locations where large fish were previously present, since larger guppies outcompete smaller guppies in competition for food and may be aggressive towards them (Chapman et al., 2008a; Laland & Reader, 1999b). Guppies were slower to enter either of the two feeding compartments on Day 45 than Day 90, supporting the idea they were less motivated by the task. Competition was also suggested to underlie age-dependent social learning in male skink lizards, *Eulamprus quoyii*, juveniles socially learned an association task but adults did not, potentially due to greater inter-male competition amongst adults (Noble et al., 2014). The relative age or size of demonstrators and observers has been investigated particularly in fish, and has been shown to influence who-learns-from-who in both guppies and sticklebacks, *Pungitius pungitius* (Duffy et al., 2009; Dugatkin & Godin, 1993; Vukomanovic & Rodd, 2007). Directed social learning (Coussi-Korbel & Fragaszy, 1995), where socially acquired behaviours only flow through a subset of the population, may thus be quite common.

In our study, only fish exposed to demonstrators that lead them to a food location later used similar social information in the social learning test, entering the demonstrated feeding compartment faster and spending more time there. This finding suggests that early experience with the reliability of social information influences adult social learning propensities, and parallels the results of the similar study of house sparrows (Katsnelson et al., 2008). Our study extends previous work by confining experience of the reliability of social cues to early life, by explicitly varying cue reliability (cf. e.g. Boogert et al., 2013; Lindeyer et al., 2013) and by measuring social learning rather than social information use. Our results thus indicate that the reliability of social cues can shape social learning, supporting the idea that social learning propensities are not simply a by-product of other developmental changes.

In the ‘avoid adults’ condition, the demonstrators provided equally reliable cues as the ‘follow adults condition’ in demonstrating the food location, except that demonstrators swam away from, rather than towards, the rewarded location. In our study, food was only available in two locations, and thus the absence of food in one location indicated the presence of food in another. That is, demonstrator behaviour during rearing provided a discriminative cue, with subjects receiving positive reinforcement for following or opposing adults in the ‘follow adults’ and ‘avoid adults’ conditions, respectively. However, we saw no indication of social learning in the ‘avoid adults’ treatment, while we suspect that they would have readily learned to make this discrimination using an asocial cue. Perhaps young guppies have an initial predisposition to follow or attend to others (Magurran, 1990a,1990b; Magurran & Seghers, 1990a), a predisposition that can be strengthened by experience, whereas it is more difficult for guppies to learn to avoid others. Numerous factors may result in such a predisposition, particularly the anti-predator and informational benefits of grouping (Krause & Ruxton, 2002). Moreover, situations where the absence of food reliably indicates exactly where food is present may be rare in nature. However, recent experience has been demonstrated to lead to non-matching behaviour in several species (Reader, 2014). For example, Dawson et al., (2013) showed that bumblebees, *Bombus terrestris*, with experience of conspecifics paired with bitter foods learned to actively avoid conspecific-demonstrated feeders. Interesting open questions are the extent to which any predispositions in social learning tendencies can be altered by experience, and whether specific experiences or developmental stages are particularly impactful in shaping social learning.

We did find clear evidence for social learning in adult fish that had been previously exposed to demonstrators that reliably indicated food. However, the strength of our conclusions is limited by the fact that the comparisons of the three different experimental treatments were not statistically significant (Nieuwenhuis et al., 2011). Further work would be needed to strengthen our conclusions, for example taking account of the considerable individual variation we observed by using increased sample sizes, a different social learning test, or more extensive training procedures. However, given the limited evidence up to now for developmental effects on social learning, we argue that the present findings offer new evidence that social learning propensities can be shaped by early exposure to social cues, and thus can act as a useful guide and impetus for future work.

Our results have both theoretical and applied implications. If social learning is shaped by both recent and early life experience, this means that observed population and species differences in social learning propensities may at least partially arise from environmental differences, possibly interacting with genetic predispositions (Carlier & Lefebvre 1997; Reader et al., 2011). Developmental plasticity in social learning would allow animals to adjust to variation in the numerous social and ecological characteristics that impact the value of social information. For example, guppy habitats differ in characteristics such as productivity and predation risk, and guppy populations differ in grouping tendencies and intraspecific competition and aggression (Magurran, 2005; Magurran & Seghers, 1991; Reznick et al., 2001). All of these characteristics could impact the value of social information and thus the development of social learning propensities. Our results caution against the interpretation of differences in social learning performance as differences in social learning capacity. Flexibility in social learning may be an adaptive feature, vital to maximising benefits, or alternatively it may reflect constraints on the evolution of predispositions affecting social learning (Reader, in press).

Conservation biologists and agricultural researchers have shown interest in social learning both as a tool to promote the success of individuals reintroduced or released into wild populations and as a consideration in conservation efforts (Brown & Laland, 2001; Reader & Biro, 2010; Suboski & Templeton, 1989; van Schaik, 2002). For example, captive-reared fish typically suffer very high mortality rates when released into the wild (Salvanes & Braithwaite, 2005), and overfishing may not just reduce population numbers but may also eliminate beneficial socially learned behaviours, such as spawning sites in herring, *Clupea harengus* (Corten, 2002). Our results suggest that animals without suitable models to learn from may not just suffer from failing to socially acquire a particular behaviour pattern, but also because social learning fails to develop at all. The take-home message from our study is that social learning propensities may change both over the lifespan and as a result of differential early-life experience, providing novel routes by which the efficacy of social learning can be fine-tuned.

ACKNOWLEDGEMENTS

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Chapter 4

Sex-dependent differences in guppy behaviour: the effects of early life predation risk and rearing density

Ioannis Leris and Simon M. Reader

(to be submitted to 'Behaviour')

ABSTRACT

Predation pressure during early life has been found to induce a variety of responses in prey animals and early social environment has been proposed to play a major role in phenotypic development. To examine whether and to what extent these factors can influence an individual's behavioural phenotype, we reared guppy fry, *Poecilia reticulata*, for 45 days in two housing densities (high and low) while exposing them to either predator or non-predator (control) cues. We then measured an array of behaviours (boldness, risk-aversion, exploration, grouping tendency and propensity to leave a group to approach a novel feeder) at two time points, one shortly after the end of the developmental manipulations and one after a 30-day period of common-garden conditions. We found strong sex differences in most of the analysed measures. Males were bolder and more exploratory than females and although they exhibited higher grouping tendencies, they were more likely to leave the group to inspect a novel feeder. Thus males presented an overall higher propensity to take risks compared to females; further illustrating the different strategies the two sexes follow to maximise fitness and survival. On the other hand, predation risk and rearing density appeared to have non-additive and generally weaker than anticipated effects in most cases. This could indicate that the developmental tuning of behavioural phenotypes may be more subtle than previously thought and dependent on complicated interactions between several factors.

INTRODUCTION

Predation is one of the biggest challenges animals have to face during their lifetime posing a strong selective force in wild populations. Failure to appropriately respond to a predator attack usually means death; therefore prey organisms have developed a variety of adaptations (morphological, physiological, life historical or behavioural) as anti-predator defences (Kats & Dill, 1998). However, there is a trade-off between engaging in anti-predator behaviours and fitness-related activities such as foraging and reproduction, rendering over-sensitivity to predation threat disadvantageous in most situations (Chivers & Smith, 1998; Kats & Dill, 1998; Lima & Dill, 1990). Hence, organisms would benefit if they could adjust their anti-predator responses to the current level of predation threat in their environment (threat-sensitive predator avoidance – Chivers et al., 2001; Helfman, 1989). Animals assess predation risk in real time primarily through visual and olfactory (or chemosensory) cues (Lönnstedt et al., 2012). The latter cues, which include damage-released (alarm) cues and predator odours (e.g. Brown et al., 2000; Elvidge & Brown, 2015), are particularly important in aquatic environments where visibility might be limited due to water turbidity or geographic complexity (Kats & Dill, 1998). In addition, although many animals can exhibit strong responses upon first encounter with an unknown predator pointing to the existence of 'hardcoded' predispositions (e.g. Scheurer et al., 2007), prior experience with predators plays an important role (Bass & Gerlai, 2008) and learning (especially chemically-mediated) can assist in fine-tuning predator recognition and avoidance (Ferrari et al., 2007). Thus, early environment conditions and experiences can provide the necessary stimuli to prime anti-predator defences in animals, allowing them to balance predation avoidance against other important activities.

Early social environment is another key factor that can shape the behavioural phenotype of an organism, with various examples ranging from primates (e.g. Newman et al., 2005), to birds (e.g. Adkins-Regan & Krakauer, 2000), and particularly relevant to our study, fish (see Jonsson & Jonsson, 2014 for a review). The presence of adult individuals during rearing, for example, can mediate the development of anti-predator defences in guppies, through agonistic and competitive interactions (Chapman et al., 2008a). In *Neolamprologus pulcher*, a cooperatively breeding cichlid, social experiences during early life can influence gene expression in the stress axis, thus influencing their social behaviour when adult (Taborsky et al., 2013). Rearing density in particular has been previously shown to influence several traits in fish, with potential applied value in fisheries management and conservation efforts. For example reduced density in a hatchery rearing environment has been found to increase social dominance, and post-release growth and survival in brown trout, *Salmo trutta* (Brockmark & Johnsson, 2010). Rearing density is also known to affect social learning and information use in guppies, with fish reared in low densities being better at following demonstrators to locate food than fish reared in high densities (Chapman et al., 2008b). Rearing in high densities should in theory result in greater competition and higher aggression levels between individuals, thus increasing the costs of grouping and decreasing their shoaling tendencies and this could in turn influence their propensities to use social cues and socially learn.

Guppies are small colourful fish inhabiting various streams and rivers in the Northern Range of Trinidad, where they encounter a wide spectrum of predation risk from very low (in the presence of small omnivorous predators that only pose a threat for the juveniles, such as Hart's Rivulus, *Rivulus hartii*) to very high (coexisting with a variety of different piscivore species including pike cichlids, *Crenicichla* spp., and wolf fish, *Hoplias malabaricus*) (Rodd & Reznick, 1997; Seghers, 1974). These low- and high-predation populations differ in various demographic characteristics, such as sex ratio, age ratio and density (Rodd & Reznick, 1997) and are known to exhibit significant behavioural differences (Endler, 1995; Ghalambor et al., 2004; Kelley & Magurran, 2003; Magurran & Seghers, 1994a). Although most of these differences in behaviour have a strong genetic basis, the environmental context experienced during early life (particularly regarding predation threat) also plays a major role (e.g. Kelley & Magurran, 2003). These features combined with the species' well-described social behaviour and the ease of its rearing and maintenance in the laboratory, constitute the guppy as an ideal study system for research on early environment influences, and particularly in our case where the effects of early predation risk and density were the main experimental questions.

In the present study, we manipulated the perceived predation threat using a combination of cues (alarm cue, predator odour and visual exposure) to simulate a high- or low-predation environment. We also manipulated the rearing density, raising guppy juveniles in low or high stocking densities, analogous to densities used in previous studies (Chapman et al., 2008b) to obtain comparable results. We then measured the impact of these factors on several aspects of the fish's behaviour using a series of standard behavioural tests. We assessed the subjects' boldness by measuring their latency to leave a refuge (Burns, 2008), exploration and risk-avoidance with an open field test (Burns, 2008; Warren & Callaghan, 1975), shoaling tendency with a dichotomous shoal choice test (Chapman et al., 2010; Paxton, 1996), and finally their propensity to leave a shoal to forage with a 'shoal versus feeder' test. In contrast to other empirical studies, we specifically confined the experimental treatments in the first 50 days of life and tested the fish in two time points, one shortly after the developmental manipulation and one after a period of 30 days of common-garden conditions to discern effects of recent and early experience, and examine whether early life influences remain fixed or change over time.

METHODS

Overview

We reared guppies in four different developmental treatments by exposing them to either predation cues (conspecific alarm cue and predator dietary and visual cues) or control cues (distilled water and non-predator dietary and visual cues), while housing them at low (~10 fish per tank) or high (~30 fish per tank) densities. After 50 days of such rearing, the fish were given a series of behavioural tests (refuge test, open field test, shoaling test and feeder test) to examine if the manipulated early life conditions had influenced any specific behavioural traits. The subjects were then transferred to new tanks and were further reared for another 30 days in standard 'common-garden' conditions. At day 80 the fish were given the same set of tests to assess if the early environment manipulation had long lasting influences or a delayed effect on their behavioural phenotype (Table I).

Table I. Experimental timeline.

Day	Action
0	Fry introduced in rearing tanks
1	Treatments begin
45	Treatments end
50	1st set of tests (boldness, open field, shoaling, shoal versus feeder) Fish moved to new tanks in common-garden conditions
80	2nd set of tests (boldness, open field, shoaling, shoal versus feeder)

Subjects and Standard Housing Conditions

For rearing and housing of guppies and stimulus fish (pike cichlids and catfish), we used 20-l rectangular glass tanks (40 cm x 20 cm x 25 cm) filled with approximately 18 l of conditioned water and equipped with thermostat heaters, air-driven sponge filters, glass thermometers, plastic plants for shelter and fine gravel as substrate. The water temperature was regulated at 27 ± 0.5 °C while other water parameters (pH, hardness, nitrites, nitrates, ammonia and oxygen concentration) were measured regularly to ensure optimum conditions. Every 15 days 30% of the water was replaced with fresh conditioned water of the same temperature using a fine siphon. The tanks were illuminated by full spectrum fluorescent ceiling lights that turned on automatically at 07:00 and off at 19:00 h, with a 30 minute dawn/dusk period where light intensity was gradually raised or lowered. Guppies came from a population of mixed wild Trinidadian populations, born and raised in captivity. Pike cichlids (*Crenicichla* sp.) were wild-caught in Trinidad, transported to McGill University, and housed in captivity for at least 12 months before the study. Sucker-mouth catfish (*Pterygoplichthys* sp.) were obtained from a commercial supplier (Aquarium du Nord, Montreal, Canada).

Alarm Cue Preparation

Alarm cue was prepared following Brown & Godin (1999) and Brown et al. (2009). A total of 61 guppies, 36 male (mean total length [TL] ± SE: 23.19 ± 0.33 mm) and 25 female (TL ± SE: 28.12 ± 0.82 mm) were sacrificed for alarm cue extraction, providing a total of 77.07 cm^2 of skin tissue. Fish were caught in their housing tanks with a net and were euthanised by immersion in ice water. Then they were decapitated and their internal organs and visceral tissue removed. We measured the surface area of the skin and underlying skeletal muscle tissue and then homogenised with 5 ml of ddH_2O. The solution thus came from multiple individuals and both sexes, thus minimising any possible effects due to sex or individual differences in alarm cue efficacy. The solution was then filtered through filter wool and diluted with ddH_2O until a concentration of $0.1\ cm^2\ ml^{-1}$ was reached. The alarm cue was then divided in aliquots of 12 and 40 ml and was placed in a freezer at -20 °C until needed. Similar aliquots of ddH_2O were also kept in the freezer to be used as controls.

Dietary Cues Preparation

Dietary cues were extracted following Brown et al. (2000). We used four pike cichlids (TL ± SE: 162.65 ± 10.39 mm) and four sucker-mouth catfish (TL ± SE: 155.8 ± 12.07 mm) to collect predator and non-predator (control) odours. The pike cichlids and catfish were initially housed individually in 20-l glass tanks under standard housing conditions, and were provided with frozen bloodworms (Hikari, Hayward, California, USA) and algae wafers (Hikari, Hayward, California, USA) respectively once per day as a staple diet. Five days prior to the cue collection, we changed their diet. Fish have been shown to react more strongly to predator dietary cues when the predator's diet includes fish of their or closely related species (e.g. Brown et al., 2000). Therefore, each pike cichlid was given an average-sized male guppy of mixed wild origin (previously euthanised by immersion in ice water) per day for four consecutive days, in order to produce a potent predator dietary cue. On the other hand, the control dietary cue should not contain any traces of same-species consumption and ideally no traces of fish consumption at all. For this, each catfish was given a blanched spinach leaf per day for four consecutive days, to produce a non-threatening control dietary cue. On the fourth day, the fish were caught with a net, rinsed with conditioned water to avoid any cue transfer and placed individually in new 20-l tanks equipped with only a thermostat heater and an airstone, for cue collection. After 48 h of remaining in the cue collecting tanks, 1 l of water from all four tanks of each species group was mixed in a plastic bucket and the water was aliquoted in 12- and 40-ml portions and placed in a freezer at -20 °C until needed. After the cue collection, all fish returned to their standard housing tank, and some were used to expose the subjects to predator and control visual cues during their rearing.

Subject Rearing and Developmental Treatments

We collected newborn guppy fry (no more than 10 days post birth) from groups of adult females of mixed wild origin that had been placed in large (35 x 23 x 31 cm) custom-made containers with mesh dividers to separate fry and adults.

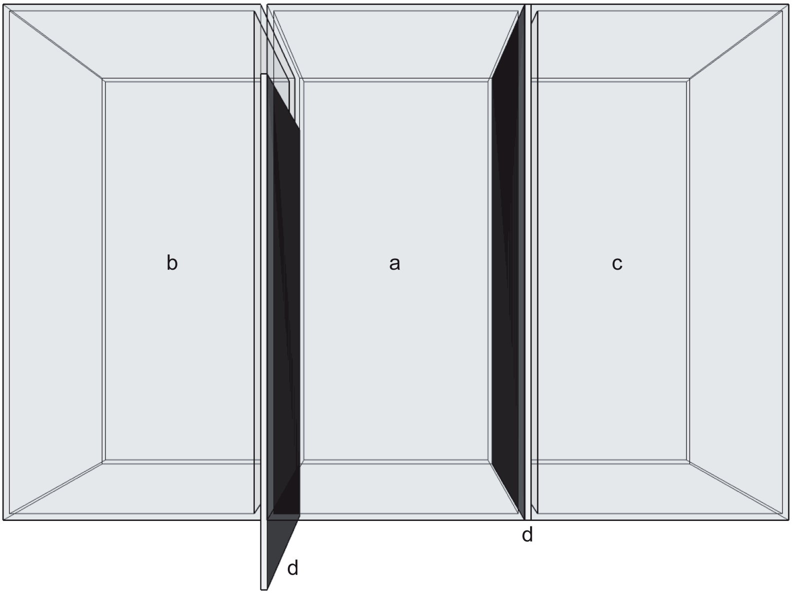


Figure 1. Rearing tanks setup, top view. Labels: a: stimulus fish tank (containing pike cichlid or catfish, depending on treatment); b: LD (or HD) guppy rearing tank; c: HD (or LD) guppy rearing tank. All housing tanks were 40 cm x 20 cm x 25 cm. d: movable plastic divider between tanks, to hide or reveal the stimulus fish when needed.

The fry were caught with a net and were transferred to their designated rearing tank with a plastic cup, where they were acclimated for 5 min and then released to the tank. The fish were fed ad libitum twice daily between 10:00 and 16:00, once with previously re-hydrated decapsulated brine shrimp eggs (Brine Shrimp Direct, Inc., Ogden, Utah, USA) and once with crushed flakes (TetraMin, Tetra, Germany). The daily feeding times and the type of food offered first were decided using a random-number-generating script in AutoIt v3 (version 3.3.10.2).

We used a 2 by 2 factorial design to examine interacting effects of early-life predator cues and housing density on the subsequent behavioural phenotype of the fish. We manipulated the perceived predation regime during early life, by daily exposing the fish to two different sets of cues. In the first experimental condition, the fish were exposed to alarm cue, predator dietary cues and predator visual cues, simulating an environment with a high predator presence. In the second (control) condition, the fish were exposed to non-predator cues, i.e. ddH_2O (the vector of the alarm cue solution) and non-predator dietary and visual cues. In addition, we reared the fish in two different densities, a low density (henceforth 'LD') of approximately 10 subjects per tank and a high density (henceforth 'HD') which was approximately three times the low one (~30 fish per tank). Each LD tank started with 12 fry and each HD tank with 33 fry, to account for unexpected mortality. The tanks were established in a staggered order depending on fry availability and to allow sufficient time to test of subjects at the appropriate later time points. The rearing tanks and the stimulus fish (pike cichlid or catfish that provided the visual cues) tanks were arranged in triplets, so that every stimulus fish tank was between a LD and a HD rearing tank (Fig. 1), thus minimising the number of stimulus fish needed and controlling for any difference in the responses they elicited. We used 3 pike cichlids (TL ± SE: 172.2 ± 5.78 mm) and 3 catfish (TL ± SE: 177.02 ± 9.45 mm) in tanks attached to 6 HD and 6 LD guppy tanks.

Exposure to Cues

Starting one day after their introduction to the tanks and for a total period of 45 days, we exposed the fish to the aforementioned cues once per day, at a random time of day that was generated using a random-number-generating script in AutoIt. This time was set at least 30 min before or after the respective feeding times for that day. The exposures took place from Monday to Friday between 10:00 and 16:00. Four days per week, the predator-exposed fish received 5 ml of predator dietary cue coupled with 5 ml of alarm cue (designed to mimic a successful predator attack) and on the remaining day they received only 5 ml of predator dietary cue. This allowed some variation in the stimuli the fish received while maintaining a high predation threat. Similarly, the control fish received 5 ml of ddH_2O coupled with 5 ml of non-predator dietary cue four days per week, and on the remaining day they received only 5 ml of non-predator dietary cue. The odd day of each week was set using a random number-generating-script in AutoIt. At the designated exposure time, we slowly removed an opaque tan-coloured plastic divider between the subject tank and the stimulus fish tank, to reveal the stimulus fish thus exposing the subjects to predator or non-predator visual cues (Fig. 1). After the divider was removed, the alarm and dietary cues or their respective controls were administered over the centre of the tank, using a 10-ml plastic syringe and a 32-cm-long transparent PVC tube attached to it with a piece of air-line, that allowed delivery from a short distance to minimise disturbance. Subsequently, 10 ml of conditioned water was administered to flush any remaining solution from the syringe and tube. Two different sets of syringe and tube were used for the two types of cues to avoid cross-contamination. Five minutes after the stimulus fish was revealed, the visual cue exposure ended and the divider was slowly returned to its place between the tanks. In total all fish received 31 exposures to either predator or control cues (25 exposures to all cues, 6 exposures to the visual and stimulus fish odour only).

Behavioural Tests

After a period of 5 days without exposure since the fish's last exposure to predator or control cues (to avoid any immediate effects on behaviour), we measured subjects' boldness, exploratory behaviour, shoaling tendency and propensity to leave a shoal to forage, with a series of short tests conducted in succession (Fig. 2). We tested the fish on their designated test day between 09:00 and 17:00. Fish were not fed prior to the test, instead treatment groups were fed after all individuals were tested and returned to their tank at the end of the day. We recorded all procedures from above using a high-definition webcam (C920 HD Pro, Logitech, USA) while observing the test behind a dark plastic sheet to minimise disturbance. We used a group of 28 adult female guppies (TL ± SE: 30.44 ± 0.58 mm) of mixed wild origin as shoal fish for the last two parts of this series of tests.

Boldness Test

The subject is introduced inside a transparent plastic cylinder (5 cm in diameter by 9 cm height), placed in the centre of a refuge (Fig. 2A) made of plastic aquarium plants (Chi boxwood ornament, Fluval, Baie d'Urfé, Canada). After 2 min of habituation, we lifted the plastic cylinder remotely using an attached fishing line, and the fish was free to leave the refuge and explore the rest of the tank (Fig. 2B). The time until the fish left the refuge was recorded. When the fish had emerged from the refuge, the test ended and the refuge was lifted using an attached string. If the subject had not emerged after 5 min, the refuge was lifted and the fish was given a maximum score of 300 s in the test.

Open Field Test

The open field test started as soon as the refuge had been lifted (at the end of the boldness test). The fish was allowed to explore the tank which was divided in 32 squares (Fig. 2C). The number of squares explored and the time the subject spent in the periphery (20 outer squares) and in the centre (12 inner squares) of the tank were recorded for 5 min 30 s.

Shoaling Test

After the open field test, the subject was placed in the transparent cylinder in the centre of the tank. We simultaneously placed two identical transparent plastic containers on both ends of the tank, one containing a shoal of 4 adult females and the other one being empty. Shortly after, the cylinder was lifted and the fish was

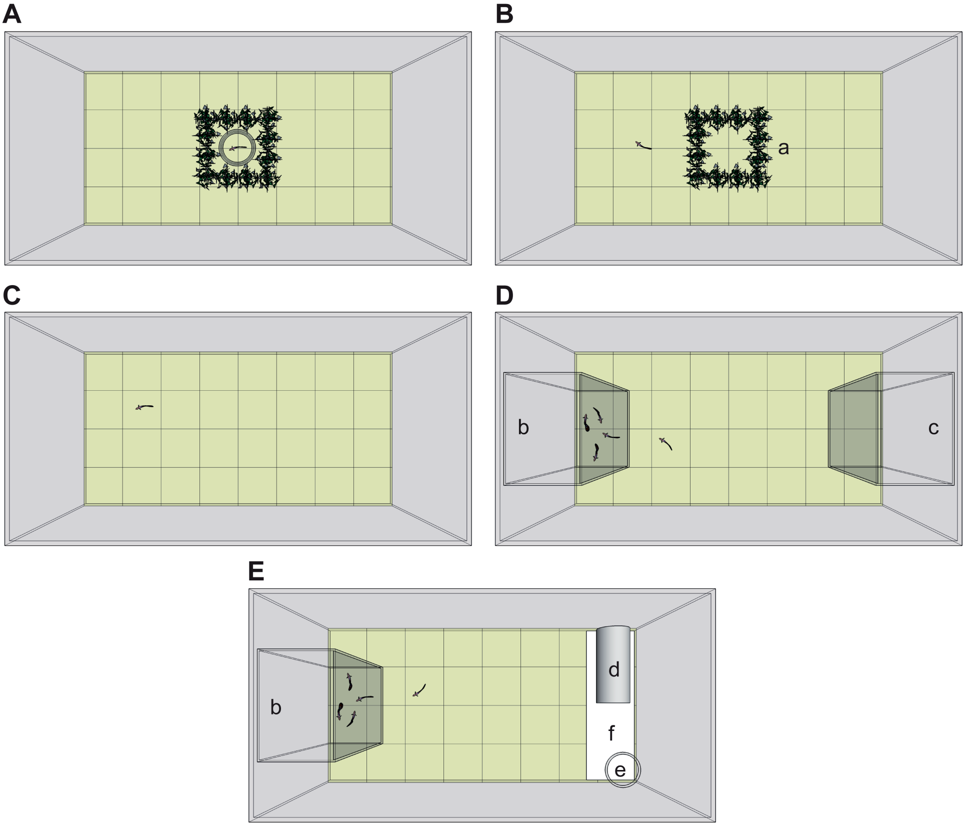


Figure 2. A: Habituation. B: Boldness test. C: Open field test. D: Shoaling test. E: Shoal versus feeder test. Tests were conducted in a 40-l rectangular glass tank (50 cm x 25 cm x 30 cm) filled to a water depth of 6 cm with fresh conditioned water of the same temperature as the subject's housing tank. The tank was covered on all vertical sides with tan plastic sheets to avoid reflections and lighting abnormalities. A tan plastic sheet with 32 squares (6.25 cm x 6.25 cm) drawn using a permanent marker, was placed under the bottom of the tank to aid with recording fish activity during the open field test. Gravel was scattered evenly on the bottom to avoid a bare substrate. A ring (approximately 10 cm x 10 cm) of green plastic plants was placed in the centre of the tank, simulating a densely planted patch acting as a refuge for the boldness test. Labels: a: refuge; b: transparent plastic container (12.3 cm x 8.5 cm x 20 cm) holding a shoal of four adult female guppies; c: identical empty container; d: grey PVC tube (5 cm in diameter x 11.6 cm in length) concealing a fake predator; e: floating feeding ring (5 cm in diameter) made of airline; f: platform the feeding device was based on (24.5 cm x 7.7 cm).

allowed to choose between the two containers (Fig. 2D). The time spent in proximity (within an approximately 4-cm-wide zone) of ('shoaling') and in contact with ('interacting') each container was measured for 5 min 30 s.

Shoal versus Feeder Test

Following the end of the shoaling test, the empty container was replaced with an apparatus concealing an artificial predator (Storm WildEye Live Perch, Rapala VMC Corporation, Vääksy, Finland) inside a PVC tube, with a floating ring feeder alongside (Fig. 2E). The fake predator was attached to a fishing line and could be pulled manually (behind the hide) to simulate an attack when the subjects approached the feeder to feed. The time spent in proximity of and in contact with the shoal container was measured as in the shoaling test. In addition, the number of approaches to the feeder (defined as a switch from the half of the tank containing the shoal to the other half of the tank containing the feeder) was recorded. Finally, we counted the number of entries to and the time spent in the feeder zone. This test lasted 5 min. In the first set of tests (at day 50), if a subject entered the feeder zone and started feeding from the floating feeder, it was exposed to a simulated predator attack. In the second set of tests (at day 80), no subjects were exposed to such an attack – even if they fed from the feeder – to avoid having adverse effects on their performance in the social learning test 5 days later.

After being tested with this set of tasks, the fish were transferred to new tanks (identical to the previous ones) and were raised in common-garden conditions (no daily cue exposures and all tanks at low fish densities) for another period of 30 days. After this period of common-garden conditions, the fish were retested (at day 80) with the same set of tests.

Data Collection and Statistical Analysis

All resulting videos from the different tests were coded in JWatcher (v1.0, Blumstein et al., 2006) by a different observer who was blind to the treatments. Data were analysed with factorial ANOVAs using SPSS v20 (IBM, USA). Normality and homoscedasticity were assessed with Shapiro-Wilk's and Levene's tests respectively. If these failed, the variables were transformed using square root or logarithmic transformations, while ratios were transformed using the arcsine transformation. If ANOVA assumptions were still not met, data were analysed with the non-parametric Kruskal-Wallis *H* test. Comparing a group mean to a single value was performed using Student's *t*-test or the non-parametric equivalent Wilcoxon signed ranks test if the *t*-test assumptions were not achieved. The Kaplan-Meier survival analysis was performed on the data obtained from the boldness test to account for the large number of subjects that had not left the refuge at the 5-min mark. Pairwise comparisons following ANOVAs were conducted with Tukey HSD post hoc tests. In comparisons following Kruskal-Wallis and Kaplan-Meier tests, *P*-values were adjusted by the statistical software to control for multiple comparisons.

Ethical Note

All procedures were carried out in accordance with Canadian law and Canadian Council on Animal Care and ASAB guidelines, and were approved by the Animal Care Committee of McGill University (Protocol #7133). Anaesthetic overdose was not used in the euthanasia of guppies for alarm substance, as this would have contaminated the alarm substance, potentially influencing experimental outcomes. Moreover, recent studies indicate that immersion in ice-cold water is a much faster, more effective and more humane method of euthanasia for small tropical fish such as the guppy than anaesthetic (Blessing et al., 2010; Matthews & Varga, 2012; Wilson et al., 2009). We used aversive stimuli in our manipulation of perceived predator threat, namely alarm and predator odour cues and visual exposure to a predator behind a transparent barrier. These stimuli were essential to our investigation of responses to predation. We limited exposure to these stimuli, used a relatively small sample size to minimise the number of animals exposed, and monitored stress-related behaviour in subjects both during and after exposure. In all cases subjects returned to normal behaviour shortly after the end of exposure. At the conclusion of the study fish were maintained in breeding populations at McGill University.

RESULTS

During the experimental manipulations, we observed the behaviour of the subjects to assess the efficacy of our exposures. All treatment groups were mildly disturbed by the removal of the opaque partition. On exposure to the pike cichlid predator, subjects were observed to shoal more tightly and perform predator inspections, whereas these effects were not observed on exposure to the catfish non-predator (IL, personal observation). Significant sex differences and treatment effects are summarised in Table II.

Table II. Summary table of the results. Significant ($P < 0.05$) results are listed. HD: high density; LD: low density.

	Day	Result
Sex differences	50	Males spent more time interacting with a shoal than females
		Males spent more time in the open field centre than females
		Males spent more time shoaling than females
	80	Males emerged from refuge sooner than females
		Males explored more squares in the open field than females
		Males spent more time interacting with a shoal than females
		Females spent more time shoaling than males in the presence of a feeder
		Males made more approaches to a feeder than females
		Males made more entries to a feeder than females
		Females spent more time in a feeder zone than males
Treatment differences	50	No pred. HD males spent more time interacting than no pred. HD females
		HD fish shoaled more than LD fish in the presence of a feeder
		LD fish spent more time in a feeder zone than HD fish
		HD males shoaled more than LD males in the presence of a feeder
		LD males made more approaches to a feeder than HD males
		No pred. LD males made more approaches to a feeder than no pred. HD males
	80	No pred. males emerged from a refuge sooner than no pred. females
		HD males emerged from a refuge sooner than HD females
		Pred. LD fish explored more squares in the open field than no pred. LD fish
		Pred. LD fish spent more time in the open field centre than pred. HD fish
		No pred. males spent more time interacting with a shoal than no pred. females
		No pred. males spent more time interacting with a shoal than pred. males
		HD males made more approaches to a feeder than HD females
		HD males made more entries to a feeder than HD females

Boldness Test

At day 50 there was no significant effect of predation risk, rearing density or sex on the ‘time to emerge’ from the refuge. At day 80, males emerged from the refuge significantly faster than females (Kruskal-Wallis test, $H_{1,n=118} = 6.52$, $P = 0.011$; Fig. 3A). The survival distributions also differed significantly between male and female subjects, confirming that males emerged faster than females (Kaplan-Meier test, $\chi^2_1 = 7.74$, $P = 0.005$; Fig. 3B). Survival analysis also revealed a significant inter-

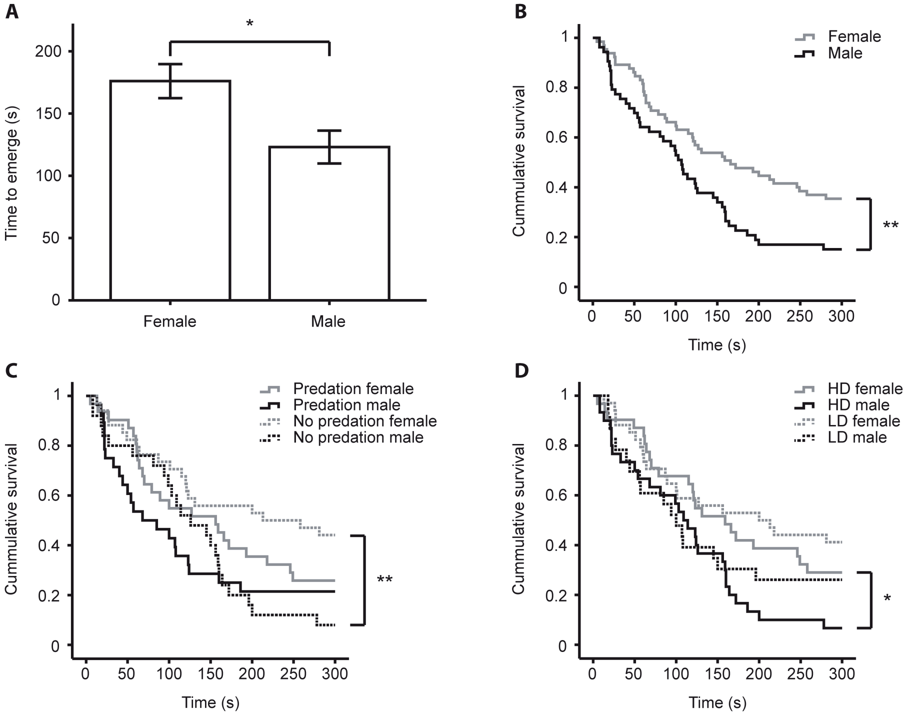


Figure 3. Boldness test results, day 80. A: Time to emerge from the refuge for males and females; B: Survival distributions (Kaplan-Meier test) for males and females; C: Survival distributions for 'predation' and 'no predation' males and females; D: Survival distributions for HD and LD males and females. *: $P < 0.05$; **: $P < 0.01$. Bars represent the standard error of the mean.

action between 'predation risk' and 'sex' (Kaplan-Meier test, $\chi^2_3 = 9.37$, $P = 0.025$). 'No predation' females spent the longest time to emerge, significantly longer than 'no predation' males ($P = 0.005$; Fig. 3C). There was also a significant interaction between 'rearing density' and 'sex' (Kaplan-Meier test, $\chi^2_3 = 9.56$, $P = 0.023$), with HD males emerging from the refuge significantly faster than HD females ($P = 0.021$; Fig. 3D).

Open Field Test

In the open field test, we predicted that the fish would generally prefer to swim near the covered walls of the tank instead of swimming in the open centre. Indeed, at day 50 and 80 the fish preferred to stay in the peripheral 20 squares compared to the central 12 squares of the tank. The observed median of this measure (Day 50: 0.16; Day 80: 0.12), differed significantly from the hypothesised value based on chance (0.375; based on the relative areas of the periphery and centre) (Wilcoxon signed ranks tests, Day 50: $Z_{n=123} = 762.5$, $P < 0.001$; Day 80: $Z_{n=119} = 292$, $P < 0.001$).

At day 50, males spent more time in the centre than females, but this effect was marginally non-significant (ANOVA, $F_{1,113} = 3.92$, $P = 0.05$; Fig. 4A). When the sexes were analysed separately, a significant interaction between 'predation environment' and 'social environment' was revealed in the case of males (Kruskal-Wallis test, $H_{3,n=52} = 8.77$, $P = 0.033$; Fig. 4B).

At day 80, males explored more squares than females (Kruskal-Wallis test, $H_{1,n=118} = 4.68$, $P = 0.031$; Fig. 4C). There was a significant interaction between 'predation risk' and 'rearing density' (Kruskal-Wallis test, $H_{3,n=119} = 11.69$, $P = 0.009$) with 'no predation LD' fish differing significantly from 'predation LD' ones (adj. $P = 0.024$; Fig. 4D). There was also a significant interaction between 'predation risk', 'rearing density' and 'sex' (Kruskal-Wallis test, $H_{7,n=118} = 17.08$, $P = 0.017$; Fig. 4E) but there were no significant pairwise comparisons. For 'preference for centre' there was a significant interaction between 'predation risk' and 'rearing density' (Kruskal-Wallis test, $H_{3,n=119} = 7.99$, $P = 0.046$), with predator-exposed LD fish differing significantly from predator-exposed HD fish (adj. $P = 0.032$; Fig. 4F). Finally, when the sexes were analysed separately, a significant interaction between 'predation risk' and 'rearing density' for number of squares explored was revealed for the males (Kruskal-Wallis test, $H_{3,n=53} = 7.88$, $P = 0.048$; Fig. 4G) indicating that it was mainly this sex driving the overall observed pattern.

Shoaling Test

The fish preferred the shoal versus the empty container, as we would predict in a basic sociability test. At both testing days, day 50 and 80, the median of the measure 'time shoaling difference' (225.07 and 263.38 respectively) differed significantly from 0 (the hypothesised value if the fish showed no preference between the two areas; Wilcoxon signed ranks tests, $Z_{n=111} = 5910$, $P < 0.001$ and $Z_{n=115} = 6581$, $P < 0.001$ respectively). The subjects also showed a preference to interact with the shoal versus the empty container, since the median of 'time interacting difference' (56.48 and 76.74 respectively) was significantly different from 0 (Wilcoxon signed ranks tests, $Z_{n=111} = 5354$, $P < 0.001$ and $Z_{n=115} = 6418$, $P < 0.001$ respectively).

At day 50, males spent significantly more time near the shoal than females (ANOVA, $F_{1,103} = 4.57$, $P = 0.035$; Fig. 5A). Males also spent more time interacting with the shoal (ANOVA, $F_{1,103} = 6.37$, $P = 0.013$; Fig. 5B) and more time interacting while shoaling than females (ANOVA, $F_{1,103} = 4.97$, $P = 0.028$; Fig. 5D). For 'time interacting difference', the interaction between 'predation risk', 'rearing density' and 'sex' is marginally non-significant (ANOVA, $F_{1,103} = 3.64$, $P = 0.059$), with 'no predation HD' males differing significantly from 'no predation HD' females (Tukey HSD test, $P =$

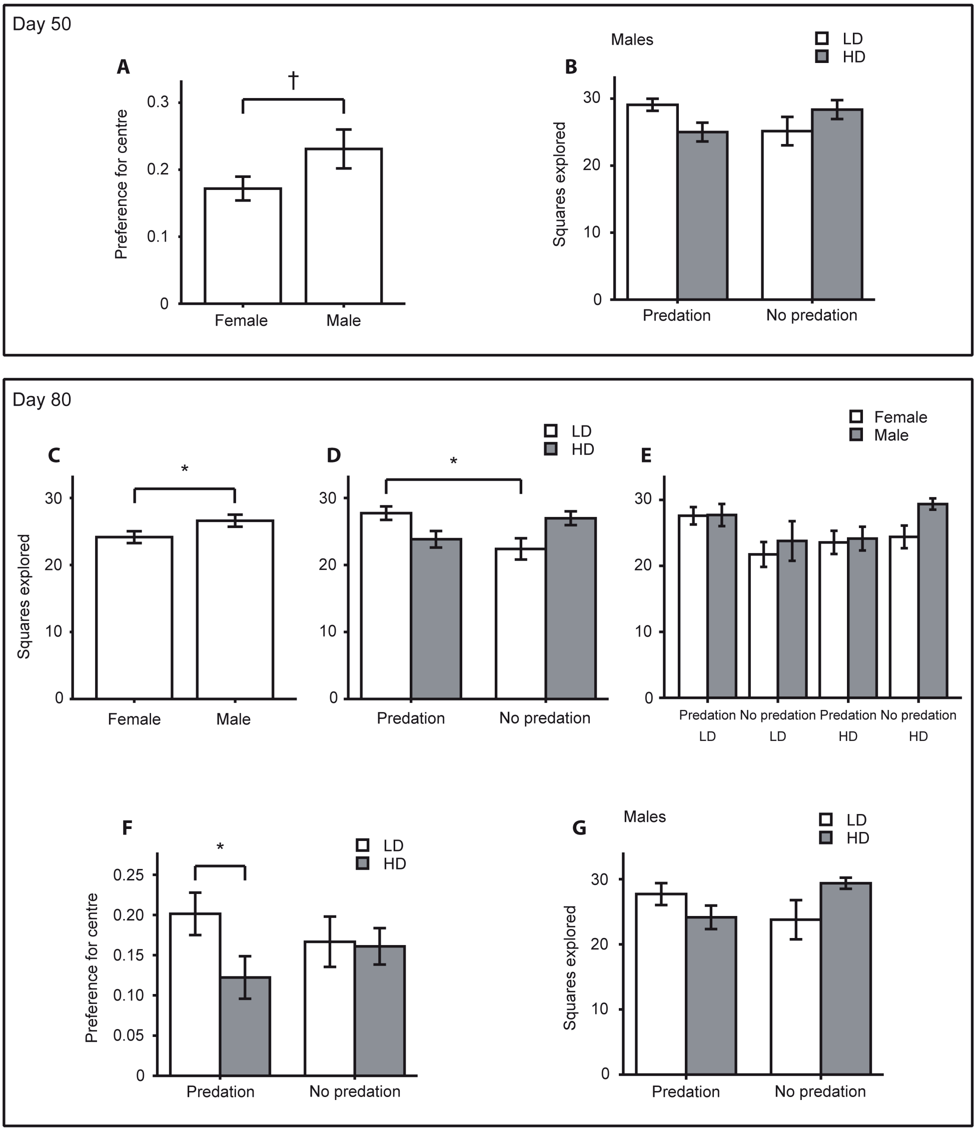


Figure 4. Open field test results. A and B: day 50; C, D, E, F and G: day 80. †: $P = 0.05$; *: $P < 0.05$. Bars represent the standard error of the mean.

0.044 Fig. 5C). Therefore we see a difference between males and females only in the 'no predation HD' treatment. When sexes were analysed separately a significant interaction between 'predation risk' and 'rearing density' was revealed for the females (ANOVA, $F_{1,63} = 4.34$, $P = 0.041$), but there were no significant differences between groups.

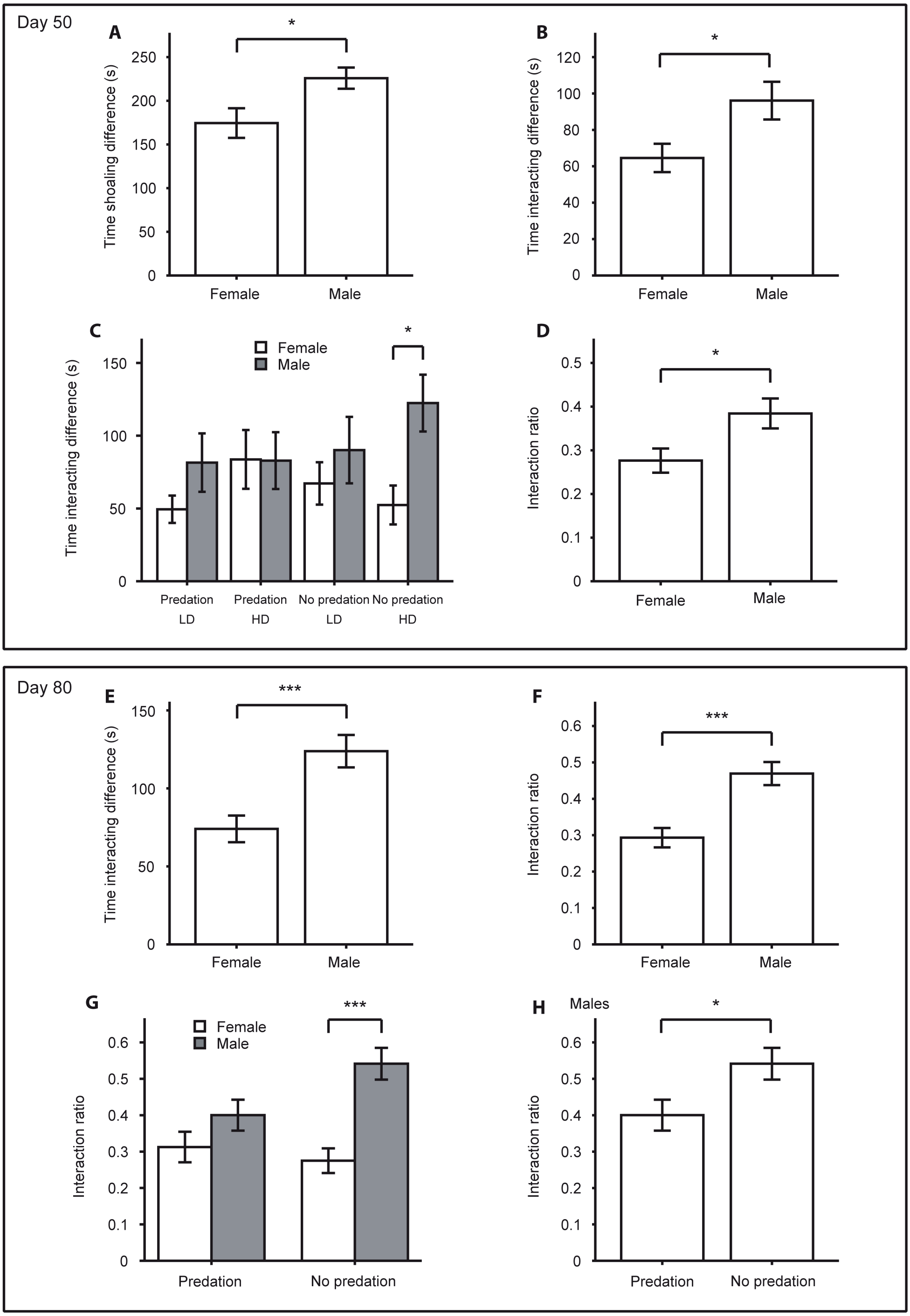
At day 80, males spent again more time interacting with the shoal (ANOVA, $F_{1,106} = 13.53$, $P < 0.001$; Fig. 5E) and interacting while shoaling than females (ANOVA, $F_{1,105} = 16.73$, $P < 0.001$; Fig. 5F). There was also a significant interaction of 'predation risk' and 'sex' for the same measure (ANOVA, $F_{1,105} = 4.92$, $P = 0.029$). The 'no predation' male group differed significantly from 'no predation' female (Tukey HSD test, $P < 0.001$) and predation female (Tukey HSD test, $P = 0.001$) groups (Fig. 5G). Hence we saw a difference between sexes in the 'no predation' but not in the 'predation' treatment. When sexes were analysed separately, we found that predator-exposed males spent more time interacting while shoaling than non-exposed males (Kruskal-Wallis, $H_{1,n=51} = 4.3$, $P = 0.038$; Fig. 5H).

Shoal versus Feeder Test

At day 50, HD fish spent more time near the shoal (Kruskal-Wallis test, $H_{1,n=117} = 9.51$, $P = 0.002$; Fig. 6A) and less time in the feeder zone (Kruskal-Wallis test, $H_{1,n=117} = 4.7$, $P = 0.03$; Fig. 6B) than LD fish. There was also a significant interaction between 'predation risk' and 'rearing density' (Kruskal-Wallis test, $H_{3,n=117} = 9.52$, $P = 0.023$) and between 'rearing density' and 'sex' (Kruskal-Wallis test, $H_{3,n=115} = 12.18$, $P = 0.007$) for 'time shoaling', but without any informative significant differences between groups. When sexes were analysed separately, we found that HD males spent more time shoaling (Kruskal-Wallis, $H_{1,n=46} = 7.4$, $P = 0.007$; Fig. 6C) and did fewer approaches to the feeder (Kruskal-Wallis, $H_{1,n=46} = 5.57$, $P = 0.018$; Fig. 6D) than LD males. There was also a significant interaction between 'predation risk' and 'rearing density' for 'feeder approaches' in the case of males (Kruskal-Wallis, $H_{3,n=46} = 8.43$, $P = 0.038$), with 'no predation LD' males performing more approaches than 'no predation HD' males (adj. $P = 0.049$; Fig. 6E).

At day 80, we found significant differences between sexes in all our measures. Females spent more time shoaling than males (Kruskal-Wallis, $H_{1,n=118} = 4.89$, $P = 0.027$; Fig. 6F). Males made more approaches to the feeder (Kruskal-Wallis, $H_{1,n=118} = 7.3$, $P = 0.007$; Fig. 6G) and entered the feeder zone more often than females (Kruskal-Wallis, $H_{1,n=118} = 7.11$, $P = 0.008$; Fig. 6H). However females spent more

Figure 5. Shoaling test results. A,B,C and D: day 50; E,F,G and H: day 80. *: $P < 0.05$; ***: $P < 0.001$. Bars represent the standard error of the mean.


Day 50
A
Time shoaling difference (s)
0
50
100
150
200
250
*
Female
Male
B
Time interacting difference (s)
0
20
40
60
80
100
120
*
Female
Male
C
Time interacting difference (s)
0
50
100
150
☐ Female
■ Male
*
Predation LD
Predation HD
No predation LD
No predation HD
D
Interaction ratio
0
0.1
0.2
0.3
0.4
0.5
*
Female
Male
Day 80
E
Time interacting difference (s)
0
50
100
150
***
Female
Male
F
Interaction ratio
0
0.1
0.2
0.3
0.4
0.5
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***
Female
Male
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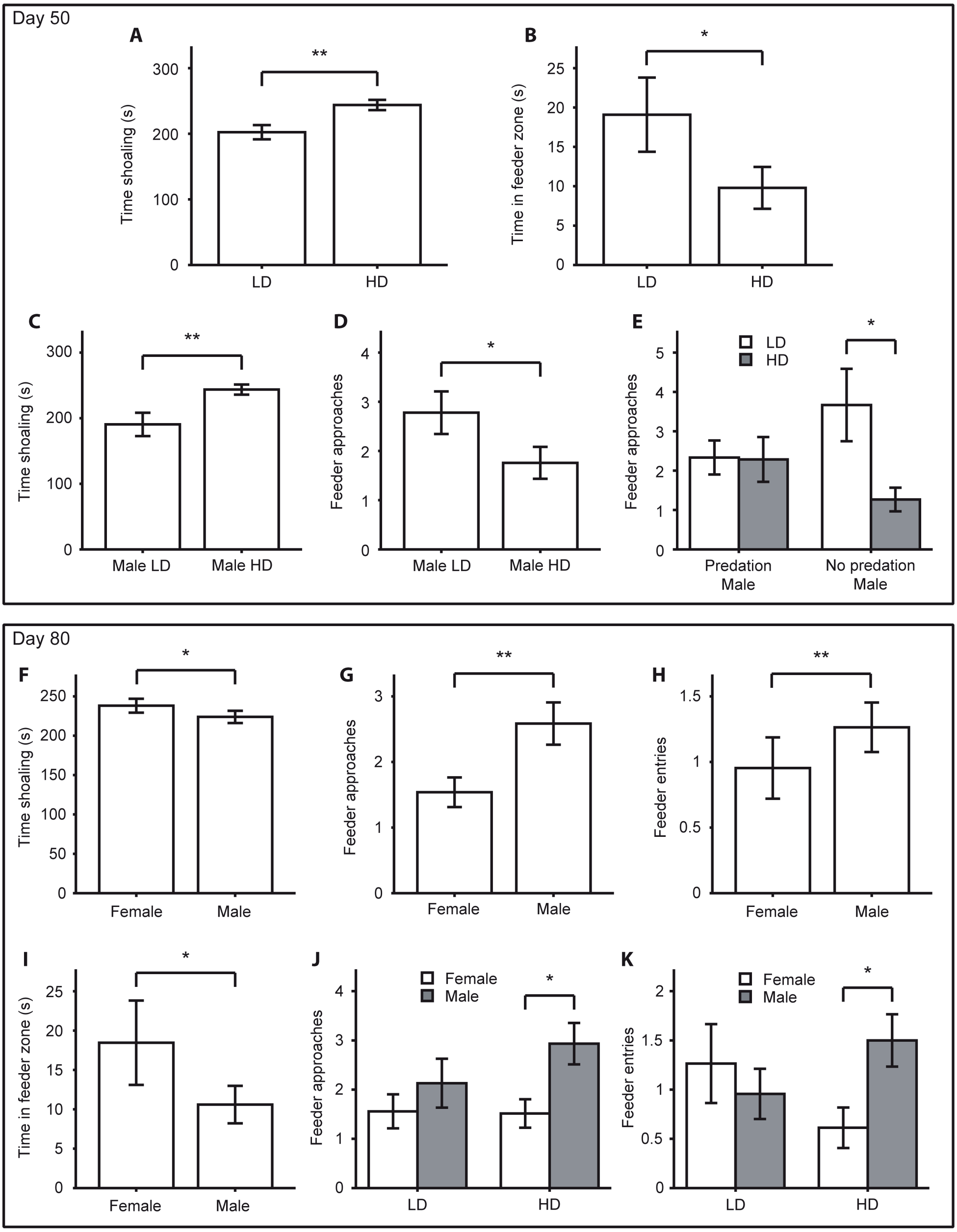

Figure 6. Shoal versus feeder test results. A, B, C, D and E: day 50; F, G, H, I, J and K: day 80. *: *P* < 0.05; **: *P* < 0.01. Bars represent the standard error of the mean.

time in the feeder zone than males (Kruskal-Wallis, $H_{1,n=118} = 5.26$, $P = 0.019$; Fig. 6I). There were significant interactions between 'predation risk' and 'sex' for 'feeder approaches' (Kruskal-Wallis, $H_{3,n=118} = 8.6$, $P = 0.031$) and 'feeder entries' (Kruskal-Wallis, $H_{3,n=118} = 8.05$, $P = 0.045$), but without any informative group differences. There were also significant interactions of 'rearing density' and 'sex' for 'feeder approaches' (Kruskal-Wallis, $H_{3,n=118} = 9.98$, $P = 0.019$; Fig. 6J) and 'feeder entries' (Kruskal-Wallis, $H_{3,n=118} = 9.07$, $P = 0.028$), with HD males entering the feeder zone more often than HD females (adj. $P = 0.026$; Fig. 6K).

DISCUSSION

Sex Differences

The most prominent outcome from our analysis is that the two sexes differ significantly in all of our behavioural tests, and in most cases these differences appear to overshadow any developmental influences. Males exhibited higher boldness than females, emerging from a refuge faster and spending more time in the centre of an open field than females. Males were also more exploratory than females, in terms of squares explored in the open field test. Males spent more time shoaling and interacting with a shoal when only a shoal was present, but when given a choice between a shoal and a feeder, they were more likely to leave the shoal and approach the novel feeder in an area that could hide a predator, again a possible indicator of a higher propensity to take risks.

The observed strong sex difference in boldness is consistent with previous empirical studies demonstrating that males are generally bolder than females in poeciliid fishes (Brown et al., 2007; Harris et al., 2010; Piyapong et al., 2009). For example, Harris et al. (2010) reported male guppies to be significantly bolder than females, as they emerged faster and spent less time near a shelter in a boldness task. Piyapong et al. (2009) found a similar pattern with males appearing bolder than females when tested in single-sexed groups in a simulated aerial predator attack test. Female guppies are generally more risk averse and show higher anti-predator reactions compared to males (Magurran & Seghers, 1994b), for example they school more and perform more predator inspections, but from a longer distance (Magurran et al., 1992). This well-documented difference of the sexes in their propensity to take risks could be explained by differences in life history strategies. Female guppies are known to grow indeterminately (Dussault & Kramer, 1981), and body size is correlated with fecundity (Magurran & Seghers, 1994b; Reznick et al., 1990). Therefore, female reproductive success would increase if they could increase longevity and fecundity by being shyer and more risk-avoiding, depending on the relationship between risk-taking and foraging success in the current environment. Males on the other hand, which cease to grow at maturation (Dussault & Kramer, 1981), can maximise their fitness only by increasing their mating opportunities, therefore they are expected to engage in more risky behaviours (Harris et al., 2010; Magurran & Seghers, 1994b).

Males visited more squares in the open field compared to females. This contrasts with the findings of Laland & Reader (1999a), who found females were more likely to complete foraging tasks that required exploration of a simple novel maze. They argued that sex differences in hunger levels and/or parental investment could result in greater exploratory behaviour in females. In the present study, the exploratory behaviour was assessed in a non-foraging context (no food cues were present in the tank) and it followed the emergence from a shelter. Thus perceived predation risk or food cues could be responsible for the different patterns observed. Female fecundity is dependent on body size (Reznick et al., 1990) and gravidity can increase energetic needs due to excess allocation of energy to the developing embryos (Ghalambor et al., 2004), thus females are potentially more motivated to explore novel foraging options than males. In addition, longevity is also crucial for female reproductive success (Griffiths & Magurran, 1998; Magurran & Seghers, 1994b; Reznick et al., 1990), therefore as predation risk increases, they are expected to reduce their exploratory behaviour to increase survival. We had some evidence for consistency in male exploratory behaviour across days 50 and 80, since we observed the same pattern between treatment groups. This is in line with previous findings demonstrating individual consistency of exploratory tendencies in birds (Dingemanse et al., 2002), fish (Nomakuchi et al., 2009), and guppies in particular (Budaev, 1997; Harris et al., 2010).

Our finding that males shoaled more than females is in contrast to previous findings reporting increased schooling tendencies in females (Magurran & Seghers, 1991,1994b; Magurran et al., 1992). Males are constantly in search for sexually receptive females which causes them to frequently switch between shoals and are thus expected spend less time shoaling than females (Croft et al., 2003; Hughes et al., 1999). However, females prefer to join shoals of familiar female individuals whereas males do not present such a preference (Griffiths & Magurran, 1998). In fact, male guppies have been shown to direct more courtship towards unfamiliar females (Kelley et al., 1999). In our study, subjects were unfamiliar with the stimulus shoal fish and this might have affected the shoaling choices of males and females in different ways. Moreover, it is possible that the observed increased shoaling tendencies of males in the shoaling test were a result of high motivation to mate with the shoal members (Griffiths & Magurran, 1998). Relative to this, in our test males spent more time interacting with the shoal compared to females at both testing time points, and this pattern was mainly driven by the 'no predation' treatment group. In fact, males from the 'no predation' treatment interacted more with the shoal than predator-exposed males. A possible explanation for this could be that predator-exposed males potentially joined the shoal to reduce predation risk and therefore spent less time interacting with it, while non-exposed males showed increased interaction ratios due to higher motivation to mate with the females of the shoal.

However, in the ‘shoal versus feeder test’ we saw a reversed pattern, with females exhibiting increased levels of shoaling compared to males. The main difference between the two tests was that in the latter, a novel apparatus was introduced in the test tank. Both male and female guppies are known to increase their shoaling behaviour in the presence of predator cues, but this effect is greater in females (Magurran & Nowak, 1991). Our novel apparatus consisted of a floating feeder and a predator model concealed inside an opaque tube. Hence, if the apparatus was indeed perceived as a potential threat, it could explain the increase in shoaling behaviour in both sexes but more so in the case of female subjects. Males made generally more approaches to the feeder and entered the feeder zone more times than females. As described earlier, male guppies are more likely to leave a shoal than females and have been found to switch between shoals more frequently compared to females (Croft et al., 2003). However, the driving force for this propensity appears to be the males’ constant tendency to find novel females, in order to increase their mating chances and not locating new food sources, which was our intended context in this test.

Developmental Effects

We did not find compelling evidence of a direct effect of predation regime on boldness or exploratory behaviour in our study. Exposure to predator cues has been shown to affect several behavioural and physiological characteristics across a wide range of taxa (Bell et al., 2011; Dingemanse et al., 2009; Gonzalo et al., 2012; Rundle et al., 2011) and particularly in fish (Bell et al., 2011; Brown et al., 2000; Dingemanse et al., 2009; Handelsman et al., 2013; O’Connor et al., 2015). High predation risk should theoretically elicit decreased activity levels and lower boldness in guppies, as risk-averse and cryptic behaviours are expected to confer selective advantages in high-predation environments (Magurran, 2005), but such clear responses were not observed here. We also did not detect significant differences between predation treatments in grouping tendency, neither in the ‘shoaling test’ nor in the ‘shoal versus feeder’ test. This is in contrast with previous findings that predict increased shoaling tendencies associated with high predation risk (Magurran, 2005; Magurran & Seghers, 1991, 1994a; Seghers, 1973, 1974). This could be due to ceiling effect in our shoaling test, with all treatment groups presenting high shoaling tendencies when encountering a novel and potentially dangerous environment, thus overshadowing any treatment effects, but it cannot explain the same pattern found in the ‘shoal versus feeder’ test where sociability could be traded off for foraging opportunities. The documented influences of predation regime on guppy behavioural, morphological and life historical traits (Endler, 1995) can be attributed to a combination of genetic predispositions shaped by selection in an evolutionary timescale and past experiences with predators in an ecological timescale (Lima & Dill, 1990). In our effort to tease these two driving forces apart we used subjects of mixed wild origin, coming from a variety of wild populations from several different river systems of Trinidad, thus creating high genetic variation regarding population history of predation and we specifically manipulated predation risk during early life by exposing them to either predator or control cues. The mixed origin of our subjects could have increased individual variation in our behavioural measures to an extent that would mask more subtle treatment effects. Another possible explanation for not detecting strong treatment effects could be that our predator cues were not sufficient to elicit the anticipated response. In contrast to previous studies (e.g. Krause & Liesenjohann, 2012) we housed guppies and stimulus fish (predators and control fish) in separate tanks to accurately control exposure to each cue and avoid habituation to the continuous presence of the predator. In addition, contrary to other studies (e.g. Dalton & Flecker, 2014) we specifically used predator dietary cues collected after consumption of euthanised guppies and frequently coupled this cue with alarm cue to simulate the presence of a highly successful predator, thus maximising the level of perceived risk. Moreover, instead of relying purely on administering chemical cues to manipulate predation risk (Dalton & Flecker, 2014; Handelsman et al., 2013; Torres-Dowdal et al., 2012), we additionally used visual cues to strengthen the subjects' experience with the predator, as visual cues have been found to also elicit strong behavioural responses (O'Connor et al., 2015). Finally, the use of sucker-mouth catfish as our non-predatory stimulus fish represented a more natural control, compared to other studies which either used species that are known to prey on juveniles (e.g. swordtails, *Xiphophorus helleri* – Krause & Liesenjohann, 2012) or did not include any control stimulus fish at all (Dalton & Flecker, 2014; Handelsman et al., 2013; Torres-Dowdal et al., 2012). All these measures, combined with the observation of the subjects' behaviour during the daily exposures, make the supposition that weak predator cues resulted in the observed weaker treatment effects, less likely.

Rearing density had a significant effect on time shoaling in the 'shoal versus feeder' test, which is in line with previous studies demonstrating changes in social behaviour in response to rearing density (Brockmark & Johnsson, 2010; Chapman et al., 2008b). However, in contrast to previous reports of low-density rearing resulting in higher grouping tendencies (Chapman et al., 2008b), we found that fish reared in high densities exhibited increased shoaling behaviour compared to fish from low densities. This could be partially explained by the difference in housing conditions between the two studies. Chapman et al. (2008b) housed guppies in densities of 1-4 in the low density treatment and 7-12 fish in the high density treatment. In the present study, we kept approximately the same densities per litre of water, but we used larger rearing tanks and thus housed our subjects in larger groups (~10 fish per tank in the low density treatment and ~30 fish per tank in the high density treatment). It could therefore be that group size and not density are actually responsible for the observed effects in shoaling behaviour. Another possible explanation for the contradicting results could be the difference in our experimental design regarding confining manipulations strictly to early life. After 45 days of rearing in the designated densities, we ceased the treatments and transferred the fish in new tanks and in low densities, whereas Chapman et al. (2008b) kept the experimental manipulations for 60 days after which they conducted the shoaling test, which makes the discrimination between past and recent experience of housing density impossible in their study.

We found evidence for significant interactions between predation risk and sex regarding time to emerge from a refuge and between predation risk and rearing density regarding preference for the centre in the open field. Our survival analysis on time to emerge from a refuge revealed that predator-exposed males are not bolder than predator-exposed females and the overall observed sex difference is driven by the non-exposed fish. Exposure to predator cues could thus result in more uniform reactions by the two sexes, while the lack of prior experiences with predators might allow the sex differences to develop. Predator-exposed fish reared in low densities were more exploratory than non-exposed fish from the same density in terms of squares explored in the open field test. Brown & Braithwaite (2005) reported a similar pattern in bishop fish, *Brachyraphis episcopi*, a poeciliid fish closely related to the guppy. They found that fish from high-predation sites spent more time exploring the test maze compared to low-predation fish which solved the maze significantly faster. They argue that under predation threat, it might be beneficial to thoroughly explore a novel environment like the test tank to gather information about shelters and escape routes or potential predators. Overall, predation risk and rearing density appeared to have non-additive effects in most of our tests. According to theoretical predictions, increased predation pressure should result in increased grouping tendencies and decreased aggression among individuals (Brown & Godin, 1999; Magurran & Seghers, 1991, 1994a; O'Connor et al., 2015; Wisenden & Sargent, 1997), while on the contrary, high population densities are associated with decreased grouping tendencies and increased aggression (and competition) between group members (Chapman et al., 2008b; Manenti et al., 2015). Finally, in natural populations predation can itself affect density by increasing the mortality rates of prey animals (Kotler & Holt, 1989) which renders the relationship between those factors even more complex.

Concluding Remarks

The present study further illustrates the existence of strong sex differences in the development of behavioural phenotypes, and supports the existing evidence that males and females follow different strategies to maximise survival and fitness. We also found limited evidence that early life predation risk and density can influence behaviour, mainly through complicated interactions between them and sex, implying that behavioural plasticity induced by early life conditions is itself flexible and dependent on other factors. These early environment influences can be expressed differentially in the two sexes, by either widening or eliminating the gap between them regarding specific traits. Our results suggest that sex differences, which are often neglected by pooling data or simply avoided by testing a single sex, should be acknowledged and controlled for in behavioural studies, in order to develop a more complete understanding of the study system's ecology. We showed that conditions confined specifically in early life can have long lasting effects in the adult phenotype, even after a period of common-garden conditions, but whether these effects reflect adaptive adjustments to different environments remains an open question. Further work involving such developmental approaches, and with the addition of tests examining the existence of developmental windows and the possibility of reversal of these early environment influences, would expand our current knowledge on this topic.

ACKNOWLEDGEMENTS

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Chapter 5

Early life predation risk and rearing density influence social information use and social learning in guppies

Ioannis Leris and Simon M. Reader

(to be submitted to 'Animal Behaviour')

ABSTRACT

Conditions and experiences in early life can influence the way animals value and use social information later on, and certain cues have been proposed to play a major role in adaptively programming juveniles to better match their environment when adult. Here, we examined whether early life predation risk and rearing density can affect social information use and learning in guppies, *Poecilia reticulata*. We manipulated perceived predation risk and housing density during the first 45 days of rearing, and after a period of common-garden conditions we measured the subjects' propensity to use a social cue (shoal cohesion) to make decisions in a novel and potentially dangerous environment. Our results suggest that fish previously exposed to predator cues and particularly male fish and fish reared in high densities used this cue to make grouping decisions (when the shoals were present) or location decisions (when the shoals were absent). We discuss the main implications of these findings, which further support the idea that reliance on social information can be developmentally plastic.

INTRODUCTION

Animals are often faced with unpredictable or variable environments where gathering individual information can be costly or dangerous. Thus, in order to minimise personal risk and effort and to maximise accuracy of estimates of quality, animals can acquire information about their environment by using social information, i.e. information from other knowledgeable individuals. For example, wood crickets, *Nemobius sylvestris*, copy predator-avoidance behaviours from informed individuals (Coolen et al., 2005a), and female guppies copy mate choices from their conspecifics (Dugatkin & Godin, 1992). Social learning, a subset of social information use where an animal's observation of or interaction with other individuals results in learning (Heyes, 1994; Hoppitt & Laland, 2013; Reader & Biro, 2010), is widespread in the animal kingdom, as it can potentially confer important adaptive benefits in various fitness-related activities such as foraging, reproduction and predator avoidance (Danchin et al., 2004; Hoppitt & Laland, 2013; Reader & Biro, 2010; Whiten et al., 2016). However, social information can be less reliable than privately gathered information, and in rapidly changing environments it can quickly become outdated. Animals would thus benefit if they were flexible in their reliance to social information to learn about their environment, depending on the current costs of acquiring individual information. Predation threat usually poses the highest risk when sampling the environment individually; therefore animals are expected to turn to social information more often when predation threat rises. Minnows, *Phoxinus phoxinus*, for example, have been found to increase their reliance on social learning to make foraging decisions when predation risk increases (Webster & Laland, 2008). There are several examples of animals following different social learning strategies regarding when and who to copy (Laland, 2004), but it still remains unclear whether early environment conditions and previous experience can directly shape an individual's tendency to seek out social information and learn from it.

There is increasing evidence, that early life conditions have paramount effects on the subsequent life of organisms. Early life predation risk poses a strong selective force and is known to induce a variety of morphological and behavioural changes in animals. Sticklebacks, *Gasterosteus aculeatus*, for example, increase their growth when exposed to cues of a size-selective predator (Bell et al., 2011) and gastropods alter the timing of developmental events in response to predator cues (Rundle et al., 2011). Moreover, it has been suggested that early life conditions and experiences can provide cues about the local environment in the form of 'adaptive programming' of the juveniles, resulting in phenotypes that are better suited for the conditions they will encounter later in life (Diorio & Meaney, 2007). It would thus be advantageous if context cues like predation risk in early life could alter the behavioural phenotype of individuals in a similar manner (for example by increasing their reliance on social information) and thus increase their survival or fitness in such dangerous high-predation environments. Furthermore, early social environment can affect several aspects of an animal's behavioural phenotype. For example, D'Andrea et al. (2007) suggest that a social enrichment in early life like communal nesting improves social competencies of mice, *Mus musculus*. Zebra finches, *Taeniopygia guttata*, reared in the absence of adult males in the colony, were less likely to choose an opposite-sex partner to mate (Adkins-Regan & Krakauer, 2000), and trout, *Salmo trutta*, reared in high-density hatchery environments become more territorial and invest more energy in contests than wild conspecifics (Sundström et al., 2003). Guppies provide an excellent study system for experiments involving manipulations of the early environment, and have been previously used extensively to examine plastic responses to predation risk (e.g. Dalton & Flecker, 2014; Handelsman et al., 2013; Torres-Dowdal, Handelsman, Reznick, & Ghalambor, 2012) or social environment (Chapman et al., 2008a,2008b; Guevara-Fiore, 2012; Magellan & Magurran, 2009).

In the study presented in Chapter 4, we manipulated the perceived predation risk and density of guppy juveniles during the first 45 days of rearing and measured their behavioural responses twice; once shortly after the developmental manipulation and once after a period of rearing in common-garden conditions. Here, in a follow-up study we presented the same fish with a new test to measure their propensity to use and learn from social cues in an anti-predator context. We specifically chose to measure these propensities after a period of no-treatment and not directly after the experimental manipulations, as we were mainly interested in early environment influences that can shape the animal's phenotype and remain fixed throughout its lifetime. There is also evidence that social learning propensities are not stable throughout an organism's life but they potentially develop gradually and in parallel to other behaviours (e.g. Takahashi et al., 2014). In a previous study (Chapter 3), we manipulated the utility of social cues during early life by exposing guppy juveniles to demonstrators providing matching or non-matching cues to foraging locations, or to no demonstrators at all. We then tested the subjects once immediately after the end of manipulation (day 45) and once after a period of rearing in common-garden conditions (day 90). We found evidence that guppy social learning propensities are indeed influenced by early exposure to reliable social cues about foraging. However, in contrast to our predictions that any effects would be strongest immediately after the developmental treatment and to previous empirical work showing that younger individuals are more likely to copy others than older ones (e.g. Noble et al., 2014), these developmental effects only appeared later in life, in the second testing. These results suggest that social learning propensities are primed by early life conditions and either develop over time or are employed discriminatorily under specific circumstances. In contrast to the study presented in Chapter 3, which involved cues about the value of social information during early life, here we examined whether more general cues can potentially have knock-on effects on the value of social information or directly on an individual's propensity to rely on social information.

In order to assess the subjects' propensity to use and learn from social information, we developed a dichotomous shoal choice test based on the behavioural ecology of guppy grouping behaviour. The most prominent and well-studied anti-predator behaviour of fish is shoaling (Magurran, 1990a,1990b) and guppies (as many other prey fish) exhibit increased shoaling tendency and form more cohesive groups when predation intensity increases (Seghers, 1974). Shoaling in tighter formations is also a common acute response that follows predator detection (Magurran & Pitcher, 1987; Morgan & Colgan, 1987; Sogard & Olla, 1997). For example, Brown & Godin (1999) reported a significant increase in shoal cohesion when they exposed guppies to conspecific alarm cue, a chemical signal released upon skin damage indicating a predation event (Smith, 1992). Therefore tight, cohesive shoals provide a potential cue of acute predation danger (but continual predator presence can also have similar or greater effects – e.g. Sogard & Olla, 1997). Moreover, visual cues of fearful or alarmed conspecifics has been found to facilitate predator recognition in fish (Chivers & Smith, 1994; Suboski, 1990). In our test, we manipulated shoal cohesion, thus presenting subjects with a choice between a tightly and a loosely shoaling group with the intention to examine whether guppies used shoal cohesion to choose shoaling partners. We subsequently retested subjects in the absence of the shoals, thus testing whether guppies used social cues of predator threat to learn about habitat locations. We predicted that if gup-

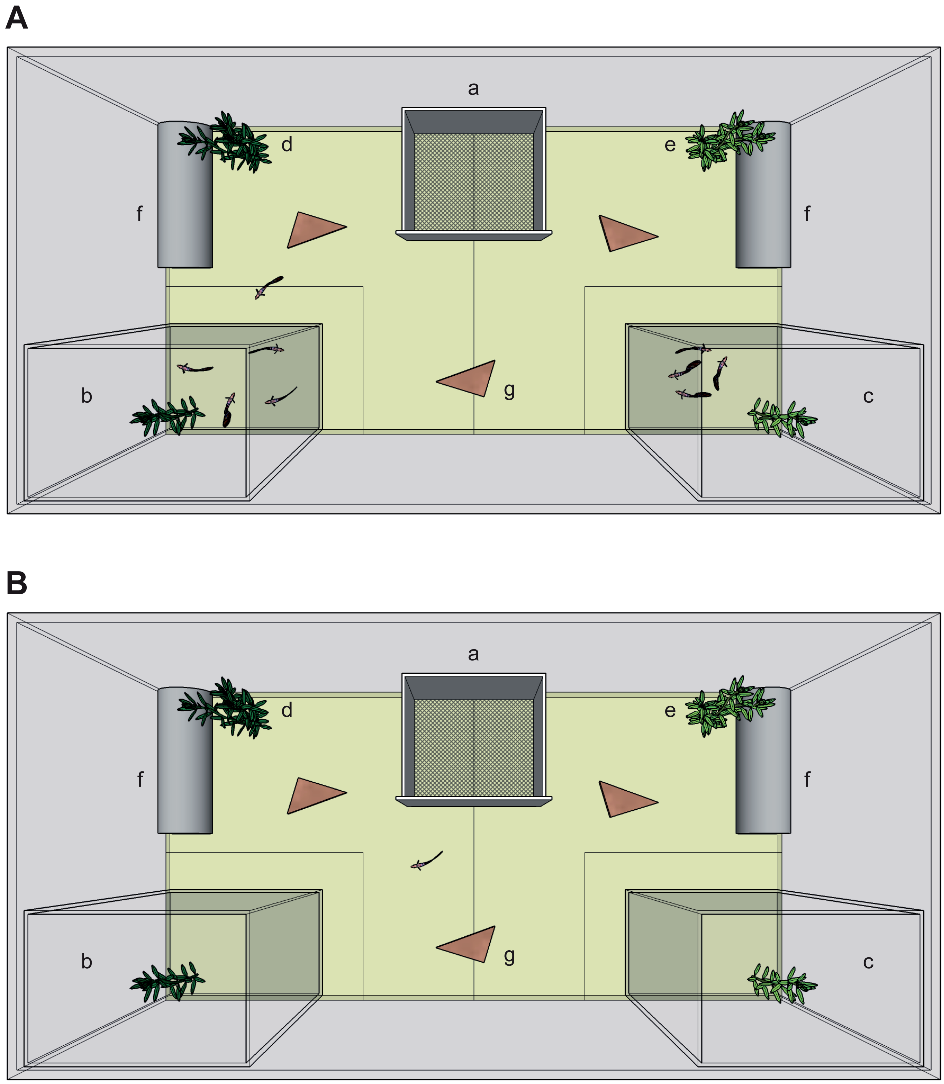

Figure 1. Shoal choice test setup. A: Shoaling phase; B: Testing phase. We used a 40-l rectangular glass tank (50 cm x 25 cm x 30 cm) filled to a water depth of 6 cm with fresh conditioned water of the same temperature as the subject's housing tank. The tank was covered with tan plastic sheets to avoid reflections and lighting abnormalities. A tan plastic sheet with rectangles drawn using a permanent marker, was placed under the bottom of the tank to indicate the locations of the shoal containers and their respective 4-cm-wide shoaling zones. The subject was introduced in a 10 cm x 9 cm x 8.5 cm container made from (continued on next page)

pies use social information to assess predation risk in a novel environment, they would approach faster and spend more time near the loose shoal over the tight one and that they would retain this information over a short time interval, returning to their previously preferred location in the absence of the shoals.

METHODS

Rearing Conditions and Experimental Treatments

Rearing conditions are described in Chapter 4. Briefly, we manipulated subjects' perceived predation risk and housing density for early life, then transferred them to new tanks and continued rearing them in common-garden conditions. Fish received the behavioural tests described in Chapter 4 before the shoaling and social learning tests described below.

Shoal Choice Test

Eighty-five days after the beginning of rearing (five days after the last behavioural test), we measured the social learning and social information use propensities of the fish with a dichotomous shoal choice test in a predation-related context (Fig. 1). The subject was introduced in the test tank in an opaque enclosure equipped with a sliding door attached to a fishing line, and mesh bottom for water circulation. After 2 min of habituation the door was lifted and the fish was able to leave the enclosure and swim freely in the tank. In the two ends of the tank, two identical transparent plastic containers were present, each of them holding a group of 4 adult females. In one of the containers, the shoal was constrained in a smaller space using a transparent mesh net; simulating a tighter shoal formation, while in the other the mesh net was larger and the shoal could use the entire container; thus appearing looser. The containers also contained two different coloured plants (dark green on the left and light green on the right container) and had two different coloured markings on their back walls (blue stripes on the left and green squares on the right container) to aid the visual discrimination be-

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(continued from previous page) white PVC sheets (a), equipped with a sliding door and mesh bottom. There were two transparent plastic containers (12.3 cm x 8.5 cm x 20 cm), one holding a loose shoal (b) and the other holding a tight shoal (c). Gravel was scattered evenly on the bottom so that it was not bare. Several pieces of ceramic pots (g), plastic plants (d: dark coloured and e: light coloured) as well as two grey plastic PVC tubes (11 cm x 4 cm) (f) were added in each side of the tank to add complexity and simulate potential predator hiding places.

tween the two sides. The time the subject spent near and interacting with each shoal was recorded for 3 min (shoaling phase – Fig. 1A). Subsequently, the subject was caught with a net and was placed back in the initial enclosure behind opaque barriers for a period of 4 min, while the two shoals and the plastic meshes were removed from the containers and the empty containers were placed back in the tank. We also mixed the water in the tank to avoid any localised olfactory cue accumulation. After this time interval, the sliding door was lifted once again and the subject was released. Again, we recorded the time the fish spent in proximity of and in contact with each container for 5 min (testing phase – Fig. 1B).

Data Collection and Analysis

All trials were recorded by a high definition camera (HD Pro C920, Logitech, USA) suspended above the test tank and connected to a laptop. An external observer blind to the treatments scored the resulting videos using JWatcher (v1.0, Blumstein et al., 2006) to extract the behavioural measures used in the analysis. The main measures were 'latency difference', defined as the latency to enter the shoaling zone of the tight shoal (henceforth 'TS') minus the latency to enter the shoaling zone of the loose shoal (henceforth 'LS'), the 'time shoaling difference', defined as the time spent shoaling with the LS minus the time spent shoaling with the TS, and 'time interacting difference', defined as the time spent interacting (touching the container wall) with the LS minus the time spent interacting with the TS. In all cases, positive values indicate a preference for the LS over the TS as hypothesised. Other measures that were examined were the 'LS interaction ratio', defined as the time interacting with the LS divided by the time shoaling with the LS, and the 'total interaction ratio', defined as the time spent interacting with both shoals divided by the total time shoaling with both shoals. These provided an alternative estimation of the subject's tendency to closely interact with conspecifics while shoaling. Data were analysed with factorial ANOVAs with early life 'predation risk', 'rearing density', and 'sex' as main factors, using SPSS (v20, IBM, USA). Normality and homoscedasticity were assessed with Shapiro-Wilk's and Levene's tests respectively. If these failed, the variables were transformed (square root or logarithmic transformations). Ratios were transformed using the arcsine transformation. If ANOVA assumptions were still not met, data were analysed with the non-parametric Kruskal-Wallis *H* test. We also examined group differences from values predicted by chance that would indicate a preference for the LS or the TS, by using Student's *t*-test or with the non-parametric Wilcoxon's signed ranks test when needed. Binomial data such as 'first choice' were analysed with Chi-squared tests.

RESULTS

Test Validation

In the shoaling phase, the fish did not spend significantly more time near the LS than the TS (*t*-test, $t_{114} = 1.12$, $P = 0.27$) nor did they approach the LS sooner than the TS (*t*-test, $t_{112} = 0.22$, $P = 0.83$), but they interacted more with the LS than the TS (*t*-test, $t_{114} = 2.1$, $P = 0.038$). In the testing phase, the fish did not spend more time near the previous location of the loose shoal versus that of the TS (*t*-test, $t_{114} = 1.33$, $P = 0.19$), nor did they interact more with the former LS container over the TS container (*t*-test, $t_{114} = 0.20$, $P = 0.84$). However, fish did approach the LS location faster than the TS location (*t*-test, $t_{109} = 2.1$, $P = 0.038$). Thus we had evidence that subjects distinguished between the two shoals on both test phases. In Table I we present the treatment groups that showed a clear preference for the LS over the TS in both phases of the test.

Table I. Summary table presenting all treatment groups that showed a significant preference ($P < 0.05$) for the LS over the TS in terms of relative latency to and relative time spent near the shoals. The remaining groups did not show a significant preference ($P > 0.05$) and are thus not presented.

Test phase	Measure	Subject group
Shoaling (shoals present)	Latency TS – LS	Predator-exposed males Predator-exposed high-density males
	Time shoaling LS – TS	Predator-exposed high-density males
Testing (shoals absent)	Latency TS – LS	Predator-exposed fish High-density fish Male fish Predator-exposed high-density fish
	Time shoaling LS – TS	Predator-exposed high-density fish Predator-exposed high-density females

Shoaling Phase (shoals present)

Latency Difference

Predator-exposed fish approached the LS sooner than the non-exposed fish (ANOVA, $F_{1,105}$ = 4.08, P = 0.046; Fig. 2A). There was also a significant interaction between 'predation environment' and 'sex' for the same measure (ANOVA, $F_{1,105}$ = 4.82, P = 0.03) with predator-exposed males approaching the LS faster than the non-exposed males (Tukey HSD post hoc test, P = 0.035; Fig. 2B), while this effect was not significant in females.

In particular, males from the 'predation' treatment approached the LS first, since the median of the 'latency difference' variable is significantly different from 0 (Wilcoxon signed ranks test, $Z_{n=26}$ = 279, P = 0.009; Fig. 2B). They also made more first choices for the LS (χ^2_1 = 5.54, P = 0.019). This effect appeared to be mainly driven by the HD males, since only the male HD predator-exposed group approached the LS significantly sooner than the TS (t-test, t_{12} = 3.18, P = 0.008; Fig. 2C). They also made more first choices for the LS (χ^2_1 = 4.5, P = 0.034).

Time Shoaling Difference

Only the predator-exposed HD male group showed a clear preference to spend more time shoaling with the LS, since its mean 'time shoaling difference' differed significantly from 0 (t-test, t_{12} = 2.47, P = 0.03; Fig. 2D).

Time Interacting Difference and Interaction Ratios

There was a significant effect of 'sex' on 'time interacting difference', with males exhibiting a higher preference to interact with the LS than females (ANOVA, $F_{1,106}$ = 5.8, P = 0.018; Fig. 3A). The male group showed a clear preference to interact with the LS over the TS (t-test, t_{49} = 2.54, P = 0.014; Fig. 3A). There was also a significant interaction of 'predation environment' and 'rearing density' on the same variable (ANOVA, $F_{1,106}$ = 4.83, P = 0.03; Fig. 3B), with 'no predation LD' fish interacting more with the LS than the TS (t-test, t_{25} = 2.65, P = 0.014; Fig. 3B). This effect appears to be driven by both males and females of this subgroup; in females, the mean 'time interacting difference' differed significantly from 0 (t-test, t_{17} = 2.23, P = 0.04; Fig. 3C) whereas in males it did not (t-test, t_7 = 1.91, P = 0.10; Fig. 3C).

There was a significant effect of 'sex' on 'total interaction ratio' (ANOVA, $F_{1,106}$ = 8.78, P = 0.004) with males exhibiting higher values (Fig. 4A). There was also a significant effect of 'social environment' on this measure (ANOVA, $F_{1,106}$ = 7.61, P = 0.007) with 'high density' fish interacting more than 'low density' ones (Fig. 4B).

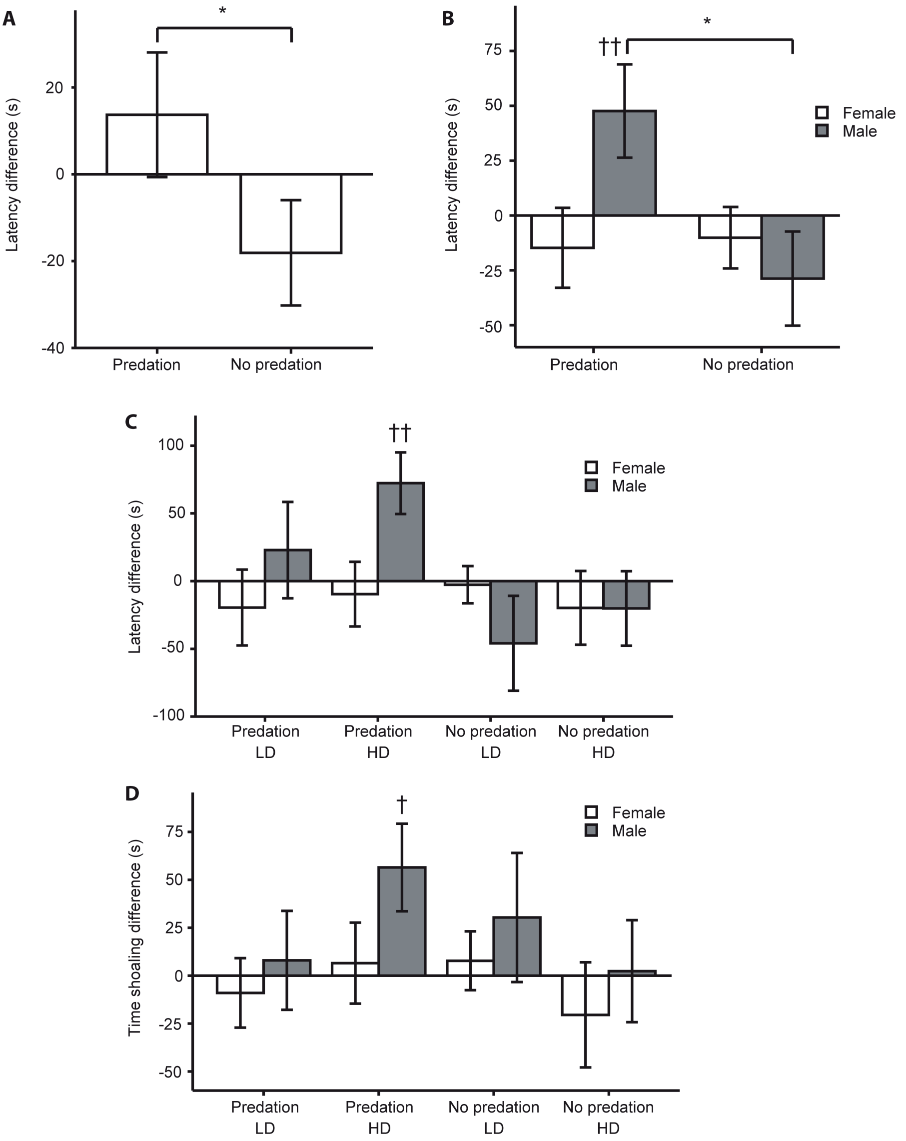

Figure 2. Shoal choice test, shoaling phase. A: predator-exposed fish approached a loose shoal over a tight shoal faster than non-predator-exposed fish. B: predator-exposed males approached a loose shoal faster than a tight shoal, and approached the loose shoal over the tight shoal faster than non-exposed males. C: predator-exposed HD males approached a loose shoal faster than a tight shoal. D: predator-exposed HD males spent more time shoaling with a loose shoal than with a tight shoal. *: significant difference between two groups, $P < 0.05$; †: significant difference from 0, $P < 0.05$; ††: $P < 0.01$. Error bars represent the standard error of the mean.

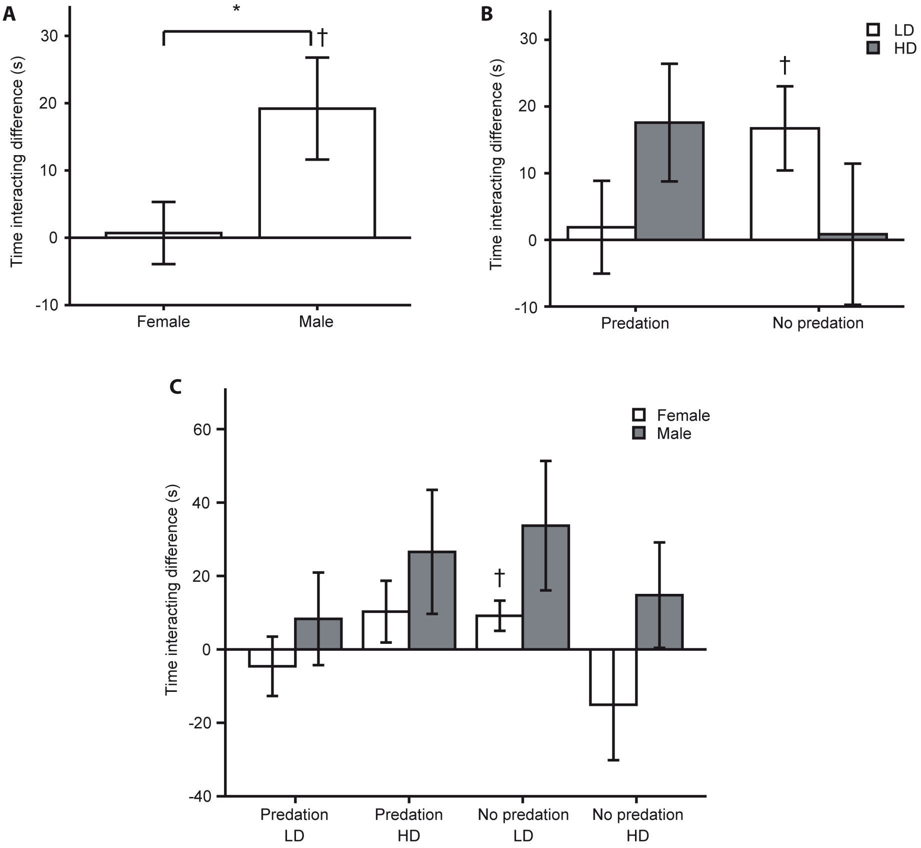

Figure 3. Shoal choice test, shoaling phase. A: Male fish interacted more with a loose shoal than with a tight shoal and this ‘time interacting difference’ was higher than that of the female fish. B: Non-exposed LD fish interacted more with a loose shoal than with a tight shoal. C: Female non-exposed LD fish interacted more with a loose shoal than with a tight shoal. *: significant difference between two groups, $P < 0.05$; †: significant difference from 0, $P < 0.05$. Error bars represent the standard error of the mean.

Finally there is a significant effect of ‘sex’ on ‘LS interaction ratio’ (ANOVA, $F_{1,106} = 9.84$, $P = 0.002$) with males spending more time interacting with the LS, than females (Fig. 4C).

When analysed separately from the females, the male HD group appeared to have spent significantly more time interacting while shoaling compared to the male LD group (Kruskal-Wallis, $H_{1,n=50} = 5.89$, $P = 0.015$; Fig. 4D).

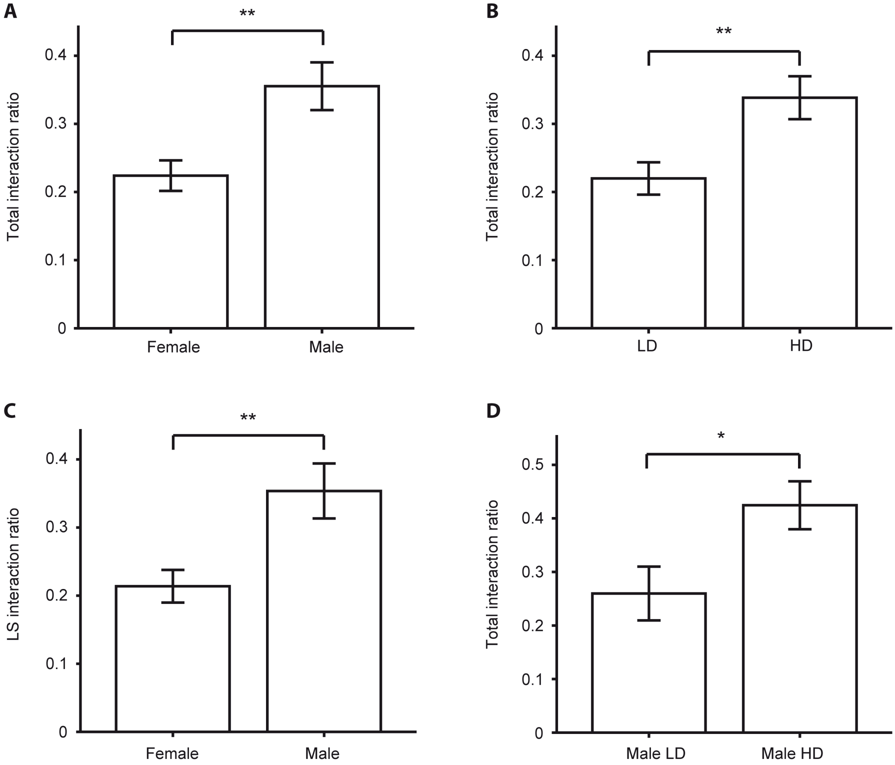

Figure 4. Shoal choice test, shoaling phase. A: Males spent more time interacting while shoaling (with both shoals) than females. B: HD fish spent more time interacting while shoaling (with both shoals) than LD fish. C: Males spent more time interacting while shoaling with a loose shoal than females. D: HD males spent more time interacting while shoaling (with both shoals) than LD males. *: significant difference between two groups, $P < 0.05$; **: $P < 0.01$. Error bars represent the standard error of the mean.

Testing Phase (shoals absent)

In this phase where shoals were absent, the terms 'latency' and 'shoaling' refer to entering and being within a shoaling zone at the previous location of either the LS or the TS.

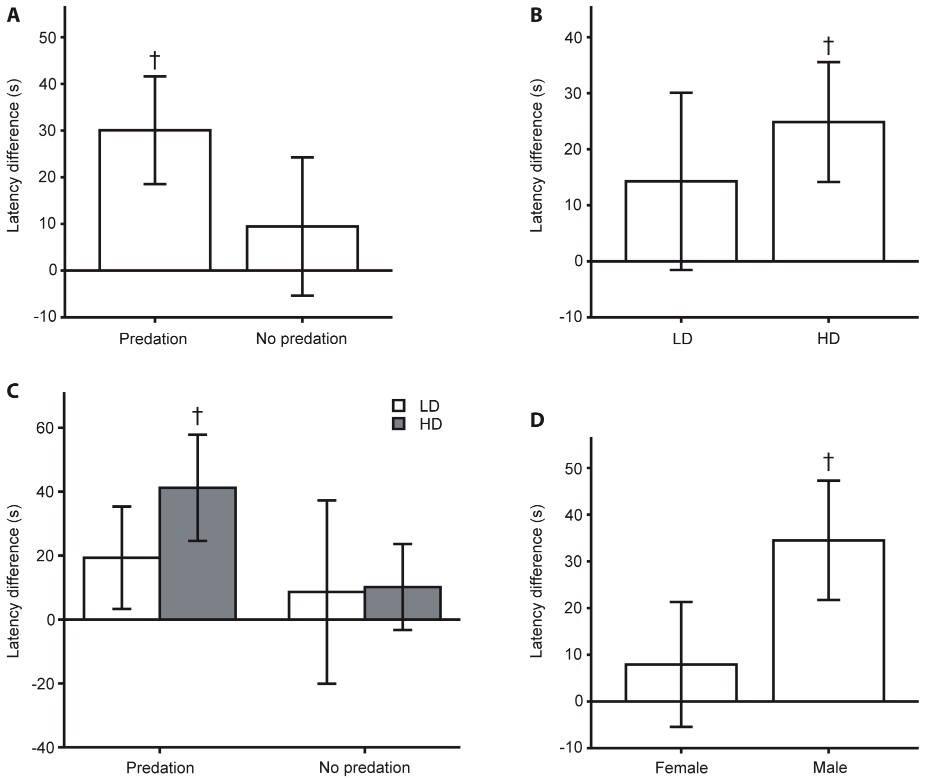

Figure 5. Shoal choice test, testing phase. A: Predator-exposed fish approached the previous location of a loose shoal sooner than the previous location of a tight shoal. B: HD fish approached the previous location of a loose shoal sooner than the previous location of a tight shoal. C: Predator-exposed HD fish approached the previous location of a loose shoal sooner than the previous location of a tight shoal. D: Males approached the previous location of a loose shoal sooner than the previous location of a tight shoal. †: significant difference from 0, $P < 0.05$. Error bars represent the standard error of the mean.

Latency Difference

Predator-exposed fish approached the previous location of LS sooner than the location of the TS (*t*-test, $t_{54} = 2.61$, $P = 0.012$; Fig. 5A). HD fish also followed the same pattern (*t*-test, $t_{56} = 2.33$, $P = 0.024$; Fig. 5B) and the HD predator-exposed group in particular appears to be driving this effect, as they also significantly approached the LS location sooner (Wilcoxon signed ranks test, $Z_{n=27} = 273$, $P = 0.044$; Fig. 5C).

Male subjects approached the previous location of the loose shoal signifi-

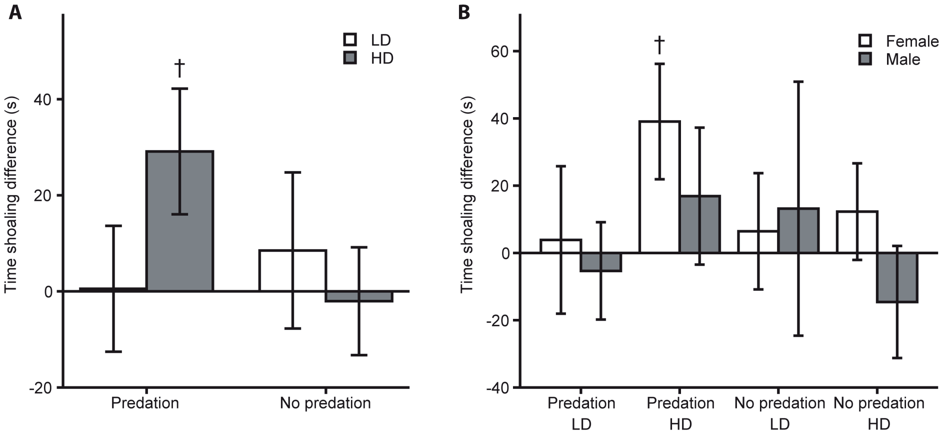

Figure 6. Shoal choice test, testing phase. A: Predator-exposed HD fish spent more time near the previous location of a loose shoal than the previous location of a tight shoal. B: Female predator-exposed HD fish spent more time near the previous location of a loose shoal than the previous location of a tight shoal. †: significant difference from 0, $P < 0.05$. Error bars represent the standard error of the mean.

cantly sooner than the location of the tight shoal (*t*-test, $t_{48} = 2.7$, $P = 0.01$; Fig. 5D). Among males, the HD fish tended to visit the previous location of the LS first, but the difference of their median from 0 was marginally non-significant (Wilcoxon signed ranks test, $Z_{n=29} = 306$, $P = 0.056$).

Time Shoaling Difference

Only the predator-exposed HD group's mean differed significantly from 0 in 'time shoaling difference' (*t*-test, $t_{28} = 2.23$, $P = 0.034$; Fig. 6A) showing a preference for the location where the LS was before. This appears to be mainly because of the female subjects of this group (*t*-test, $t_{15} = 2.28$, $P = 0.038$; Fig. 6B).

DISCUSSION

In the shoaling phase of the test, we found that predator-exposed fish approached the LS significantly sooner than the non-exposed fish, with the male individuals mainly driving this effect as predator-exposed males approached the LS significantly faster than non-exposed males. Predator-exposed males also approached sooner and made more first choices towards the LS, showing a clear preference over the TS. This effect was mainly driven by individuals reared in high densities, which preferred the LS over the TS both in terms of latency and time spent shoaling. Our results suggest that fish reared in the high predation risk environment, particularly males and males reared in high densities used a social cue, shoal cohesion, to choose between two groups, in a novel and potentially dangerous environment. In the second phase of the test, we examined whether guppies that used social cues in the first part, could retain this information and return to the same location after a short time interval and in the absence of the shoals. Although we did not find significant effects of our developmental treatments on our measures, we did find evidence for social learning in some of the groups, indicated by their preference for the previous location of the LS. Predator-exposed fish, fish reared in high density and male fish all approached the previous location of the LS sooner than the location of the TS. Predator-exposed high-density fish returned to the LS location faster and spent more time there than at the previous location of the TS.

Early life exposure to predator cues has been reported to affect a series of behavioural, life history and physiological traits in a variety of animal taxa (e.g. Ayers et al., 2015; Daly et al., 2012; Ferrari & Chivers, 2009), and more relevant to our study, in guppies (Dalton & Flecker, 2014; Handelsman et al., 2013; Torres-Dowdal et al., 2012). For example guppies reared in the presence of predator cues developed lower resting metabolic rates and lower growth rates than others reared without predator cues (Handelsman et al., 2013). Predator-exposed guppies have been also shown to reduce food intake but were more efficient in nutrient conversion than non-exposed conspecifics (Dalton & Flecker, 2014). Hence, one would predict that in their effort to balance out predation risk with foraging efficiency, guppies reared in the presence of predator cues, would have increased propensity to use social cues for foraging decisions. However, predator-induced plasticity in behavioural responses has received little attention and social learning about predators is not studied to the same extent as social learning in foraging contexts (Griffin, 2004). Although environments with high predation risk have been commonly linked to the development of anti-predator behaviours, such as the increased shoaling tendency in guppy populations experiencing high predation in the wild (Magurran & Seghers, 1991,1994a; Seghers, 1973,1974), our study presents novel evidence that predation risk specifically confined in early environment can result in adult phenotypes with increased reliance on social information.

However, only male individuals seemed to convincingly use this social cue for their shoaling decisions in the first phase of the test. Female guppies have been previously reported to react more strongly to predator cues than males and they are generally considered a more preferable prey for predators due to their larger size (Magurran & Seghers, 1990b; Seghers, 1973). Males are thus thought of being less at risk of predation due to their small size and in addition it has been proposed that they balance out predation risk with increased chances for sneaky mating. As females typically respond more strongly to predator presence compared to males (in terms of schooling behaviour and predator inspections initiated), males exploit this change in behaviour by increasing their sneaky copulation attempts (Magurran & Nowak, 1991). It is possible that in our test males made decisions based on reproductive opportunities, since we used all female groups, and the fact that males also showed increased interaction ratios with both shoals compared to females supports this supposition. However, if males used social cues in order to maximise their mating chances and not to avoid a risky situation, we would expect a preference for the TS where females would be perceived as engaged in anti-predator behaviour and thus more vulnerable to sneaky mating attempts. In addition, guppy predators preferentially attack individuals with brighter colours (Godin & McDonough, 2003), therefore male guppies could potentially be more vulnerable to predation than females due to their ornamentation and conspicuous courting behaviours (Agrillo et al., 2008), thus explaining the observed increased reliance on social cues in risky circumstances.

The finding that individuals reared in high densities were more likely to use social cues for such decisions and their increased interaction ratios with both shoals is in contrast with previous findings. Guppies reared in high densities have been reported to exhibit lower shoaling tendencies than others reared in low densities (Chapman et al., 2008b). They were also less adept at learning a route to a foraging location after repeated trials with trained demonstrators in a maze, indicating a decreased propensity to socially learn in comparison with fish reared in low densities. Hence, one would expect the low-density-reared fish to be more social in general and rely more on social information in our test. The main differences between this study and ours are the experiences confined to early life and the context of the test itself. We manipulated the subjects' early life predation risk and examined their propensity to use social cues (shoal cohesion) to avoid potential threats and risky situations in a spatially complex novel environment, whereas the previous study focused on social learning in a foraging context and without any prior exposure to predator cues. There is a well-described trade-off between benefits and costs of grouping (reviewed by Krause & Ruxton, 2002). Hence, in the absence of early experience of predation and in situations where the perceived threat levels are low, as in the case of the earlier study by Chapman et al. (2008b), the costs of grouping due to competition and aggression can overshadow any benefits, thus resulting in reduced shoaling tendency and consequently reduced propensity to socially learn in a foraging context. In our case, early exposure to predator cues and higher real-time perceived risk during the test could cancel this effect, but this does not explain the observed reverse pattern with evidence for social information use in the high-density-reared individuals but not in the low-density-reared ones.

We cannot exclude the possibility that preferences for the LS over the TS could be due to the TS being perceived as less numerous, for example because fish in the TS blocked the view of each other. Guppies have been reported to prefer large over small shoals (Lachlan et al., 1998; Ledesma & McRobert, 2008), but there are reports that this occurs only under predation threat (Weetman et al., 1999) and cases where this pattern is not observed at all (Dougherty et al., 2010). To control for this possibility, we also measured time spent near each shoal, which ensured that even if the subject made a first choice for one of the shoals by chance, we still had an alternative measure of grouping preference. The test was designed in such a way that switching between shoals was easy, therefore encouraging the subject to sample both ends of the tank and examine the two choices more closely if needed.

Interestingly, in the second phase of the test, it was the female individuals that significantly preferred the previous location of the LS over the TS. Female guppies are known to use social information to make decisions, particularly when choosing mates (Dugatkin & Godin, 1992; Dugatkin & Godin, 1993; Vukomanovic & Rodd, 2007), and they have been used extensively and preferentially over males in empirical studies of social learning (Kendal et al., 2004; Lachlan et al., 1998; Laland & Williams, 1998, 1997). It is plausible that these fish have indeed learned about this location, without showing a clear preference for the corresponding shoal in the first phase. A possible explanation for this lack of significant preference could be that in the presence of both shoals, fish switched between containers to sample both parts of the tank and to assess the condition of the shoals (and perhaps any cues provided by them) more closely. Guppies have been reported to frequently leaving their shoal in the wild in order to increase phenotypic assortment and males in particular, often switch between shoals or leave their shoal to search for novel receptive females (Croft et al., 2003). In addition, the duration of this first shoaling phase was relatively short and perhaps insufficient for any preference (in terms of time spent shoaling) for a particular shoal, to emerge. Furthermore, the second phase of the test started after a 4-min period during which the focal fish was kept inside its original holding container. Isolation from conspecifics can substantially increase the stress levels of guppies (Laland & Williams, 1997). Our subjects might have thus been in a more stressful state at the beginning of the testing phase, leading them to choose the location they perceived as the safest, based on social information acquired earlier. However, the time near shoal measure could be considered less relevant in the testing phase where shoals were absent compared to the shoaling phase. Thus the relative latency measure, revealing that (similarly to the first part of the test) mainly the male individuals approached the previous location of the LS first, could provide more compelling evidence in this part of the test.

However, the observed effects were more subtle than anticipated. Most of our findings regarding evidence for social information use and social learning derive from the presence (or absence) of a significant preference for the LS over the TS in the various treatment groups and subgroups, compromising firm conclusions on the effects of our developmental manipulations (Nieuwenhuis et al., 2011). In addition, this approach required a considerable number of comparisons of group means with values hypothesised by chance. Thus these results should be interpreted with caution and further work is needed to strengthen our conclusions, for example taking account of the high individual variation we observed by increasing sample size or by utilising a different social learning test. However, due to the limited evidence for developmental effects on social information use and social learning in the literature, our findings can serve as a valuable guide for further investigations.

To conclude, we found evidence that exposure to high predation risk during early life increased the reliance on social information to make grouping decisions, particularly in male guppies and male guppies reared in high densities. We also found limited evidence in some of the groups, that they can potentially retain this information and use it after a short time interval. These results support the idea that social information use and social learning propensities are plastic and can be dependent on conditions and cues specifically confined in early life. Plasticity certainly plays an important role in dispersing or newly established populations, allowing individuals to adjust to novel conditions and environments (Handelsman et al., 2013; Thibert-Plante & Hendry, 2011). However, to what extent such early life influences reflect a form of adaptive developmental programming, shaping the juveniles' behavioural phenotype to better match their local environment when adults, remains an open question for future work.

ACKNOWLEDGEMENTS

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Chapter 6

General Discussion

The main aim of this thesis was to examine how aspects of social behaviour such as social learning propensities and grouping tendencies are shaped by conditions and experiences confined in the early life of an organism. I also examined the individual consistency and heritability of these propensities as well as other individual behavioural traits such as boldness and exploratory behaviour in order to examine how these traits change alongside social behaviour. My main findings are discussed within each chapter. Here, I discuss how these results fit together and with previous work and provide some future perspectives.

Consistency and Plasticity

In this thesis, we examined two different hypotheses; the first one suggested that social behaviours like shoaling tendency and social learning propensity are individually consistent traits with strong genetic components, while the other one posed that they are plastic and can be shaped by early life conditions and experiences. However, these hypotheses are not mutually exclusive, as it is widely acknowledged that phenotypes are the outcome of complex interactions between genes and the environment. Moreover, there may be consistent individual differences in the exhibited behavioural plasticity of organisms, and plasticity itself can be heritable (e.g. Nussey et al., 2005). In our first experimental study, described in Chapter 2, we hypothesised that grouping tendencies would be consistent and heritable as suggested by previous empirical findings (Breden et al., 1986; Budaev, 1997; Magurran & Seghers, 1990a; Paxton, 1996; Seghers, 1974), and we expected that social learning propensities would follow a similar pattern. We found evidence for individual consistency in shoaling tendencies but we found no evidence for consistent reliance on social information. In addition, we found limited evidence for a genetic component in grouping tendencies, in the form of sibling group clustering (which does not exclude the possibility of maternal effects), but we saw no evidence for the heritability of social learning propensities. In the literature social learning and living in groups are often assumed to be directly linked, and it has been proposed that social learning is an adaptive specialization to social living (e.g. Templeton et al., 1999 but see Reader & Lefebvre, 2001). In guppies, previous empirical studies have repeatedly linked social learning and social information use to shoaling tendencies and propensities to follow conspecifics. For example, Laland & Williams (1997) demonstrated that guppies were able to socially learn foraging routes by swimming with informed conspecifics and attributed this to their general tendency to group. In addition, Bates & Chappell (2002) showed that naïve guppies followed trained conspecifics into taking a long, costly route to a feeding location over a short route, but this occurred only when they were tested in groups, indicating the existence of a relationship between using social cues and being in a group. Chapman et al. (2008b) found that guppies with increased tendencies to follow the demonstrators were more adept at finding food at the end of a maze and thus learned the route faster than guppies with lower grouping tendencies. Our social learning tests differed partially from the tests in the aforementioned studies, in that they relied more in the subject learning foraging locations by observing the demonstrator behaviour rather than by closely interacting and following the demonstrators in the tank. There is evidence from other fish species that grouping tendencies and social learning propensities appear with the same developmental timing (Takahashi et al., 2014), which further supports the idea that these two elements are strongly linked. Our failure to detect any evidence for consistency or heritability of social learning in subjects that showed consistency and heritability of shoaling, combined with the outstanding lack of evidence for heritability of social learning propensities in the literature, could indicate that these two characteristics can be decoupled and expressed differentially. Thus animals are potentially flexible in employing social learning and possibly switch between relying on personal or social information according to the current associated costs.

In Chapters 3-5, we investigate the idea that social learning propensities and other behavioural traits are developmentally plastic, influenced to a certain extent by early life social cues (Chapter 3), predation risk and rearing density (Chapters 4 and 5). First we examined whether the utility and reliability of social cues could influence social learning usage later in life. Our hypothesis suggested that fish reared in the presence of adult demonstrators would show higher social learning propensities (and copy or avoid demonstrated location according to their early life experiences), compared to individuals that were raised in the absence of adults. We found that only fish reared in the presence of adult demonstrators that reliably led subjects to the location of food delivery in the tank successfully copied the demonstrated locations in our social learning task later on, illustrating that early experiences with reliable and valuable social cues can lead to increased social learning propensities later in life. This finding is in line with the results of a previous study by Katsnelson et al. (2008), who showed that house sparrows, *Passer domesticus*, raised in the presence of an artificial parent that provided reliable foraging cues, were more likely to use social information to forage later in life. Our study extends previous work by confining experimental manipulations to early life, by explicitly varying cue reliability and utility (cf. e.g. Boogert et al., 2013; Lindeyer et al., 2013) and by measuring social learning rather than social information use. In our second treatment, the adult demonstrators provided equally reliable cues about where the food would not be delivered, and since there were only two alternative locations of delivery, these cues could also be used by guppies to locate food. Our intention was to replicate situations where the presence of individuals in a foraging patch might indicate that this patch will be rapidly depleted, especially in similar cases where juveniles observe adults, since older – thus larger – individuals can outcompete smaller ones (Laland & Reader, 1999b). Recent experience with social cues has been previously demonstrated to lead to non-matching behaviour in several species (Reader, 2014). For example, bumblebees, *Bombus terrestris*, with previous experience of demonstrators associated with bitter foods, learned to actively avoid demonstrated choices (Dawson et al., 2013). However, situations where the absence of food in one patch reliably indicates its presence in a different patch are possibly rare in nature. The fact that we saw no evidence for social learning in this treatment supports existing evidence that juvenile guppies possibly have an initial predisposition to follow or attend to individuals (Magurran, 1990a,1990b; Magurran & Seghers, 1990a), that confers many anti-predator and informational benefits in young individuals (Krause & Ruxton, 2002). This predisposition appears to be further strengthened by experience, in the case of our first treatment, but it is possibly more difficult to abolish, in the case of our second treatment.

In Chapter 4 we discuss evidence that early life predation environment and conspecific density influence various behavioural traits like boldness, exploratory and grouping tendency and propensity to leave a group to explore novel foraging options. However the effects of our developmental treatments were limited and strongly interacted with sex, and will be thus further discussed in the next section. In Chapter 5, we showed that guppies raised in an environment of high perceived predation threat were more adept at using a social cue, shoal cohesion, to make grouping decisions in a dichotomous shoal choice test later in life, compared to control fish. This effect was particularly evident in the case of male individuals. Shoaling in tighter formations is a well-established reaction of prey fish to increasing predator danger (Brown & Godin, 1999; Brown et al., 2000; Seghers, 1974) and fish often rely on information provided by their conspecifics to make informed decisions instead of personally sampling the environment (Hoare & Krause, 2003). Particularly regarding anti-predator responses, fish are reported to change their behaviour after observing conspecific behaviour indicating the presence of a potential threat nearby (Magurran & Higham, 1988; Mathis et al., 1996). Early predation environment can have a great impact on behavioural, life historical and physiological characteristics animals, and exposure to predator cues during early life can induce the development of anti-predator defences (Trussell & Smith, 2000). For example freshwater snails, *Helisoma trivolvis*, are known to develop particular morphological defences (i.e. larger shells and apertures) when exposed to predatory water bugs, *Belostoma flumineum* (Hoverman & Relyea, 2007). Particularly in guppies, exposure to predator cues and its effects have been extensively studied in several traits (Dalton & Flecker, 2014; Handelsman et al., 2013; Torres-Dowdal et al., 2012), and high-predation environments are typically linked to the development of anti-predator responses like increased grouping tendencies (Magurran & Seghers, 1991,1994a; Seghers, 1973, 1974), however our study expands previous knowledge by providing novel evidence on how early life experiences with predators can shape social information use. It should be mentioned that the observed effects of our developmental manipulations were more subtle than expected. Predation risk and conspecific density appeared to have non-additive effects and there were complex interactions between them and with the factor sex in many behavioural measures. However, the effects of predation pressure on prey behaviour, particularly when studied in combination with other important factors (such as food availability and parasitism), are often found to be more complicated and thus challenging to disentangle and interpret (Jacquin et al., 2016; Krause & Liesenjohann, 2012). Thus, further work is needed with increased sample sizes or different tests to cope with the high individual variation we observed, in order to draw more firm conclusions.

These influences of early conditions and experiences on social behaviour presented so far were long-lasting and persisted well into adulthood. In the experiment described in Chapter 3, we tested the subjects two times, once immediately after the end of our developmental manipulation and once after a period of no-treatment. Interestingly, we did not find evidence for social learning in the first testing but we did so in the second testing, suggesting that an adult individual's reliance on social information is influenced by early life and this propensity develops over time. In a different fish species, jack mackerels *Trachurus japonicus*, social learning propensities were found to develop over time and alongside the development of grouping tendencies (Takahashi et al., 2014), further supporting this idea. Another possibility is that social learning propensities are primed by early life conditions but are only employed under specific circumstances or conditions. Several social learning strategies have been proposed, regarding when animals turn to social learning (Hoppitt & Laland, 2013; Laland, 2004; Rendell et al., 2010). For example, minnows *Phoxinus phoxinus*, were shown to increase their reliance on social cues when predation danger and thus the cost of individual sampling increased (Webster & Laland, 2008). Since size plays a major role in competition for food (Laland & Reader, 1999b), our observer fish could preferentially rely on social learning later in life, when they are old/large enough to withstand competition or aggression from their conspecifics demonstrating foraging locations in the test. In the study presented in Chapter 5, we did not test the fish immediately after our early life treatments, but in a later time point, again after a period of common-garden conditions. Once again we found evidence that early environment had long-term effects on the propensity to use social information later in life, but these effects appeared only in certain treatment groups.

Sex Differences

One of the clearest outcomes of our studies was the existence of strong sex differences on multiple measures. Sex differences in behaviour are widespread in a range of animal taxa (Dammhahn, 2012; Darden et al., 2009; Harris et al., 2010;

Hedrick & Kortet, 2012; King et al., 2013; Magurran & Nowak, 1991). For example, male great tits, *Parus major*, appear to be bolder than females as they have been found to return faster to a feeding location after a mild startle (van Oers et al., 2005). These differences between sexes are often found to interact with other factors like age and reproductive stage (Dammhahn, 2012; Hedrick & Kortet, 2012) or body mass (Dehnhard et al., 2015). Here we present several examples from our experiments that further illustrate the existence of strong sex differences in the behaviour of guppies and discuss the implications of these differences for the ecology of our study system.

First, in Chapter 2, we saw that males and females differ in their individual consistency of grouping behaviour. Males showed evidence for both short-term consistency, between two parts of a shoaling test separated by a short time interval, and long term consistency, between two similar but distinct shoaling tests conducted several days apart. These findings are in agreement with previous reports of consistent grouping tendencies in male guppies (Budaev, 1997). Male guppies have also been previously found to exhibit consistent exploratory and mating behaviours (Harris et al., 2010; Kelley et al., 2013; Magellan & Magurran, 2007). On the other hand, female guppies have been previously found to show individual consistency in boldness and mate choice tests (Harris et al., 2010; Kodric-Brown & Nicoletto, 1997) but there are no previous reports of consistency in their shoaling tendencies. In our case, the female subjects showed no evidence for consistency in their shoaling tendency. However, distinguishing grouping tendencies from motivation to mate is difficult in the case of male individuals. In this study we specifically used groups of stimulus fish that consisted of only females, as previous findings from other poeciliid fishes have demonstrated that both males and females prefer to shoal with female or female-biased shoals to increase mating chances and avoid harassment respectively (Agrillo et al., 2006,2008). In the wild, male guppies are constantly in search of novel females and they are more likely to join shoals with high numbers of receptive females to increase reproductive success (Croft et al., 2003; Lindström & Ranta, 1993). Moreover, in our study male subjects were separated from females for several months prior to the tests, and we often observed courtship behaviour and mating attempts during the tests. Thus the consistency in shoaling we observed in male subjects could reflect consistent differences in the motivation to seek mates.

In Chapter 4 we found significant differences between males and females in all of the standard tests we conducted, at one or both time points of testing. Males were bolder and more exploratory than females in the emergence and open field tests, they spent more time shoaling and interacting with the shoal in the shoaling test but they were more likely to leave the shoal to inspect a novel feeding apparatus in the 'shoal vs. feeder' test, compared to females. There is a well-described difference between sexes in poeciliids, regarding their propensity to take risks, with males typically exhibiting bolder behaviours (Barbosa et al., 2016; Brown et al., 2007; Harris et al., 2010; Piyapong et al., 2009). Our results are in line with previous studies reporting that male guppies emerge sooner from refuges and are generally bolder compared to females (Harris et al., 2010; Piyapong et al., 2009). Females generally avoid risks and show stronger anti-predator responses than males (Magurran & Seghers, 1994b), by shoaling more when threatened and initiating more predator inspections (Magurran & Nowak, 1991; Magurran et al., 1992). We also saw that males explored significantly more squares than females in the open field. Barbosa et al. (2016) found that male guppies started exploring significantly sooner than females, but this should be considered a boldness-related feature, rather than indicative of exploratory behaviour. In contrast to our result, (Laland & Reader, 1999a) attributed the higher innovation rates of female guppies in foraging tasks to increased exploratory tendencies and argued that differences in level of hunger and/or in parental investment might underlie this difference between sexes. In our case however, exploration was assessed in a different context and thus the observed difference might be influenced by other factors, such as differences in perceived danger or the extent of disturbance by our handling. Regarding grouping tendencies, males spent more time shoaling and interacting with a group of conspecifics than females, in contrast to previous empirical work demonstrating higher shoaling tendencies in females compared to males (Magurran & Seghers, 1991,1994b; Magurran et al., 1992). However, in the ‘shoal vs. feeder’ test, females spent more time near the shoal, while males were more likely to leave the group and approach a newly-introduced feeding apparatus which contained a concealed predator model. This again suggests that males are more ready to take risks, leaving a safe or familiar area to enter a novel one (Barbosa et al., 2016; Harris et al., 2010).

Finally, in Chapter 5, we saw that males and females differed in their reliance on social cues, as only males appeared to make choices between shoals based on group cohesion in a novel and potentially dangerous environment. Grouping (shoaling or schooling) is one of the most well-established anti-predator responses found in fish (Magurran, 1990a), and guppies are known to typically increase their shoaling cohesion when predation threat increases (e.g. Brown & Godin, 1999; Seghers, 1974). Although male guppies are more ornamental and often engage in elaborate courtship behaviours, increasing their conspicuousness to predators (Agrillo et al., 2008; Godin & McDonough, 2003), female guppies have previously been reported to be more responsive to predator cues than males, and are usually considered to be more at risk of predation, being a more preferable prey due to their size compared to males (Magurran & Nowak, 1991; Magurran & Seghers, 1990b; Seghers, 1973). In addition, female guppies have generally provided more convincing evidence for using social cues in other contexts like foraging and mate choice (Dugatkin & Godin, 1992,1993; Kendal et al., 2004; Lachlan et al., 1998; Laland & Williams, 1998,1997; Vukomanovic & Rodd, 2007). We would thus expect females to be more likely to use social cues than males to make shoaling decisions in risky situations. As in previous tests, we used all-female stimulus shoals as these are more attractive for both sexes, hence we cannot exclude the possibility that mating motivation could underlie the male subjects' group choices. Magurran & Nowak (1991) found that females generally invest more time in anti-predator behaviours like shoaling, and tend to initiate more predator inspections than males. In response to this change in behaviour, males switch their mating tactics and increase their sneaky mating attempts, thus trading off the increased risk to be eaten by not engaging in anti-predator behaviours, with increased reproductive opportunities. Therefore, if males made choices aiming to increase their mating opportunities, we would expect them to choose the tight group of females, as these would be perceived as engaged in anti-predator behaviour and thus more vulnerable to sneaky mating attempts (Magurran & Nowak, 1991). In the present study male individuals showed a preference for the loose over the tight shoal, thus motivation to mate with the shoal members cannot explain the observed sex difference and the alternative explanation that they indeed relied on the available social cues about a potential danger to pick a group seems more likely.

These findings further support existing evidence on the presence of strong sex differences in guppy behaviour. The observed differences in individual consistency, boldness, exploration, grouping and reliance on social cues between males and females described above potentially reflect differences in life history strategies. For example, there are different reproductive rewards regarding risk-taking behaviour between males and females (King et al., 2013). Female guppies grow indeterminately (Dussault & Kramer, 1981), and their fecundity is strongly correlated with body size (Magurran & Seghers, 1994b; Reznick et al., 1990). Thus females would benefit, in terms of reproductive success, from increased longevity and thus by being generally less bold and more risk-averse they substantially increase their chances of survival (Griffiths & Magurran, 1998). On the contrary, males essentially stop growing after maturation (Dussault & Kramer, 1981), and the only way to maximise fitness is through increasing their chances of mating. Thus, due to their increased variance in reproductive success compared to females (Barbosa & Magurran, 2006), males exhibit a higher propensity to engage in risky but potentially more rewarding behaviours (Barbosa et al., 2016; Harris et al., 2010; Magurran & Seghers, 1994b). This strategy has also been reported in other taxa, for example risky behaviour was found to increase reproductive success in fiddler crabs, *Uca mjoebergi*, (Reaney & Backwell, 2007). We also found evidence that early environment influences can be expressed differentially in the two sexes, by either widening or eliminating the gap between them regarding specific traits. For example, we found a significant difference between sexes in the time to emerge from a refuge (Chapter 4), but predator-exposed males and females did not differ and the overall observed difference between sexes was driven by the non-exposed fish. Exposure to predator cues early in life could therefore lead to more uniform responses by males and females, while the absence of predator cues might allow certain sex differences to develop. Guppies have been shown to alter their reproductive strategy according to predation threat. In high-predation locations both sexes mature faster and have smaller body size at maturity (Reznick et al., 1997). Females from high-predation sites devote more time to anti-predator behaviours compared to males from the same areas, or females from low-predation sites (Magurran & Seghers, 1994b). High-predation-origin females also invest more resources and produce larger litters of smaller offspring, while producing litters more frequently compared to those from low-predation areas (Reznick & Endler, 1982; Reznick et al., 1997). On the other hand, males from high-predation areas show decreased foraging rates compared to males in low-predation sites, and invest more time in searching for females (Magurran & Seghers, 1994b). Thus both males and females are influenced by the predation environment but on different aspects of their behaviour, and this could be the main driver of the observed interactions between sex and predation risk in our tests. Such interactions between sex differences and other factors are not uncommon. For example, male grey mouse lemurs, *Microcebus murinus*, are typically bolder than females, but their boldness varies with age, with young individuals exhibiting bolder behaviours than old individuals (Dammhahn, 2012), while field crickets, *Gryllus campestris*, show sex differences which interact with age, in their boldness consistency, with females showing consistent boldness throughout their metamorphosis, but males become shyer when they reach maturity (Hedrick & Kortet, 2012). Although predation risk and other early life factors have been extensively examined separately from each other, few studies have investigated their combined effects and interactions (e.g. Bakker et al., 2005; Krause & Liesenjohann, 2012). Developmental approaches like ours, manipulating more than one factor simultaneously represent a more natural situation, and can provide a more complete insight on effects of early life conditions and their possible interactions with other important factors (e.g. sex or age).

In the present thesis, we provide new evidence that further emphasise the strong sex differences regarding the development of behaviour and behavioural flexibility. Male and female guppies follow different strategies to maximise fitness and survival and this is further supported by the overall higher risk-taking propensity of the males we observed in our tests. Moreover, we found that sex differences in behaviour can be partially influenced by early life conditions and experiences and these might affect each sex differently. Our results suggest that sex differences are pervasive and should be thoroughly investigated further and taken into consideration when designing and conducting behavioural studies. Moreover, in order to reach a better understanding of the behavioural ecology of guppies, or any other animal system, it is necessary to study the effects of multiple factors simultaneously and examine their potential interactions and their subsequent impact, not only at a few specific developmental points but over the lifespan of the individual.

Implications and Future Perspectives

The findings presented in this thesis add to the increasing body of empirical evidence that early environment can significantly influence the development of social behaviour and particularly reliance on social information (Boogert et al., 2013; Chapman et al., 2008b; Farine et al., 2015; Katsnelson et al., 2008; Levy et al., 2003; Lindeyer et al., 2013). This implies that observed differences between populations and variation both between and within species regarding social learning propensities, can be – at least partially – attributed to differences in their environments possibly interacting with genetic underpinnings (Carlier & Lefebvre, 1997; Reader et al., 2011). Mesoudi et al. (2016), present a valuable approach to explain individual variation in social learning. They categorise existing evidence on social learning differences in four main groups: cases about heritable individual differences in social learning, cases where developmental cues and reproductive state influence social learning propensities, cases where animals individually learn about social learning and the reliability of social cues, and finally (and possibly restricted to humans) cases where social learning propensities are socially acquired. They then place these groups of evidence on a phenotypic plasticity continuum, with the heritable fixed differences in one end, reflecting low phenotypic plasticity and the socially learned social learning in the other end, reflecting high phenotypic plasticity (Fig. 1).

Our study touches on the three first approaches, since we examined the consistency and heritability of grouping and social learning in Chapter 2, developmental cues with possible knock on effects on social information use in Chapter 5, and the influence of the reliability and utility of social cues on social learning propensities in Chapter 3. However, variation in social learning propensity does not necessarily imply variation in social learning capacity. Relying on social rather than individual information to make foraging, mating, anti-predator and other fitness-related decisions is dependent upon the associated costs of each option and the current environmental conditions (Boyd & Richerson, 1985; Rendell et al., 2010; Webster & Laland, 2008). In relatively stable or slowly changing environments, animals benefit from acquiring information socially as they avoid personal costs, however in unpredictable or rapidly changing environments, social information could be unreliable or might quickly become outdated (Boyd & Richerson, 1985; Krause & Ruxton, 2002). In addition, the efficiency of social learning decreases when the number of copiers increases (Rendell et al., 2011). Therefore, being flexible in utilising and learning from social information can offer adaptive

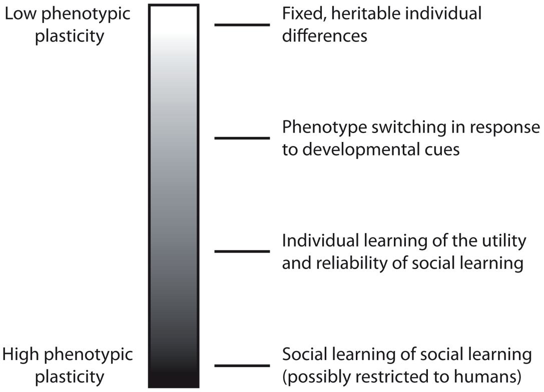

Figure 1. Continuum of phenotypic plasticity in social learning variation. At the top, representing the lowest end of phenotypic plasticity, there are cases where genetic variation results in different social learning phenotypes fixed from birth. In Chapter 2 of this thesis we examine the consistency and heritability of individual differences in grouping and social learning. Next are the cases where different social learning phenotypes are generated in response to specific developmental cues. We explore the effect of such developmental cues (predation risk and stocking density) in social behaviour in Chapters 4 and 5. Further down the spectrum, there are cases where individuals learn about the value of social information asocially. In Chapter 3 we examine how the reliability and utility of social cues early in life can influence social learning propensities later on. Finally at the bottom, representing the highest degree of phenotypic plasticity, there are the cases where social learning is acquired from others via social learning, a phenomenon possibly only observed in humans. Figure modified from Mesoudi et al. (2016).

benefits (Reader, 2014), for example for living in variable environments or adjusting to novel conditions after dispersal (Handelsman et al., 2013; Thibert-Plante & Hendry, 2011). This flexibility is potentially reflected in the individual variation in social learning propensities commonly observed in the literature.

Our results also have applied implications, since social learning can pose a useful tool for the successful release or re-introduction of captive-reared species in their natural habitats and should be considered in conservation efforts (Brown & Laland, 2001; Griffin, 2004; Reader & Biro, 2010; Suboski & Templeton, 1989; van Schaik, 2002). As fish stocks continue to deplete globally due to overfishing and habitat degradation, developmental approaches like ours can be particularly useful. Overfishing, apart from reducing population numbers, can also lead to the elimination of crucial socially transmitted information. For instance, overfishing can result in disruption of the effective ratio between demonstrators and observers in herring, *Clupea harengus*, populations, thus eliminating important social information regarding spawning and foraging routes (Corten, 2002). There have been efforts to enhance natural stocks by introducing hatchery-reared fish to the wild, but usually these individuals lack the necessary skills to cope with novel conditions and thus suffer very high mortality rates when released in the wild (Salvanes & Braithwaite, 2005). Manipulating the rearing conditions and providing the fish with the required stimuli (e.g. experiences with novel foods and predators), could facilitate the development of a more elaborate behavioural repertoire, which would potentially increase the chances of survival of the released or reintroduced species. For example, Atlantic Salmon parr reared in enriched environment and with previous experience of foraging on live prey, showed significant improvements in their ability to handle a novel live prey later on (Brown et al., 2003). Similarly, cod juveniles reared in spatially variable environments developed better behavioural skills associated with survival in the wild, such as refuge usage and anti-predator responses (Salvanes & Braithwaite, 2005). The benefits of exposure to novel cues during rearing are expected to be even higher for species with high social learning propensities, as even naïve fish can potentially acquire new and useful information rapidly from informed conspecifics (Brown & Laland, 2001). Furthermore, social learning is one of the most important social-cognitive skills that humans have evolved for social living (Cultural Intelligence Hypothesis - Herrmann et al., 2007). Studying social learning in animal systems can improve our understanding about the evolution of human culture (Hill, 2010) and empirical work on developmental influences on the adult social phenotype can find important applications in research regarding normal and abnormal human social and cognitive development (Lindeyer et al., 2013).

The take-home message from this thesis is that social learning propensities can change both over the lifespan and as a result of different early life conditions and experiences. Developmental approaches like ours are essential to further understand the phenotypic plasticity of social behaviour and its associated adaptive benefits or incurring costs. Although there have been numerous studies exploring social learning mechanisms and adaptive function both in the field and in the laboratory, relatively little is known about when and how social learning propensities develop (Reader & Biro, 2010). Several important questions remain unanswered, for example which early environmental factors are the most influential on the development of social behaviour and learning in particular. We found that reliable social cues to foraging locations during rearing were able to promote social learning in a foraging task, whereas early life exposure to predation cues induced increased reliance on social information in a novel and potentially dangerous environment. However, further investigation is necessary to identify the key elements of the early environment that drive the observed differences. Another interesting question is how these influences interact with genetic predispositions, developmental trajectories and learning. For example, certain anti-predator responses have a strong genetic component (Magurran, 1990b; Scheurer et al., 2007), but early life experiences and learning about predators are necessary to further optimise them (Bass & Gerlai, 2008; Ferrari et al., 2007; Lönnstedt et al., 2012). Can the same be true for social behaviours like grouping and social learning? We presented some evidence about consistency, heritability and developmental influences of grouping tendencies, but further research is required to establish how initial predispositions in social behaviour can be fine-tuned during development. Furthermore, are there sensitive developmental windows that allow these responses to form, and to what extent are these responses plastic and potentially reversible? Freshwater snails, for instance, have wide developmental windows to change the morphology of their shells when exposed to predation by water bugs, and this change can be reversed if triggered early in development (Hoverman & Relyea, 2007). Could such patterns also apply to inducible behavioural defences against predators, including social learning? All these interesting topics remain open objectives for future work.

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English Summary

One of the many advantages of group living is the swift transfer of information between group members. Social learning – learning from others – offers a quick and economical way to acquire information about the local environment and is considered adaptive under a wide range of circumstances. However, social information can be unreliable or quickly become outdated in rapidly changing environments. Thus flexibility in the utilization of social learning, depending on the associated costs and benefits, would be expected to be advantageous in most situations. Early environment plays a major role in shaping the morphological, physiological or behavioural phenotype of an animal, and conditions and experiences during early life can potentially prepare (or program) a developing individual for the environment it will encounter when adult. In this thesis we examined whether social behaviours such as grouping and social learning could be influenced by early life conditions and to what extent. For this purpose, we used two different strains of the guppy *Poecilia reticulata* (domestic and wild-origin), a popular study system in behavioural and evolutionary ecology with well-described social behaviour.

In the study presented in Chapter 2, we investigated whether grouping tendencies and social learning propensities were consistent and heritable traits. First, we tested individuals in similar social learning and shoaling tests to assess individual consistency in their performance. We found evidence for consistency in the male subjects' shoaling behaviour but not in their social learning propensities. We found no evidence for individual consistency in the case of females. Subsequently, we paired male and female subjects at random, obtained their offspring, reared them in common-garden conditions until adulthood and then tested them in similar social learning and shoaling tests to examine the heritability of these traits. Again we found evidence for the existence of a genetic component in shoaling behaviour, expressed as differences between sibling groups, but not in the subjects' propensity to socially learn.

In Chapter 3, we describe our first developmental approach, in which we directly manipulated the perceived value of social information during early life and examined if this influenced our subjects' social learning propensities later on. We reared fry in the presence of adults demonstrating which of two food delivery locations contained food, with adults demonstrating the opposite location to food delivery, and without any adults. Only subjects exposed to demonstrators that led them to food in early life reliably copied demonstrator choices in a social learning test later in life. These results provide novel evidence that the usefulness of social cues to foraging locations during early life can drive social learning propensities in similar contexts later on. Moreover, social learning was not detected in our first tests immediately after the end of our developmental manipulations (day 45) but only in our second tests after a period of rearing in common-garden conditions (day 90), suggesting that social learning propensities develop over time or are expressed under specific circumstances.

We continued with another developmental study examining the effects of early life predation risk and housing density on various behavioural traits such as boldness, exploration and grouping tendency (presented in Chapter 4). We tested the subjects at two time points, one shortly after the end of experimental treatments (day 50) and one after a period of common-garden conditions (day 80) to establish if the observed influences were long-lasting. We found significant differences between sexes in all standard tests at both testing dates. Males were bolder and more exploratory; compared to females they spent more time shoaling and interacting with a group of stimulus fish but were more likely to leave the shoal to approach and enter a novel feeder. We also saw significant treatment effects in some of the behavioural measures but these were often confined within certain subgroups, indicating the existence of complex interactions between the analysed factors. Shortly after the second series of behavioural tests (day 85), we assessed the propensity of these fish to use (and retain) social information in a two-part shoal choice test within a potentially dangerous novel environment, the results of which are reported in Chapter 5. We found that predator exposed fish, and particularly male individuals, were more adept at using a social cue – shoal cohesion – to make shoaling decisions. These fish showed a preference for a loose shoal over a tight shoal (which in nature would typically indicate the presence of nearby potential threats). Predator-exposed fish appeared to also retain this information after a short time interval and made similar choices regarding tank locations in the second part of the test when the shoals were absent.

Overall, we found evidence for consistency and heritability in grouping behaviour, but although grouping tendencies were (to some extent) correlated with social learning propensities, we found no evidence for consistency and heritability in social learning. Further work is needed to examine if this was driven by the subjects' poor performance on the social learning tasks or if it reflects a higher degree of flexibility in utilization of social learning compared to grouping behaviour. Furthermore, we saw that early environmental conditions and experiences play a major role in shaping the behavioural phenotype of an individual. We found that both cues about the value of social information and context cues like predation risk and population density in early life can influence an individual's reliance on social information later in life. Moreover, as males and females differ significantly in their strategies to maximise fitness and survival, these early life cues can affect the behaviour of the two sexes in different ways.

Nederlandse Samenvatting

De snelle uitwisseling van informatie is een van de belangrijkste voordelen van het leven in een groep. Sociaal leren – het leren van anderen – biedt een snelle en voordelige manier om informatie over de lokale omgeving te verzamelen en is een strategie om gedrag aan te passen in uiteenlopende omstandigheden. Daarentegen kan sociale informatie ook onbetrouwbaar zijn of achterhaald worden in een snel veranderende omgeving. Dus, afhankelijk van bijbehorende kosten en baten, kan van flexibiliteit in het gebruik van sociaal leren verwacht worden dat het voordelig is in de meeste situaties. De vroege omgeving speelt een belangrijke rol in het vormen van het morfologische, fysiologische of gedragsmatige fenotype van een dier. De omstandigheden en ervaringen gedurende het vroege leven kunnen mogelijkerwijs een zich ontwikkelend individu voorbereiden (of programmeren) op de omgeving die het zal tegenkomen als volwassene. In deze thesis hebben we onderzocht of sociale gedragingen, zoals groepsgedrag en sociaal leren, beïnvloed kunnen zijn door omstandigheden in het vroege leven en in welke mate. Hiervoor namen we twee stammen van de guppy *Poecilia reticulata* (gedomesticeerde en van wilde origine), een populair modelsysteem in gedrags- en evolutionaire ecologie waarvan het sociaal gedrag goed beschreven is.

In Hoofdstuk 2 presenteren we de studie waarin we onderzochten of neigingen tot schoolgedrag en sociaal gedrag consistent waren en of het erfelijke eigenschappen zijn. Ten eerste onderzochten we de individuen in vergelijkbare testen voor sociaal leren en schoolgedrag om de individuele consistentie in hun prestaties na te gaan. We vonden bewijs voor de consistentie van het schoolgedrag van de mannelijke guppy's maar niet voor hun neigingen tot sociaal leren. We vonden geen bewijs voor individuele consistentie bij de vrouwelijke vissen. Vervolgens hebben we mannetjes en vrouwtjes willekeurig in paren ingedeeld, en hun nakomelingen opgekweekt tot volwassenheid in standaard omstandigheden. Daarna werden de nakomelingen getest op vergelijkbare sociaal leren en schoolgedrag testen om de erfelijkheid van deze eigenschappen te onderzoeken. Opnieuw vonden we bewijs voor het bestaan van een genetische component in schoolgedrag - er bestonden verschillen tussen groepen broers en zussen - maar niet in de neigingen van de guppy's voor sociaal leren.

In Hoofdstuk 3 beschrijven we de eerste ontwikkelingsaanpak, waarin we de waargenomen waarde van sociale informatie manipuleerden gedurende het vroege leven en onderzochten of dit in een later stadium de neigingen voor sociaal leren beïnvloedde. We kweekten jonge vissen op in drie verschillende condities. In de eerste waren volwassen guppy's aanwezig, die demonstreerden welke van twee voedsellocaties daadwerkelijk voedsel bevatte. In de tweede conditie toonden de volwassen vissen juist de tegenovergestelde locatie waar geen voedsel aanwezig was. In de laatste conditie waren er geen volwassen guppy's aanwezig. Alleen guppy's die opgroeiden met volwassen vissen die de correcte voedsellocatie toonden, kopieerden op betrouwbare wijze de keuzes van de demonstrerende vissen in een sociaal leren test in een latere situatie. Deze resultaten bieden nieuw bewijs dat het nut van sociale aanwijzingen naar foerageerlocaties gedurende het vroege leven kunnen leiden tot een vergrote neiging tot sociaal leren in vergelijkbare contexten op latere momenten. Bovendien werd sociaal leren niet gedetecteerd in onze eerste tests direct na het einde van de ontwikkelingsmanipulaties (dag 45) maar alleen in onze tweede tests na een periode van kweek in standaard omstandigheden (dag 90), wat suggereert dat neigingen tot sociaal leren zich na verloop van tijd ontwikkelen of worden uitgedrukt onder specifieke omstandigheden.

We vervolgden met een andere ontwikkelingsstudie waarin we de effecten van predatierisico en populatiedichtheid in het vroege leven op verschillende gedragskenmerken zoals neigingen tot vrijmoedigheid, exploratie en scholingsgedrag (gepresenteerd in Hoofdstuk 4). We testten de guppy's op twee tijdpunten, één kort na het einde van de experimentele behandeling (dag 50) en één na een periode van standaard omstandigheden (dag 80) om vast te stellen of de geobserveerde invloeden langdurig waren. We vonden significante verschillen tussen de geslachten in alle standaard testen op beide testmomenten. Mannetjes waren vrijmoediger en exploreerden meer; vergeleken met vrouwtjes spendeerden zij meer tijd in scholingsgedrag en interacteerden meer met een groep stimulus-vissen, maar maar ook verlieten zij de school vaker om een nieuwe voedsellocatie te benaderen en binnen te gaan. We zagen ook significante behandelingseffecten in sommige gedragsmetingen, maar deze waren vaak beperkt tot bepaalde subgroepen, wat wijst op het bestaan van complexe interacties tussen de geanalyseerde factoren. Kort na de tweede reeks gedragstesten (dag 85), onderzochten we de neiging van deze vissen om sociale informatie te gebruiken (en te behouden) in een tweedelige schoolkeuze test binnen een potentieel gevaarlijke nieuwe omgeving, de resultaten hiervan worden gerapporteerd in Hoofdstuk 5. We vonden dat aan roofdieren blootgestelde vissen, en in het bijzonder mannelijke individuen, meer bedreven waren in het gebruik van sociale signalen – schoolcohesie - bij het maken van beslissingen in scholingsgedrag. Deze vissen toonden een voorkeur voor een losse school boven een dichte school (in de natuur zou dit doorgaans wijzen op de aanwezigheid van nabijgelegen potentiële bedreigingen). Aan roofdieren blootgestelde vissen leken deze informatie ook te behouden na een korte tijdsperiode en maakten vergelijkbare keuzes over aquarium locaties in het tweede deel van de test, toen de scholen afwezig waren.

Over het geheel genomen hebben we bewijs gevonden voor consistentie en erfelijkheid van scholingsgedrag, maar hoewel de neiging tot groeperen (tot op zekere hoogte) gecorreleerd waren met neigingen tot sociaal leren vonden we geen bewijs voor de consistentie en erfelijkheid van sociaal leren. Verder onderzoek is nodig om na te gaan of dit werd veroorzaakt door de slechte prestaties van de guppy's in de testen voor sociaal leren, of dat het meer flexibiliteit in de benutting van sociaal leren vergeleken met scholingsgedrag weerspiegelt. Verder zagen we dat de vroege omgevingsomstandigheden en ervaringen een belangrijke rol spelen in het gedragsfenotype van een individu. We vonden dat een individu's afhankelijkheid van sociale informatie op latere leeftijd afhangt van zowel signalen over de waarde van sociale informatie als contextuele signalen, zoals predatierisico en populatiedichtheid in het vroege leven. Aangezien de mannetjes en vrouwtjes significant verschillen in hun strategieën om fitness en overleving te maximaliseren, kunnen deze signalen in het vroege leven het gedrag van de twee geslachten op verschillende manieren beïnvloeden.

Curriculum Vitae

I, Ioannis Leris, was born on October 23rd 1983 in Agrinio, Greece. I graduated from Arsakeio Psychikou High School in 2001 and begun my BSc in Biology in University of Crete at Heraklion. During my undergraduate studies, I joined Prof. Maroudio Kentouri's Aquaculture laboratory and participated in various research projects on fish physiology and aquaculture. I conducted my BSc research project entitled: The effect of rearing temperature on body shape differentiation in zebrafish, *Danio rerio*, in the same lab. I graduated in 2006 and enrolled in University of Crete's MSc programme entitled 'Environmental Biology - Management of Terrestrial and Marine Resources'. During my postgraduate studies I conducted three short term research projects. I examined the phylogeography of the Mediterranean darkling beetle (genus *Dendarus*) in the Aegean area, with the use of molecular techniques in Dr. Nikos Poulakakis' lab in the Natural History Museum of Crete. Then I conducted research on industrial food preferences in farmed common dentex, *Dentex dentex*, in collaboration with Dr. Stavros Chatzifotis in the AquaLabs of Hellenic Centre for Marine Research. Finally, I studied morphological deformities of the lateral line in intensively reared gilthead sea bream, *Sparus aurata*, in Prof. Maroudio Kentouri's lab back at the University of Crete. I conducted my MSc research project, entitled: The effect of sex and rearing temperature on swimming performance of zebrafish, *Danio rerio*, in the same lab. After obtaining my MSc degree, I did my mandatory 12-month military service in Arta, Kos and Athens, Greece. In 2010, I started my PhD research project on the development of social behaviour in guppies, in the Behavioural Biology group of Utrecht University and Helmholtz Institute, under the supervision of Dr. Simon Reader and promoted by Prof. Johan Bolhuis. Part of this research was conducted at McGill University in Montreal, Canada.

Publication List

1. Sfakianakis, D. G., **Leris, I.**, & Kentouri, M. (2010). Effect of developmental temperature on swimming performance of zebrafish (*Danio rerio*) juveniles. *Environmental Biology of Fishes*, *90*, 421–427.

2. Sfakianakis, D. G., **Leris, I.**, Laggis, A., & Kentouri, M. (2011). The effect of rearing temperature on body shape and meristic characters in zebrafish (*Danio rerio*) juveniles. *Environmental Biology of Fishes*, *92*, 197–205.

3. Sfakianakis, D. G., **Leris, I.**, & Kentouri, M. (2012). Exercise-related muscle lactate metabolism in zebrafish juveniles : The effect of early life temperature. *Italian Journal of Zoology*, *79*, 568–573.

4. Sfakianakis, D. G., **Leris, I.**, Mylonas, C. C., & Kentouri, M. (2012). Temperature during early life determines sex in zebrafish, *Danio rerio* (Hamilton, 1822). *Journal of Biological Research*, *17*, 68–73.

5. **Leris, I.**, Sfakianakis, D. G., & Kentouri, M. (2013). Are zebrafish *Danio rerio* males better swimmers than females? *Journal of Fish Biology*, *83*, 1381–1386.

6. Reader, S. M., & **Leris, I.** (2014). What shapes social decision making? *Behavioral and Brain Sciences*, *37*, 96–97.

7. **Leris, I.**, & Reader, S. M. (2016). Age and early social environment influence guppy social learning propensities. *Animal Behaviour*, *120*, 11–19.

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"Those who have handled sciences have been either men of experiment or men of dogmas. The men of experiment are like the ant, they only collect and use; the reasoners resemble spiders, who make cobwebs out of their own substance. But the bee takes the middle course: it gathers its material from the flowers of the garden and field, but transforms and digests it by a power of its own. Not unlike this is the true business of philosophy; for it neither relies solely or chiefly on the powers of the mind, nor does it take the matter which it gathers from natural history and mechanical experiments and lay up in the memory whole, as it finds it, but lays it up in the understanding altered and digested. Therefore, from a closer and purer league between these two faculties, the experimental and the rational (such as has never been made), much may be hoped."

Francis Bacon, 1620. Novum Organum.