PHENOTYPIC PLASTICITY IN SOCIAL INFORMATION USE AND STRESS RESPONSES IN TRINIDADIAN GUPPIES

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ABSTRACT

Many animals navigate an environment where resources and threats fluctuate rapidly, and while phenotypes evolve, they can also adapt within a lifetime through phenotypic plasticity. Since obtaining appropriate information about resources and strategically responding to stressors are crucial to survival and reproduction, we expect these phenotypes to adjust to local conditions. In this thesis, I examined the contribution of phenotypic plasticity to social information use propensities and stress responses in a small prey fish, the Trinidadian guppy, *Poecilia reticulata*. In Chapter 2, I used a comparative field experiment to demonstrate that wild guppy populations vary in their tendency to use social information and to socially learn about food resources, and in whether they copied or avoided other individuals. In Chapter 3, I followed up Chapter 2 by investigating the developmental basis of social information use with a laboratory experiment. I found that early experience of conspecific density, but not simulated predation cues, can shape social information use. Together, these two chapters suggest that 1) social information use and social learning tendencies are flexible; and 2) developmental and evolutionary processes shape these abilities. In Chapter 4, I investigated a potential mechanism, the stress axis, through which early life experience could affect propensities to use social information. I measured cortisol release in fish with different early life experiences. Males released high levels of cortisol stable over time and regardless of rearing experience, indicative of a fast life history but of low reactivity to stressors. Females reacted strongly to a mild stressor but quickly habituated, except for females reared under stressful conditions. Behaviours measured in Chapter 5 paralleled these results: males approached a predator more closely than females. Early life experience of predation cues resulted in both males and females distinguishing between a predator and a non-predator. Chapters 4 and 5 together show that 1) hormonal and behavioural stress responses are shaped by developmental experience, 2) sexes, which vary considerably in life history in guppies, also employ different risk managing strategies. My thesis, in sum, demonstrates the dramatic impact of developmental experience on guppy phenotypes, provides one of the first demonstrations that social learning tendencies differ across natural populations and social information use can be shaped partially by development, as well as demonstrating that early experiences and life-histories affect the development of the stress response.

RÉSUMÉ

Les animaux naviguent un environnement où les conditions fluctuent rapidement. Bien que les phénotypes évoluent, la plasticité phénotypique permet une adaptation rapide aux conditions locales. Acquérir de l'information sur les ressources et réagir stratégiquement aux dangers sont des habiletés cruciales à développer pour survivre et se reproduire. Ces phénotypes sont donc susceptibles de s'ajuster aux conditions locales. Dans cette thèse, j'examine la contribution de la plasticité phénotypique sur la tendance à utiliser l'information sociale et la réponse au stress chez le guppy de Trinité *Poecilia reticulata*. Dans le Chapitre 2, je démontre par une étude comparative que les populations sauvages des guppys diffèrent dans leur tendance à utiliser l'information sociale et à apprendre socialement, et s'ils copient ou évitent le choix des autres individus. Le Chapitre 3 est un suivi du Chapitre 2, où j'évalue la base développementale de la tendance à utiliser l'information sociale avec une expérience en laboratoire. J'ai démontré que l'expérience en début de vie de densité sociale, mais pas celle d'indice de prédation, façonne l'utilisation d'information sociale. Ces deux chapitres démontrent que 1) l'utilisation d'information sociale et l'apprentissage social sont flexibles; et 2) que les processus développementaux et d'évolution façonnent ces habiletés. Dans le Chapitre 4, j'ai investigué un mécanisme potentiel, l'axe du stress, qui pourrait affecter la tendance à utiliser l'information sociale. J'ai mesuré deux fois le cortisol sécrété par les poissons avec différentes expériences en début de vie. Tous les mâles sécrètent un haut taux d'hormone, mais qui reste constant. Ceci indique des traits d'histoire de vie rapides et une faible réaction au stress. Par contre, les femelles réagissent fortement au stress mais s'habitue rapidement, sauf celles avec les expériences de début de vie les plus stressantes. Les comportements mesurés dans le Chapitre 5 sont parallèles: les mâles approchent plus que les femelles les prédateurs. De plus, l'expérience avec des indices de prédation tôt dans la vie permettent de distinguer les prédateurs des non-prédateurs. Ensemble, les Chapitres 4 et 5 démontrent que 1) les réponses comportementales et hormonales au stress sont façonnées par les expériences lors du développement, 2) les deux sexes, qui diffèrent dans les traits d'histoire de vie, ont des réponses au stress différentes et alignées avec leur stratégie. Ma thèse démontre l'impact des expériences lors du développement sur les phénotypes des guppies, offre une des premières preuves que l'apprentissage social diffèrent entre les populations naturelles et que l'utilisation d'information sociale est façonnée lors du développement, et démontre que les expériences en début de vie affectent le développement des réponses au stress.

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CONTRIBUTION TO ORIGINAL KNOWLEDGE

All the chapters and linking statements of this thesis are original scholarship and were created for the partial fulfillment of the degree of Doctor of Philosophy.

This thesis is manuscript based, and all chapters contribute distinct contributions to scientific knowledge. In Chapter 2, I investigated population differences in social information use and social learning, that populations with different ecological conditions differ in whether they use social information, and in whether they copy or do the opposite to other individuals. In Chapter 3, I demonstrated that the differences in Chapter 2 might be minimally attributed to developmental experiences and if so, by the rearing social environment rather than by simulated predation pressure. In Chapter 4, I demonstrated that the same developmental experiences as addressed Chapter 3 influences the physiological stress responses (cortisol release) in females, and that these responses are greatly influenced by sex. In Chapter 5, I showed that the developmental experiences of simulated predation, influence how individuals respond to predators later in life, while developmental experience of the social environment influences grouping tendencies.

Chapter 4 has been published in the journal Royal Society Open Science and the dataset is accessible in the Dryad Digital Repository at https://doi.org/10.5061/dryad.22mh6. All other datasets will be made available in an online repository.

CONTRIBUTION OF AUTHORS

Chapter 2: Laura Chouinard-Thuly designed the study with input from Simon M. Reader. L.C.-T. ran the experiment, coded the videos (with help from Lauren Redies), analyzed the data and wrote the manuscript with input from Simon M. Reader.

Chapters 3, 4, and 5 were part of an experiment on the effects of early life experiences. Ioannis Leris designed and ran the developmental manipulations, with input from Simon M. Reader, Adam R. Reddon and Laura Chouinard-Thuly. Our parental fish generously came from Kiyoko Gotanda.

Chapter 3: Laura Chouinard-Thuly conceived the experiment with input from Simon M. Reader. L.C.-T. and Lisa Xu ran the experiment and coded the videos. L.C.-T. ran the statistical analyses and wrote the manuscript with input from Lisa Xu, Ioannis Leris, and Simon M. Reader.

Chapter 4: Laura Chouinard-Thuly, Adam R. Reddon, Ioannis Leris, Ryan L. Earley and Simon M. Reader conceived and designed the study, planned analyses and contributed to the manuscript. L.C.-T. and A.R.R. collected the hormones. R.L.E. extracted and assayed the hormones. L.C.-T. and A.R.R. ran the analyses. L.C.-T. wrote the manuscript with input from all co-authors.

Chapter 5: Laura Chouinard-Thuly conceived and designed the study with input from Simon M. Reader. L.C.T. conducted the predator approach tests, coded the videos, planned and ran the analyses, and wrote the manuscript with comments from S.M.R. and I.L.

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CHAPTER 1: GENERAL INTRODUCTION

Environmental conditions can change rapidly. They can vary between populations of a single animal species, but also within and between generations, changing the adaptive value of phenotypic traits. Phenotypic plasticity allows the fine tuning of genotypes to local conditions. Such responses can even create individuals that are so morphologically distinct they were originally classified as distinct species. For example, predation cues trigger the development of a helmeted *Daphnia pulex* morph (Krueger & Dodson 1981), or seasonal photoperiod and temperature induces different colour morphs in the European map butterfly *Araschnia levana,* (Windig & Lammar 1999). Most often, environmental conditions lead to more subtle changes in life-history, morphology, physiology, or behaviour (West-Eberhard 1989; Monaghan 2008). Since phenotypic plasticity may allow the expression of optimal phenotypic traits in changing conditions quicker than does genetic adaptation, it confers an advantage that can be subjected to selection. It is however still debated if plasticity plays a role in the evolutionary processes leading to novel phenotypes, and whether it speeds or slows evolutionary change (Gilbert et al. 2015). The acquisition of information about the environment, either directly by personal sampling, or indirectly, through the observation of or interaction with other individuals (Danchin et al. 2004) is central to phenotypic plasticity (Schmidt et al. 2010). Theoretically, as more information is gathered, the better an organism can adapt its phenotype to local conditions, but information is costly to gather. One taxonomically widespread alternative to reduce these costs is to use information produced by other individuals ('social information'; Danchin et al. 2004). As phenotypes result from the integration of evolutionary history with previous and current experience (Dall et al. 2005), individuals might possess genetic predispositions (e.g. "reaction norms"; Via et al., 1995), and/or constraints on how well they can and should adjust to local conditions to maximize benefits (Schmidt et al. 2010). And while some phenotypes remain plastic through adulthood and keep adjusting to current conditions, many phenotypes exhibit a particularly sensitive period during early life in which information about the environment is most likely to affect the organization of these phenotypes (Bateson 1979). The early life conditions may lead to increase in fitness in adulthood, but can also lead to suboptimal phenotypes if there is a mismatch in the environment or if early conditions imposed a constraint on the development of animal phenotypes (Monaghan 2008).

The aim of my thesis is to examine the phenotypic plasticity of social information use and stress responses. Responding adequately to stressors, for example to the presence of a predator, is an important skill to develop for prey species. To investigate this question, I took a range of approaches. I compared wild populations living in different ecological conditions. I then followed-up with a series of laboratory experiments to examine how manipulations of early life conditions shaped adult phenotypes. I was specifically interested in examining the different responses to social information that exist in wild population and how local conditions contribute to their development, and physiological and behavioural responses to external stressors.

ENVIRONMENTAL CONDITIONS AND THE DEVELOPMENT OF PHENOTYPES

We observe a great deal of variation in phenotypes between individuals of the same species of animals or the same population that is not necessarily fully due to differences in genotypes. While some phenotypes may be canalized, or present little variation in different environments, phenotypes most often represent the outcome of complex interactions between genes and the environment (Mameli & Bateson 2011). The evolution of phenotypic plasticity, or the ability of a single genotype to produce different phenotypes depending on the environment (West-Eberhard 1989), has costs and limitations, such that genotypes might constrain what can develop ("reaction norms"), but often phenotypic plasticity allows the development of phenotypes that are most suited to the local conditions. Moreover, we expect certain phenotypes, such as many behaviours, to retain plasticity through life (referred to as "behavioural flexibility"; Ghalambor et al. 2010), while some other traits may be organized early in life with little room to adjust later in life. Depending on the ecology of the species – and sometimes the population – different characteristics of the environment may be influential for the development of phenotypes, although predation risk and local social density stand out as particularly important and widespread influences.

PREDATION RISK

Predators act as a major selective and developmental force (Lima & Dill 1990). Predators can have direct effects on the selection of phenotypes through consumption, such as the negative selection of brightly coloured guppies *Poecilia reticulata* by predators (Godin & McDonough, 2003). The threat of predation also creates non-lethal changes in prey that can be short-term, such as freezing behaviour or grouping, and which comes to a cost to other activities such as foraging and mating (Lima & Dill 1990). When predators are present, individuals might reduce overall activity and increase time spent in a refuge (Turner 2004), or optimize trade-offs by, for example, taking more risk when hungry than when not (Lima 1998). As the presence of predators is variable in time, predation cues can promote phenotypic plasticity, which allows fine-tuning to the environmental conditions (DeWitt et al. 1998, Agrawal 2001). The changes produced by nonlethal effects of predation can be large (Cresswell 2008) and are often reflected in life-history, morphology, physiology and behaviour (Peckarsky et al. 2008). Such non-consumptive effects, and lifetime experience with predation threat can also trigger long-lasting changes ("developmental plasticity"; Snell-Rood, 2013) that reduce the risks of living under high predation risk, but that would be suboptimal to express in environments without predation. This is seen in the dipteran *Chironomus tentans,* for example, that adopts a different life-history in which individuals molt at a smaller size under cues of predation threat, enabling them to escape quickly the high-risk larval stage but thereby reducing the opportunity for energy storage (Ball & Baker 2014). Therefore, individuals can benefit from appraisal of the local risk, which they can form from non-lethal encounters with predators, or through indirect cues or signals coming from other individuals that encounter the predators. Indirect cues may come from intergenerational parental effects, or from current experiences of other individuals in the same environment (Gilbert et al. 2015). Many species use alarm cues from conspecifics or heterospecifics to gain information about predation risk (Sherman 1977). In fish, a chemical released from the skin of injured individuals ("alarm substance") serves as an alert that there is a predator present in the environment. Thus, this cue can serve to learn about novel predators or simply trigger an anti-predator response (Pfeiffer 1977). How much experience with cues of predation changes different phenotypes is investigated in Chapters 3, 4, and 5.

In most cases of developmental plasticity, the presence of predators impacts a combination of life-history, stress reactivity, and behaviours. Life-history theory has well established how and when predation pressure should trigger a correlated development between the reproductive output and physiological systems of individuals to maximize fitness (Nonacs & Blumstein 2010). It has however proven difficult to predict accurately how predation pressure (and other early life experiences) shapes behaviour, and when different behaviour patterns would co-vary. The Pace-Of-Life Syndrome (POLS) hypothesis suggests that individuals should display phenotypes that align with their reproductive strategies, such that individuals with fast life-histories should also be risk takers, but that individuals with slow life histories and a high future reproductive output should exhibit behaviours that maximize longevity (Réale et al. 2010). It is also hypothesized that not all phenotypes or phenotypic combinations can be produced because of constraints in the genetic architecture (Dingemanse et al. 2009; Fawcett et al. 2013), creating suboptimal behaviours when arising through a genetic, neurodendocrine, or other physiological correlation with other traits (Quinn & Cresswell, 2005). Research (mostly in fish) has however demonstrated that otherwise correlated behaviours can be uncoupled, suggesting that behaviours co-vary in animals only when beneficial (Bell 2005; Dingemanse et al. 2007). It is possible that behaviours retain more plasticity than other physiological systems that are established early to allow adjustment if conditions change in lifetime, or that local conditions change the correlation between traits through evolutionary and developmental pressures (Bell 2005; Bell & Sih 2007). I evaluate the results of chapters 3, 4, and 5 in light on this hypothesis in the general discussion.

SOCIAL ENVIRONMENT

The composition and density of the social environment also shapes phenotypes. At one extreme, rearing rats in social isolation results in a pathology where morphology, brain development, physiology, and behaviour are impaired (Heidbreder et al. 2000), possibly because of high experienced stress which constrains the development of normal behaviour. The social environment can also affect the optimality of different phenotypes through density-effects. For example, in *Schistocerca emarginata* grasshoppers, and other insects, the development of the aposematic colouration is density dependent (Sword 1999). The perceived social density is also an indication of competition for resources, and can impact population dynamics through changes in adult life strategies or by affecting allocation of resources (Reznick & Yang 1993). Competition can be a selective agent, selecting for larger offspring size for example (Bashey 2008), but also affects social structures by altering for instance dominance hierarchies (Ward et al. 2006), or courtship strategies (Jirotkul 1999). In marbled salamanders, *Ambystoma opacum,* density during their larval stage determines the age at which they first reproduce, as well as clutch size, suggesting a strong impact of social density on life-history (Scott 1994). In sexually dimorphic species, mating strategy differences can alter the effect of social density. In guppies, for example, females pay higher reproductive costs, and a high perceived competition for mates forces male guppies to change their courtship behaviour (Price & Rodd 2006). Thus, even though high density can offer benefits, it may also be a synonym of competition for resources.

Intraspecific competition for resources brought by high densities can also bring more subtle phenotypic changes (Ward et al. 2006). In crowded situations, many fish develop high aggressiveness, in turn affecting shoaling tendencies (Olla et al. 1998). But although a high density can bring competition, high densities provide anti-predator advantages, and can be advantageous too if resources are not overly limited, as more individuals can share information about the environment. This is notably the case of species that use vigilance to detect the presence of predators (Beauchamp & Ruxton 2003). While the density is important for developing courtship behaviours, the composition of the social environment also plays a role, and can affect sexes differently. Milkweed beetle *Tetraopes tetraophthalmus* males disperse when the patch is male biased (Lawrence 1987). The presence of adults can be a predation risk for the young of some species, and guppies reared with many adults develop phenotypes akin to those reared under predation risk (Chapman et al. 2008). How animals gather information about the density or composition of their social groups remains however uncertain, and the effects of density are investigated in Chapters 3, 4, and 5.

RESPONDING TO THE ENVIRONMENT

As highlighted above, while natural selection provides a mechanism for long-term adaptations to stable or predictable conditions, local conditions in the environment may change between and within generations, and rapid phenotypic adaptations through phenotypic plasticity might be required. Gathering information about local conditions is central to this process, as it reduces uncertainty about the state of the environment. Making informed decisions can have a tremendous impact on individuals' fitness and thus how animals gather information and how information influences the development of phenotypes is important to investigate (Dall et al. 2005).

Information is difficult to define operationally within organismal biology, although most definitions rely on the central concepts that information 1) reduces uncertainty by improving knowledge of the probability of different events in the current environment (Stephens,1989; Danchin et al., 2004) and 2) changes the state of the receiver in a functional way (Jablonka, 2002; Dall et al., 2005; both concepts in Schmidt et al., 2010). My thesis is oriented towards questions of trade-offs in the expression of phenotypic traits and thus I focus on the changes in the receivers of information ("observers") and operationalize the definition by focusing mainly on concept 2.

USING AND ACQUIRING INFORMATION

Typically, researchers categorize information based on the sources it is acquired from. On the one hand, animals can interact directly with resources, thus collecting "personal information". An example of this would be 'prospecting behaviour', where birds such as Northern wheatears (*Oenanthe oenanthe*) assess features such as ground vegetation as a quality indicator (Pärt et al. 2011). On the other hand, animals also collect indirect information by monitoring other individuals, or "demonstrators" ("public information"; Danchin et al. 2004). Collared flycatchers *Ficedula albicollis,* for example, use the success of other individuals at a specific breeding patch to asses patch quality (Doligez et al. 2002). In some cases, the information collected from other individuals will also be retained to inform later decisions, and thus results in "social learning", i.e. learning that is influenced by interaction with other individuals or their products (Hoppitt & Laland 2013).

For animals to engage in social learning, it implies that they possess the ability to learn. Learning is costly (Johnston 1982), requiring investment in the development and maintenance of cognitive systems, which can come at the expense of other tissues like reproductive tissues or fat storage (Navarrete et al. 2011). Learning can however offer fitness benefits, when the environment is not stable enough between generations to promote genetic adaptations, but not variable enough within generations to make learned information useless quickly (Dunlap & Stephens 2009). Whether social learning requires specialized mechanisms or is simply derived from individual learning processes using social cues is still debated (Heyes, 2012; Lefebvre & Giraldeau, 1996; Reader, 2016). The latter hypothesis, that social learning relies on individual learning processes, has received most empirical support, with the demonstration that many cases of social learning can be explained by simple learning mechanisms such as associative learning (Alem et al. 2016), and since we observe a correlation between general cognitive abilities and social learning propensities (Reader 2003; Reader et al. 2011; Reader 2016). Conversely, complete maternal care deprivation during rearing affects social but not other forms of learning in adult rats (Lévy et al. 2003). This suggests that asocial and social learning processes can be uncoupled, at least developmentally. This could also be explained if social abilities correlate with social learning propensities and the former are affected by low maternal care. In the case of *Neolamprologus pulcher,* rearing in isolation or with restricted social contact impairs the proper development of social abilities which may in turn affect how well they attend to social cues (Fischer, Bessert-Nettelbeck, Kotrschal, & Taborsky, 2015; van Leeuwen, Mulenga, & Chidester, 2014). Regardless of whether it requires specialized mechanisms or not, social learning has consequences that are distinct from individual learning. It can allow the spread of novel behaviour patterns or discoveries –innovations- that could be difficult or impossible to discover for some individuals. By enabling the transmission of those innovations, social learning underlies the spread of traditions seen in animals groups like lobtail feeding in humpback whales, which originated from a single individual (Allen et al. 2013). The creation of traditions through social learning processes underlies the development of human culture (Boyd & Richerson 1985; Morgan et al. 2015).

While social information can provide fitness benefits, it could also provide maladaptive information (Laland & Williams 1998; Giraldeau et al. 2002). Trade-offs between social and asocial information largely determine which type of information individuals decide to rely on. In itself, the value of social information is dependent on the ecological, temporal, and spatial distance between the observer and the demonstrator of the information (Seppänen et al. 2007). Social information can lead to unsuitable (i.e. suboptimal) information if gathered from individuals that are ecologically remote or if the information was gathered too long ago (Giraldeau et al. 2002). The trade-offs between personal and social information are often a function of the current situation. Ninespine sticklebacks (*Pungitius pungitius)* rely on the most recently acquired information to make foraging decisions when they conflict, thus using the most reliable information (Bergen et al. 2004). In general, animals seem to follow so-called social learning strategies, or rules-of thumb, that guide when and from who to use social information to maximize benefits (Laland, 2004). There is considerable evidence that animals adhere to "when" strategies, selecting to follow social information when it would be optimal (Kendal et al. 2005). For example, under predation threat, social information would be particularly beneficial as acquiring personal information is costly, as has been demonstrated in minnows *Phoxinus phoxinus* (Webster & Laland 2008). The value of social information is also density dependent, where for example using the same resources as another individual may lead to depletion (Hoppitt & Laland 2013a; Seppänen et al. 2007). If the decision is made to copy, there are also biases regarding who animals chose to copy, possibly preferring for example familiar individuals (Swaney et al. 2001). These strategies seem to be influenced as well by developmental experiences (e.g. brood size: Riebel et al. 2012; cortisol levels: Farine et al. 2015; maternal care: Lindeyer et al. 2013). This suggests that experience is important to determine social learning strategies, rather than the use of strategies being a fixed trait. How populations with different conditions use social information is the question of interest of Chapters 2 and 3.

There is also the possibility that social information use and social learning tendencies are influenced by characteristics of individuals that change the probability of attending to social information or copying it (Mathot & Giraldeau 2008). Personality traits can correlate with the ability to acquire or use social information or directly influence the trade-offs between the types of information, though not much work has yet been done. Raising guppies in low densities resulted in a greater shoaling tendency, and a faster discovery of food in a maze trial following a demonstration (Chapman et al. 2008). Captive great tits labeled as "fast" explorers preferred the feeder demonstrated by a tutor compared to the previously known feeder (Marchetti & Drent 2000). Individual characteristics might affect the use of social information at different stages: the successful use of social information implies different steps, as individuals need to acquire, retain and retrieve, and successfully use the information (Lindeyer et al. 2013). Each of these steps might be constrained differently by individuals' phenotypic traits, for example if rank status makes it difficult for an individual to obtain a resource (Carter et al. 2016). The evolution of correlated behaviours is suggested to be explained by trade-offs in life-history, where individuals with high future expectations for reproduction are more risk averse, creating a between-individuals difference in the degree and extent of behavioural plasticity affecting life strategies and fitness

(Dall et al. 2004; Wolf et al. 2007; Dingemanse & Réale 2005; Dingemanse & Wolf 2013; Réale & Festa-Bianchet 2003). An hypothesis is therefore that social learning and social information use propensities are underpinned by the stress axis, possibly explaining correlations found between risk taking tendencies and social information use (Trompf & Brown 2014, Harcourt et al. 2010).

STRESS RESPONSES

The stress axis, controlled by glucocorticoid hormones, is a central player in responses to stressors, or outside stimuli that provoke a change in homeostasis (Reeder & Kramer, 2005). Acute changes in hormone concentrations bring about a suite of integrated behavioural responses collectively known as the stress response (Boulton et al. 2015). Glucocorticoid hormones are typically understood as regulating energy allocation between mundane activities like foraging and mating at background levels, and emergency situations when a stressor occurs (Sapolsky et al. 2000). Acute elevated levels trigger a mobilization of energy that translates into a behavioural response to the stressor (Boulton et al. 2015).

Activation that is beyond the normal scope of reaction (either too high or for too long) brings about pathologies that can be lethal or result in decreased reproduction (Romero et al. 2009). High stress conditions in snowshoes hares *Lepus americanus* can lead to cyclical population patterns through a decrease in reproduction, while intergenerational effects on the stress axis explain delayed recovery of the populations (Sheriff et al. 2015). High social densities can also lead to similar patterns, by inducing increasing stress levels due to competition for food or agonistic social interactions (Creel et al. 2013). To avoid going outside the normal scope of reaction, the stress axis is thus sensitive to the conditions in the environment (Romero et al. 2009). For example, laboratory rodents who are habituated young to be handled, a mild stress, seem to be better able to cope with stressors later in life because of a quick and efficient deactivation of the system (Clinchy et al. 2013).

Whether early life conditions adaptively prepare individuals for a similar future is however still ambiguous. On the one hand, the predictive adaptive response (PAR) hypothesis suggests that difficult early life conditions have benefits in adult life as they shapes phenotypes to operate optimally in these conditions, assuming there is a high match between early and adult environment. One the other hand, systems may not be able to develop optimally under stressful conditions due to cumulative stress, such that individuals with little early life stress benefit from a "silverspoon" effect and fare better in any conditions than individuals who developed in stressful conditions (Clinchy et al. 2013).

MODEL SYSTEM

The Trinidadian guppy offers an extremely suitable study system to investigate questions related to the responses to information and environmental conditions, due to their rapid generation time, quick evolutionary responses, and the ability to perform tests in both the field and the laboratory (Magurran, 2005). Guppies are a small tropical fish of the poeciliid family, distinguished by their livebearing reproductive system. Guppies exhibit a wide variety of phenotypes, and are generally recognized by their very colourful pattern, which makes their domestic strain extremely popular in the aquarium trade. Guppies are sexually dimorphic; females carry the larger cost of reproduction through livebearing, and thus present a much slower life history and are more risk averse than males (Magurran & Nowak, 1991). Males devote relatively little time to foraging, instead devoting most of their energy on reproductive effort. Males and females also experience different predation pressures in the wild. While females are larger and thus a more profitable prey item, their anti-predator behaviour reduces the actual predation rate. Through sexual selection, males have become brightly coloured and bold and thus easier to capture for predators; males therefore suffer a greater mortality from predation than females (Godin & Dugatkin, 1996; Reznick & Endler, 1982).

Originally, guppies inhabited small tropical streams in the south of the Caribbean islands (notably Trinidad) and in Northern South America (such as in Colombia). Their habitat in Norther Trinidad is characterised by distinct rivers with little to no connection between the basins (Crispo et al. 2006; Becher & Magurran 2000). Most of the rivers in Northern Trinidad, where guppies are particularly well studied, are characterised by two types of habitats. In the upper part of the rivers, habitats generally contain no fish predators that prey on adult guppies, narrow streams, low light levels, high biomass, and low primary productivity, while the lower habitats contain significant fish predators, large streams, high light levels, low biomass, and high primary productivity (Reznick et al. 2001). The combination of higher resources in lower river habitat with the pressure of predation shapes guppy life-history patterns and behaviour: in lower river habitats, guppies have a fast life-history characterised by rapid growth, high reproductive investment, dull colouration in males, as well as high shoaling tendencies, low aggressiveness, and low risk taking tendencies (Magurran & Seghers, 1994, Harris et al. 2010, Budaev & Brown 2011). The specific contribution of environmental conditions and predation to the evolution and development of life-history is still debated. Some traits, such as male colouration, are mainly determined by the dichotomous presence of predators (Millar et al. 2006), while others are also influenced by the density and composition of the predator species present (Rodd & Reznick 1991; Torres Dowdall et al. 2012). Overall, competition and predation stand out as important contributors to guppy phenotypes.

While guppies were originally found in south and central America, they have since colonized more than 70 countries, either through feralisation of escaped domestic individuals, or by a deliberate import for control of mosquito larvae. Guppies fare extremely well in a variety of regions and climate, possibly due to their reproductive system and high social mixing with local populations (Sievers et al. 2012; Deacon et al. 2011). While mixing with local populations provides protective benefits, it also provides access to information about local resources and anti-predator behaviours. Guppies readily use and learn from social information in the wild (Reader, Kendal, & Laland, 2003), even from heterospecifics, potentially contributing to a successful invasion (Camacho-Cervantes et al. 2015). Evidence that the propensity to rely on social over personal information is affected by the boldness and sociality of individuals suggests plasticity in information gathering strategies (Trompf & Brown 2014), that can be altered by recent experience of the social environment (Chapman et al. 2008). The propensity to socially learn also seems developmentally plastic to some extent, with only fish having experience that social cues are beneficial using them as adults (Leris & Reader 2016). In guppies, sex differences are also observed in learning, with females learning more readily than males how to get to food in a group setting, although being hungry had more impact on males than on females (Reader & Laland 2000). Moreover, risk of predation changes grouping tendencies and thus how social information spreads (Hasenjager & Dugatkin 2017). Trinidadian guppies are thus an excellent system to investigate questions about the plasticity of social information use and social learning tendencies in the laboratory and particularly in the field, where very few studies have investigated social information and social learning.

The responses to stressors, and predators in particular, have been the subject of many inquiries in guppies. Guppies from populations with predators are better at handling encounters with predators, avoiding particularly dangerous areas of the predators such as the head zone (Magurran & Seghers, 1990), and tend to approach potential predators in larger groups (Magurran & Seghers, 1994). Those approaches are usually made to gather information about the threat posed by the potential predator (Pitcher 1992). Guppies can indeed recognize whether a predator is satiated or hungry, although guppies from populations with predation do so better than those from populations without predation (Licht 1989). The behavioural responses to stress, and particularly the tendency to display risky behaviours ("boldness") often correlates with the reactivity of the stress axis (Boulton et al. 2015). Guppies with evolutionary and developmental experience of predators that usually display risk averse behaviours have lower waterborne cortisol levels (Fischer et al. 2014), and they also tend to recover faster from stressful events. Guppies are thus a useful model system to investigate the role of developmental plasticity in the development of stress responses.

PHENOTYPIC PLASTICITY IN RESPONSES TO INFORMATION AND STRESS IN TRINIDADIAN GUPPIES

The previous section reviewed how local conditions can influence phenotypes, with possible long-lasting effects of early life conditions on adults' phenotypes. In this thesis, I investigate the influence of local conditions and sex on social and stress phenotypes in Trinidadian guppies. In **Chapter 2**, I investigated the hypothesis that socio-ecological factors can shape social information use and social learning propensities. I provide evidence that those propensities vary between natural populations with different local conditions. While this shows that these propensities are flexible, they could be shaped by evolutionary, early, or current experience. Moreover, these effects could be due to predation, or to other ecological conditions that co-vary with predation risk, such as social density. In the following chapters, I investigated the contribution of early life experience of predation and social density on adult phenotypes known to vary in the field, using a 2x2 laboratory design manipulating developmental conditions of fry guppies. Thus in **Chapter 3**, I investigated the hypothesis that social information use and social learning are developmentally plastic. I provide evidence that propensities to use social information are shaped by developmental experience of social density, but not of predation and that sex has a major effect on the type of information individuals attend to. I found no evidence of social learning, and suggest that those abilities might also be flexible. As local conditions also influence how fish respond to stressors, and social information use and social learning propensities are hypothesised to be influenced by the stress response, I investigated in **Chapter 4** the hypothesis that sex, and early life conditions shape the development of the stress axis. I provide evidence that males are less reactive to stressors, and that early life conditions affect the ability of females to habituate to stress. Since physiological stress responses often correlate with behavioural responses, I investigated in **Chapter 5** the hypothesis that sex and early life experiences would affect how adult fish reacted to a predator and non-predatory fish. I provide support for the idea that females are more cautious than males, and that early experience with predation helps guppies to distinguish predatory from non-predatory fish. I thus demonstrate the impact of developmental experience on phenotypes, provide one of the first demonstrations that social learning tendencies differ across natural populations, as well as demonstrating that early experiences and life-histories affects the development of hormonal and behavioural stress responses.

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CHAPTER 2: POPULATION DIFFERENCES IN HOW WILD TRINIDADIAN GUPPIES USE SOCIAL INFORMATION AND SOCIALLY LEARN

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ABSTRACT

Animals frequently have access to information produced by the behaviour of other individuals, which they may use ("social information use") and learn from ("social learning"). However, the benefits of using such information, and the way it can be adaptively employed are likely to differ with socio-ecological conditions. As a result, population differences in social information use and social learning may occur. We tested this possibility with a comparative study of social learning across five wild populations of Trinidadian guppies (*Poecilia reticulata*) known to differ in their local ecology and social behaviour. Using a field experiment, we found population differences in how guppies used and learned from social information, with only fish from one of the three rivers studied showing evidence of social information use and social learning. Within this river, populations differed in how they employed social information: fish from a high-predation regime where guppies exhibit high shoaling propensities (Lower Aripo) copied conspecifics, choosing the same foraging location as conspecifics, while fish from a low-predation regime with reduced shoaling propensities (Upper Aripo) chose and learned the opposite foraging location to that demonstrated by conspecifics. We speculate that these differences are due to differences in predation risk and conspecific competition, possibly mediated via changes in grouping tendencies. Thus, not all populations used social information or socially learned in our test, and populations within the Aripo river differed in how they used the available social information. Our results provide evidence that propensities to employ social information and social learning can differ across animal populations.

INTRODUCTION

As environments change, animals that gather information about resources and threats can be at an advantage. This information can be gathered by interacting directly with the environment and thus acquiring "personal" information (Dall et al. 2005). Alternatively, individuals can gather information from the behaviour or products of other individuals, a process termed social information use. Animals may learn from personal information (i.e. asocial learning) or social information (i.e. social learning; Hoppitt & Laland, 2013). Social information use and social learning are taxonomically widespread, being observed in invertebrates (e.g. Leadbeater & Chittka

2009), fish (Brown & Laland 2011), birds (e.g. Davies & Welbergen, 2009), and mammals (Hoppitt & Laland, 2013; Thornton & Clutton-Brock, 2011). Moreover, social information use has been described in a diversity of contexts, such as using social information about foraging locations (e.g. Aplin et al., 2015), foraging techniques (e.g. Whiten, Horner, & de Waal, 2005), predators (Griffin 2004), breeding habitats (e.g. Doligez et al. 2002), or potential mates (e.g. Witte & Ryan, 2002).

Choosing whether to employ asocial versus social information is dependent on multiple factors. Social learning has been shown to be used strategically to optimize net benefits (Kendal, Coolen, Bergen, & Laland, 2005; Laland, 2004; Rendell et al., 2011), although flexibility in both social information use and social learning can be constrained (Toelch et al. 2014; Rosa et al. 2012; Reader 2015). Using social cues can reduce the energy required to acquire information (Weigl & Hanson 1980), or reduce risk related to personally sampling a resource (Boyd & Richerson 1985; Webster & Laland 2008). In other cases, using social cues can be maladaptive or suboptimal if the information gathered is outdated or irrelevant to the observer (Giraldeau, Valone, & Templeton, 2002; Feldman, Aoki, & Kumm, 1996; Laland & Williams, 1998), and using social information may increase competition if individuals thus converge on a limited resource (Seppanen et al. 2007). Environmental or individual characteristics may inform the decision on which type of information is most likely to be beneficial (Laland, 2004) by modifying the costs and benefits of social information use and social learning (Bouchard et al. 2007; Reader 2003; Reader 2015). For example, the current presence of predators can increase the cost of personally exploring the environment, thus increasing the net benefits of using social information (Boyd & Richerson 1985; Webster & Laland 2008).

As well as local and current conditions being a likely influence on how animals utilise social information, their recent experience, early-life experience and evolutionary history may also play a role. For example, bumblebees *(Bombus terrestris)* will learn to copy or avoid other individuals' foraging choices depending on whether following these social cues was previously rewarded, demonstrating the effect of recent experience (Dawson et al.2013). Early-life experiences can also shape adult social information use, either due to direct experience with the value of following social cues (e.g. Leris & Reader, 2016), or to broader differences in social experience such as maternal care (e.g. Melo et al. 2006; Lindeyer et al. 2013). Species differences in social information use and social learning have also been described (e.g. birds: Templeton et al. 1999; mammals: Herrmann et al. 2007; Reader et al. 2011) which could be the result of evolved and/or developmental influences. For example, ninespine sticklebacks (*Pungitius pungitius)* who are under high predation pressure display increased propensities to socially learn than the less predated but closely related threespine stickleback (*Gasterosteus aculeatus;* Coolen, van Bergen, Day, & Laland, 2003). Furthermore, individual behavioural phenotypes that themselves could be shaped by experience and evolution, such as the speed to explore a novel environment or to solve a novel problem, can also predict social information use (Marchetti & Drent 2000; Rosa et al. 2012; Trompf & Brown 2014; Bouchard et al. 2007).

Given these influences on social information use, differences in social information use between populations dwelling in different socio-ecological environments are likely. However, very little work has investigated such population differences, particularly with experimental tests. A notable exception is the finding that populations of Zenaida doves *(Zenuida aurita)* differ in how they learn from a Carib grackle (*Quiscalus lugubris)*, a finding that has been explained by differences in foraging ecology shaping differences in social behaviour between these populations (Carlier & Lefebvre 1997; Dolman et al. 1996). Here, we investigated population differences in social information use and social learning by comparing multiple replicate populations tested in the wild, with the aim of identifying ecological factors that shape social information use.

We tested wild Trinidadian guppies to investigate this question. Guppies have successfully invaded over 70 countries (Deacon et al. 2011), colonizing rivers that are extremely diverse in geography and ecology. Guppies readily learn from conspecifics and hetereospecifics in both the field and the laboratory, which may partially explain why they thrive in diverse and new conditions (Camacho-Cervantes, Ojanguren, & Magurran, 2015; Reader, Kendal, & Laland, 2003). The ecology and evolution of Trinidadian guppies is particularly well studied, with differences in physiology, morphology, life history and behaviour found between populations that are partially separated by natural barriers, driven mostly, but not only, by the presence, density, and composition of predators (Reznick, Rodd, Cardenas, et al. 1996; Endler 1984; Torres Dowdall et al. 2012). Upper river habitats in northern Trinidad typically contain fewer predators of adult guppies, as well as a weaker current, and more access to invertebrates than lower river habitats (Magurran 2005). Trinidadian guppy populations differ on numerous behavioural measures: guppies from the upper river populations display lower shoaling tendencies, higher intraspecific aggressiveness and competition, and bolder phenotypes than in the lower river (Fraser & Gilliam 1987; Magurran et al. 1994; Brown et al. 2009; Magurran & Seghers 1991). High shoaling tendencies could increase the propensity to rely on social information since individuals are in close proximity to conspecifics, while high aggression and competition may increase the net costs of social information use and social learning. Trinidadian populations provide a valuable opportunity to test natural variation in the transmission of social information between populations exposed to varying environments.

We compared propensities for social information use and social learning using a foraging task in five populations of wild Trinidadian guppies from the Aripo, Marianne, and Paria rivers, testing fish in enclosures within their home river. Testing multiple rivers allowed us to investigate not only if there are population differences, but also whether within-river differences were paralleled across different rivers, which would provide support for socio-ecological conditions shaping population differences in a consistent manner. Naïve fish experienced two feeder locations, but could view and interact with conspecific "demonstrators", i.e. individuals with prior information, foraging at one of these two locations. Subjects could access food during the first phase when the demonstrators were present, and during the second phase when demonstrators were removed. This way, the experiment allowed us to investigate the differences between populations in the readiness to feed with conspecifics (social information use), as well as the propensity to retain this information (social learning). Broadly, we predicted guppies tested in their home rivers to prefer to forage at the same location as conspecific demonstrators, and to retain this preference when demonstrators were removed, as previously shown (Reader et al., 2003). However, we expected these tendencies to vary across populations. In fish from the Lower Aripo, known to display high shoaling tendencies and low interspecific aggression (Magurran 2005), we predicted subjects would copy the demonstrated location. We expected the same finding in Lower Marianne fish, a population which is classified as similar to Lower habitats of other rivers, and thus are expected to shoal in large group and show low interspecific aggression (Magurran 2005). In comparison, we expected guppies from the Upper Aripo, Upper Marianne and Paria, known to display low shoaling tendencies and expected (Marianne) or shown (Upper Aripo, Paria) to show high interspecific aggression (Magurran 2005), to either avoid the demonstrated location or to be unaffected by social cues. Guppies from the Paria site show particularly low shoaling tendencies and high interspecific aggression, making it an interesting comparator (Magurran & Seghers 1991) We expected similar population differences between the Upper and Lower sites in the Aripo and Marianne rivers, although we note that recent literature suggests that rivers may not be perfect replicates (Grether et al. 2001). This comparative study of social information use and social learning propensities thus allows us to determine (1) whether populations differ in these propensities, as might be predicted from hypotheses that evolutionary and developmental processes shape social information use; (2) why and when propensities change, and (3) whether these propensities change in similar manner, thus providing evidence for specific socio-ecological factors shaping social information use.

METHODS

OVERVIEW

We used a foraging test to compare how five guppy populations used social information and learned from conspecifics. We assessed social information use and social learning by (1) comparing subjects' responses to conspecific "demonstrators" at two feeding locations in a counterbalanced design and (2) comparing these responses to control subjects not exposed to demonstrators. Social information use was measured during a demonstration phase, when demonstrators were present (except in the control), while social learning was measured during a subsequent test phase, when demonstrators had been removed. Social influences on behaviour would result in subjects being more or less likely to feed at the demonstrated location than the alternative location.

STUDY SITES AND SAMPLING

We tested in three rivers located in different watersheds of the Northern Range Mountains in Trinidad: the South slope Aripo river (Caroni watershed; tests run June 2013), the North slope Paria river (June 2013), and the North slope Marianne river (July 2014). We tested at previously studied locations, detailed in Jacquin et al. (2016) and Gotanda et al. (2013). Guppy lineages from these rivers are genetically differentiated (Willing et al. 2010). In both the Aripo and Marianne rivers, we tested at a "Upper" and "Lower" river location: the Ap2, Ap4, Ma14, and Ma8 sites (site numbers follow Gotanda et al. 2013), and in the Paria river we tested at site Pa14. "Upper" and "Lower" river locations are separated by waterfalls, with large teleost fish predators absent from upper but not lower locations, and numerous other ecological differences between the locations (Magurran 2005). There is no similar "Lower" location in Paria, so we thus sampled only one site, with no large teleost fish predators (like other "Upper" locations), but where large predatory prawns *Macrobrachium crenulatum* are present (Reznick, Rodd & Cardenas 1996; Rodd & Reznick 1991). We chose sites where the ectoparasite *Gyrodactylus* has been recorded (Gotanda et al. 2013, Jacquin et al. 2016), so that *Gyrodactylus* presence/absence was the same for all study sites. To ensure independent fish were sampled, we typically selected subsequent sampling pools by going upstream, or by selecting physically separated pools. We used butterfly nets to gently collect female guppies, and ran our tests in enclosures within rivers. Fish were held in a water-filled enclosure placed in the river for a maximum of 5 hours. During this time, we presented them with the social information use and learning tests, then moved them to an enclosure for tested fish, with fish released at their capture site at the end of a testing day.

TESTING APPARATUS

The testing apparatus consisted of a small floating box made of mosquito net (23 cm high, 38 cm wide and long; figure 2.1), which allowed stream water to flow freely through the apparatus, with the front and back of the apparatus made of transparent plastic. Since fish were tested in an enclosure, they were physically separated from any local predators and the experiment was not a field test of social learning on free-living animals (Reader & Biro 2010). However, they were in field conditions until the experiment began, were tested in their local environment, and were exposed to olfactory and visual cues from outside the enclosure. We mounted a waterproof camera (1080p at 30fps, GoPro3 Black Edition, San Mateo, California) on one wall to record behaviour at the removable feeder (36 cm width) positioned on the opposite side. This feeder consisted of two feeding locations separated by 10 cm, with each location made up of two vertical 5 cm wide feeding columns placed 3 cm apart, creating patches of food that were accessible to multiple individuals simultaneously. The feeding columns were made of food sprinkled on gelatin (KNOX, Treehouse Foods, New York State, USA) mixed with food colouring (Club House, McCormik Canada, London Ontario, Canada) poured on a patterned background (figure 2.1). We used two types of feeding column. One was made of freeze-dried bloodworms (*Chironomus* spp., Omega One, Omegasea Ltd, Sitka, Alaska) sprinkled on green-coloured gelatin, placed on a black-striped background. The other was made of flake food (TetraMin, Tetra, Germany) sprinkled on yellow-coloured gelatin placed on a black-dotted background. We used this variety of food, pattern, location and colour cues to provide multiple discriminatory cues for the subjects and to increase differences between the feeding columns. For demonstrations by conspecifics, we put "demonstrator" fish in a small "demonstration box" (10 cm height, 5 cm width and depth) made of perforated transparent plastic so that demonstrators reliably fed on one column without requiring extensive training. We placed the box directly in front of one column, with a similar but smaller feeding column inside the box.

EXPERIMENTAL METHODS

Each trial consisted of a 1) habituation, 2) demonstration, and 3) test phase. In the 1) habituation phase, we placed a group of four fish in the testing apparatus without the feeding wall for 10 minutes. We tested fish in groups as guppies are typically highly social and may show stress responses when placed in isolation (Depasquale et al. 2016). Group testing is particularly important since isolation stress may vary between populations, given that populations vary in grouping tendencies (Magurran & Seghers 1991), potentially impacting the social information use we examine here. Simultaneously, two fish from the previously tested subject group, selected at random to act as demonstrators, were habituated to the demonstration box outside of the apparatus. All demonstrators fed during this phase. Between the habituation phase and the demonstration phase, we inserted an opaque partition between the fish and the foraging area. With the partition in place, we inserted the feeder wall and demonstration box out of view of the subjects. The demonstration box was placed in front of one of the four columns, and thus at one of the two locations and at one of the two column types, except for the control groups which viewed no demonstrators. By being placed in front, it partially obscured one of the two feeding columns of that location. The control groups were run twice per testing day, as the first test each day (thus providing demonstrators for the first demonstration of the day) and a second test chosen at random.

We counterbalanced the demonstration groups, so that if possible an equal number of demonstrations were conducted at each of the four columns every day. The 2) demonstration phase started upon lifting of the partition and lasted 6 minutes and was used to determine the propensity of subjects to use social information. During this phase we allowed fish to freely move and access the food resources. This procedure differs from many social learning tests where subjects only observe feeding behaviour (but see Reader & Biro, 2010 for similar procedures). We considered it important to maintain ecological relevance and match much guppy foraging in the wild. Moreover, blocking subject access to food could represent a situation where conspecifics prevent foraging access. Between the demonstration and test phase, the opaque partition was reinserted, the feeding sheet rinsed to remove any odour cues and placed inverted (to reverse the order of the columns and further remove odour biases), and the demonstration box was removed. The 3) test phase started upon lifting of the partition and lasted 8 minutes, and was used to evaluate if social learning had occurred. As on the demonstration phase, the subjects could feed and were rewarded at any foraging location.

From the video recordings, one of two observers blind to the population tested counted the number of feeding pecks (Dussault & Kramer 1981) on each food column. Since we could not discriminate individuals, we summed the feeding pecks of the four subjects tested together as a group. No feeding pecks were observed away from the food columns. Inter-observer reliability was measured for 30 videos and was high (ICC= 0.81, 95% C.I. = 0.73 < ICC < 0.86). In total, we tested 82 groups with demonstrators and 25 control groups. Of these, 17 were from the Lower Aripo, 15 from the Upper Aripo, 33 from the Lower Marianne, 30 from the Upper Marianne, and 12 from the Paria.

STATISTICAL ANALYSES

All statistical analyses were performed using R version 3.2.2 (R Core Team 2015) and the packages ggplot2 (Wickham 2016) and lme4 (Bates et al. 2015). Model results are reported following Zuur, Ieno, Walker, & Smith (2009) and Zuur & Ieno (2016). We found no evidence that the demonstrated feeding column type affected foraging behaviour (unpublished data), and thus below we examined feeding locations and feeding rate only.

Population differences

We wanted to investigate if and why populations differed in social information use and social learning. We thus examined the influence of demonstrator location on subjects' foraging location choices for the fish exposed to demonstrators. We ran generalized linear mixed-effect models (GLMMs) with a binomial distribution with the distribution of pecks between the demonstrated and the undemonstrated location as the response variable for the demonstration phase, and for the test phase. This approach, compared to examining the total or percentage of pecks at the demonstrated location, accounts for differences in groups' propensities to feed.

We investigated population differences between the five sites we tested: Lower Aripo, Upper Aripo, Lower Marianne, Upper Marianne, and Paria. We did not analyse the data hierarchically with "Upper" and "Lower" regimes within each river due to the different sampling years, to avoid assuming "Upper" and "Lower" regimes are similar across drainages, and because no "Lower" site exists within Paria. The model also included an observation-level random effect to correct for overdispersion (Harrison 2015). In this model, an effect of site indicates that populations differed in in their proportion of pecks at the demonstrated location. The reference for the site model was the Lower Aripo, so this site model already provided a population comparison within the river Aripo. We followed by specifically investigating population differences within the Marianne river, by running a GLMM that included the main effect "population" ("Upper" or "Lower"). We only had one population in the river Paria, so we did not do any follow-up analysis.

Social information use and social learning

While the site model above examined whether populations differed in their reaction to the demonstrators, we also need to know how they reacted to the demonstrator. If demonstrator location had no influence, we would expect subjects to peck equally at both locations. We therefore tested whether the observed distribution of pecks differed from chance expectation, which we set at 50% assuming fish randomly feed at both feeders. We did this by removing the intercept of the site model, thus forcing the model to compare the population's estimates to zero on the latent scale or 50% on the original scale.

Feeding rate

To investigate whether demonstrator presence changed the total number of pecks subjects performed (i.e. feeding rate), we ran generalised linear mixed effect model (GLMM) with a Poisson distribution for each river and each phase. Rivers rather than populations were analysed so that an adequate amount of control data was available. The models had the response variable "total pecks", and the main effect "demonstration" ("control" or "with demonstration") to compare the absolute number of pecks of fish from the control group to the fish with a demonstration. We included as random effects population and group as well as an observation-level random effect to correct for overdispersion. A significant main effect of demonstration with a positive estimate would indicate that exposure to demonstrators increased feeding rate.

Feeding location consistency

To analyse whether control fish acquired a preference about feeding locations regardless of social cues, we analysed whether the group random effect significantly helped explain a significant part of the variation. We did this by creating an overall river model for the control trials. In the model, we included the main effect "river" and "phase" to create a repeated measure model. Using a likelihood ratio test (LRT), we compared the overall river model with the same model from which we removed the group random effect, to evaluate if a significant amount of variation is explained by groups.

RESULTS

SITE DIFFERENCES

Demonstration phase: During the demonstration phase, which examined differences in social information use, sites varied in the proportion of pecks at the demonstrated location: fish from the Upper Aripo, Lower Marianne and Paria pecked significantly less (*P* = 0.0046; *P* = 0.028; *P* = 0.018, respectively; table 2.1; figure 2.2) at the demonstrated location than our reference site Lower

Aripo. Examining the two Marianne populations alone, there were no significant differences in the proportion of pecks at the demonstrated location during the demonstration phase (table 2.2).

Test phase: During the test phase, which examined differences in social learning, Upper Aripo fish pecked significantly less at the demonstrated location compared to Lower Aripo fish (*P* = 0.0093; table 2.1, figure 2.2). Other sites did not differ in the proportion of pecks at the demonstrated feeder during the test phase (*Ps* > 0.2; table 2.1). Examining the two Marianne populations alone, there were no significant differences in the proportion of pecks at the demonstrated location during the test phase (table 2.2).

SOCIAL INFORMATION USE AND SOCIAL LEARNING

Demonstration phase: During the demonstration phase, when demonstrators were present, fish from the Lower Aripo pecked significantly more than expected by chance at the demonstrated location, with 97% of pecks (*P* = 0.033; figure 2.2, table S2.1). In contrast, fish from the Upper Aripo pecked significantly less than expected by chance at the demonstrated location, with only 8% of pecks (*P* = 0.05; table S2.1). Fish from the Lower Marianne, Upper Marianne, and Paria did not peck at the demonstrated location significantly more or less than the chance expectation of 50%.

Test phase: During the test phase, when demonstrators had been removed, fish from the Lower Aripo made 86% of pecks at the previously demonstrated location, but this was not significantly different from chance (*P* = 0.14; figure 2.2; table S2.1). Upper Aripo fish made only 9% of pecks at the previously demonstrated location, significantly different from chance (*P* = 0.020; table S2.1). Fish from the Lower Marianne, Upper Marianne, and Paria did not peck at the previously demonstrated location significantly more or less than the chance expectation.

FEEDING RATE

Demonstration phase: In the Aripo river, exposure to demonstrators increased slightly the total number of feeding pecks compared to the control condition, but this was not significant (*P* = 0.055;

figure 2.3). Exposure to demonstrators did not significantly increase the total number of feeding pecks in the other rivers (*P*s > 0.3; figure 2.3).

Test phase: Exposure to demonstrators did not significantly increase the total number of feeding pecks in any river (*P*s > 0.1; figure 2.3).

FEEDING LOCATION CONSISTENCY

There was no evidence that fish from the control groups, without a demonstration, had a consistent preference for a feeding location over the two experimental phases, suggesting that the feeding locations were not strongly dissimilar. That is, the model with a group random effect that accounted for repeated measures was not a significantly better fit than the model without for control groups (LRT $X^2 = 1.17$, df=1, $P = 0.28$). We did find evidence that groups with demonstration had a consistent preference for a feeding location. The model with the group random effect was significantly better at explaining variation than the model without for groups with a demonstration (LRT $X^2 = 7.45$, df = 1, $P = 0.006$). In other words, only fish with a demonstration showed a consistent preference for a certain feeder.

DISCUSSION

Using an experimental test within wild habitats, we compared the effect of a social demonstration on foraging rate and foraging location across guppy populations. We found that the response to social information varied between populations. We only found evidence for social information use and social learning in fish in the Aripo river. Moreover, within the Aripo river, populations differed in how they reacted to social information: fish from the Upper Aripo avoided the location where conspecifics were seen feeding and retained this bias after the removal of the demonstrators, while fish from the Lower Aripo foraged at the demonstrated location, but this bias was not statistically significant (although still substantial) when demonstrators were removed. Our results show population variation in social information use and social learning, suggesting that evolutionary and/or developmental experiences shape social information use and social learning propensities.

Perhaps our most interesting finding is that Aripo populations reacted differently to social information. Their habitats differ on multiple characteristics, such as food productivity, light levels, and predation pressure, providing multiple possible explanations for the differences we observed. However, predation pressure and competition provide the most likely explanations. The Lower Aripo population is characterised by very cohesive and large shoals, a result of the local predation regime, with little intraspecific aggression (Magurran & Seghers 1991; Magurran 2005). In contrast, in the Upper Aripo, predators of adult guppies are mostly absent, and food is more scarce than in the lower reaches (Grether et al. 2001), with fish displaying lower shoaling tendencies and higher aggression (Magurran & Seghers 1991). Thus fish in the Upper Aripo will suffer intraspecific competition if foraging in a group, will gain little in terms of anti-predator benefits, and resource patches may be more rapidly depleted, potentially explaining their tendencies to avoid locations where conspecifics are or were foraging (Seppanen et al. 2007). While most work on social information use has focused on animals matching demonstrator behaviour, animals can employ social information in a variety of ways, including avoiding the choices of others (Boogert et al. 2013; Seppanen et al. 2011; Dawson et al. 2013). Fish in the Lower Aripo suffer increased risks of individual exploration and leaving the group, suffer little intraspecific competition when foraging where others forage, and have easy access to social information, potentially explaining their copying behaviour. Previous work has linked between-individual variation in shoaling tendency with social information use in fish (Chapman et al. 2008; Trompf & Brown 2014), and with sociality more broadly in corvids (Templeton et al. 1999). Competition and limited resources availability has been proposed as an important influence on social information use in species as varied as Japanese quail (*Coturnix japonica*) (Boogert et al. 2013) and fruitfly *Drosophola melanogaster* larvae (Durisko & Dukas 2013).

Fish from the Aripo, but not the Marianne or Paria rivers, showed evidence for social information use and social learning. Thus, we did not find evidence of parallelism between rivers in this foraging test. A parallel response would have been indicative of a strong effect of specific socio-ecological factors like the presence of predators. Recently, work has highlighted important differences between rivers and drainages in the flow, productivity, and canopy cover (Millar et al. 2006). So while certain traits, like coloration, may be selected independently of the composition of the predator community (Rodd & Reznick 1991; Reznick, Rodd & Cardenas 1996), some are particular responses to the type, composition, temporal heterogeneity, and density of predators (Crispo et al. 2006; Torres Dowdall et al. 2012; Millar et al. 2006; Grether et al. 2001; Deacon et al. 2018, Barbosa et al. 2018). Additionally, other habitat characteristics and how they interplay with predation may be important. For example, guppy density strongly impacts competition and mate choice (Jirotkul 1999), and light spectrum affects mating tactics (Gamble et al. 2003). Environmental characteristics that shape competition are particularly likely to shape social information use (Seppanen et al. 2007).

Much research on social learning investigates cases of observational learning, in which subjects are unable to access the food resource during the demonstration phase. Somewhat atypically, our fish could access the food resources during the demonstration phase of the test, mimicking usual foraging conditions in the wild. Thus, shoaling or avoidance could have mediated the discovery of a food location, and a learned association between the food reward and its location would lead to fish subsequently favouring this location (Atton, Hoppitt, Webster, Galef, & Laland, 2012; Heyes, 1994; Laland & Williams, 1997). The mechanisms underlying different social learning processes are an open question (Heyes 2012, Leadbeater 2015, Reader 2016). However, from a functional viewpoint, the social learning we describe here and observational learning have the same outcome: both result in individuals' foraging choices being biased depending on the choices of other individuals. We note that fish without a demonstration, our "control" group, did not form a strong preference for one feeder over the other through repeated feeding, suggesting that demonstrators may have not only biased learning to a particular location but also facilitated learning of that location.

We found extensive population variation in the response to social cues. Depending on the population, social demonstration resulted in copying, avoidance, or no detectable effect on behaviour. Further work is needed to establish the relative contributions of evolution and development to the differences we observed, the underlying neurobehavioural mechanisms, and the question of whether differences in social information use are a byproduct or an adaptive specialization. Our results suggest that it may be beneficial to forage with others but not to learn a foraging patch preference from this experience. The differences we observe could have sizable impacts on community dynamics, by shaping and maintaining population-specific foraging preferences or avoidances (Schmidt et al. 2010; Thorogood et al. 2018). Our findings also suggest that social learning researchers should pay close attention to the origin and developmental history of their study subjects.

ETHICS

We minimized handling time and released fish immediately after testing at their capture location. All procedures were carried out in accordance with Trinidadian and Canadian law, with Animal Behavior Society and Canadian Council for Animal Care guidelines, and were approved by the Animal Care Committee of McGill University (Protocol # 2012-7133).

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TABLES

Table 2.1: Site differences in proportion of pecks at the demonstrated location. Estimates and standard error of fixed parameters and their interaction for the GLMM looking at the effect of site on the proportion of pecks at the demonstrated location, defined as a binomial variable of number of "successes" (proportion pecks at demonstrated location) and number of "misses" (proportion pecks at the undemonstrated location). Estimates are presented on the logit scale. The reference level was Lower Aripo for "site". The model also included an observation-level random effect to correct for overdispersion.

	Demonstration phase				**Test phase**			
parameter	**estimate**	**s.e.**	**z-value**	***p*-value**	**estimate**	**s.e.**	**z-value**	***p*-value**
Intercept	3.64	1.70	2.14	**0.033**	1.81	1.23	1.47	0.14
Upper Aripo	-6.07	2.14	2.83	**0.0046**	-4.16	1.60	2.60	**0.0093**
Paria	-5.53	2.33	2.38	**0.018**	-2.13	1.70	1.26	0.21
Lower Marianne	-4.41	2.01	2.20	**0.028**	-0.75	1.50	0.50	0.62
Upper Marianne	-3.33	2.01	1.66	0.098	-1.35	1.50	0.90	0.37

Table 2.2: Population differences in the Marianne river. Estimates and standard error of fixed parameters and their interaction for the GLMM looking at the effect of population on the proportion of pecks at the demonstrated location in the Marianne river, defined as a binomial variable of number of "successes" (proportion pecks at demonstrated location) and number of "misses" (proportion pecks at the undemonstrated location). Estimates are presented on the logit scale. The reference level was Lower Marianne for "population". The model also included an observation-level random effect to correct for overdispersion.

	Demonstration phase				**Test phase**			
parameter	**estimate**	**s.e.**	**z-value**	***p*-value**	**estimate**	**s.e.**	**z-value**	***p*-value**
Intercept	-0.70	0.85	0.82	0.41	0.94	0.69	1.35	0.18
Population (Upper Marianne)	1.01	1.23	0.82	0.41	-0.56	0.99	0.56	0.58

Table 2.3: Effect of having a demonstration on total number of pecks of fish. The estimates are presented on the log scale for the demonstration phase (left) and the test phase (right) for the river Aripo (top), Marianne (middle) and Paria (bottom). Our reference levels were no demonstration for the demonstration factor. The GLMM included also a correction for overdispersion in the random effects. Significant p-values (P<0.05) are presented in bold.

	Demonstration phase				**Test phase**			
parameter	**estimate**	**s.e.**	**z-value**	***p*-value**	**estimate**	**s.e.**	**z-value**	***p*-value**
Aripo								
Intercept	-0.71	1.09	0.65	0.52	0.63	1.05	0.60	0.55
Demonstration (demonstration)	2.24	1.16	1.92	0.055	1.74	1.15	1.51	0.13
Marianne								
Intercept	0.28	1.03	0.27	0.79	0.057	1.03	0.056	0.96
Demonstration (demonstration)	-0.22	1.14	0.20	0.84	0.61	1.10	0.55	0.58
Paria								
Intercept	0.079	1.70	0.047	0.96	2.20	1.53	1.44	0.15
Demonstration (demonstration)	1.64	1.82	0.90	0.37	0.041	1.73	0.024	0.98

Table S2.1: Comparison against chance expectation (50%). Models were fitted without intercept. Effect of the combined variable of river and population on the proportion of pecks directed at the demonstrated location for the demonstration (left) or test (right) phase. The estimates are presented on the logit scale. The GLMM included also a correction for overdispersion in the random effects. Significant p-values (P<0.05) are presented in bold.

	Demonstration phase				**Test phase**			
Parameter	**Estimate**	**s.e.**	***z*-value**	***P*-value**	**Estimate**	**s.e.**	***z*- value**	***P*-value**
Upper Aripo	-2.43	1.24	1.96	**0.050**	-2.35	1.01	2.32	**0.020**
Paria	-1.89	1.54	1.23	0.22	-0.33	1.16	0.28	0.78
Lower Aripo	3.64	1.70	2.14	**0.033**	1.81	1.23	1.47	0.14
Lower Marianne	-0.77	1.05	0.73	0.46	1.06	0.84	1.26	0.21
Upper Marianne	0.31	1.07	0.29	0.77	0.46	0.86	0.53	0.60

FIGURES

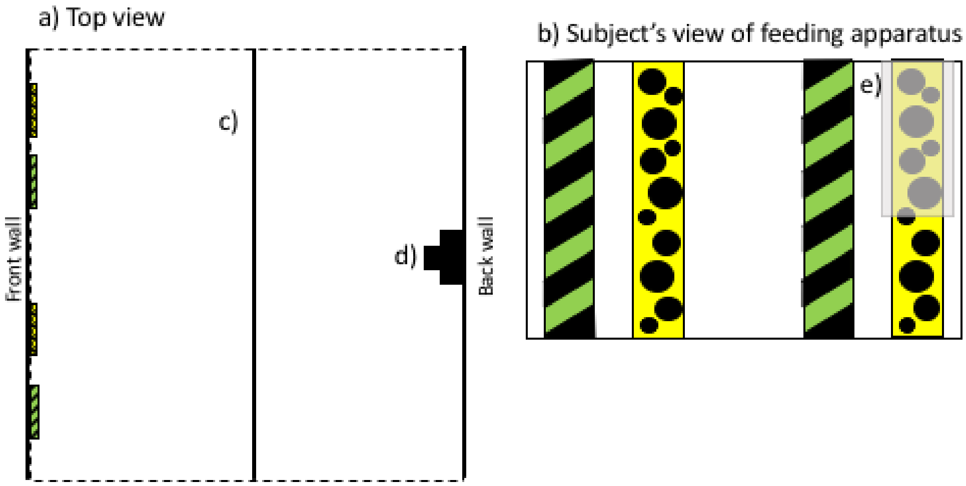

Figure 2.1: Top view of the a) testing enclosure and b) subjects' view of feeding apparatus. The front and back walls were made of Plexiglas (solid lines), while the right side, left side, and bottom were made of mesh allowing water flow (dotted lines). A removable opaque partition c) was inserted in the center. The feeding apparatus on the front wall was made up of two sets of two 5 cm wide columns, separated by 3 cm. We defined "feeding location" as each set of two columns (the two locations were separated by 10 cm). The yellow feeders were marked with 11 filled black circles ranging from 1.3 cm to 2.5 cm in diameter, while the green feeders were marked with six 1.9 cm-thick black stripes. The camera d) was placed on the back wall. The demonstrator's box e) was made of transparent perforated plastic and could be placed in front of one of the four feeding columns. In the figure, it is placed as an example in front of the yellow column at one location.

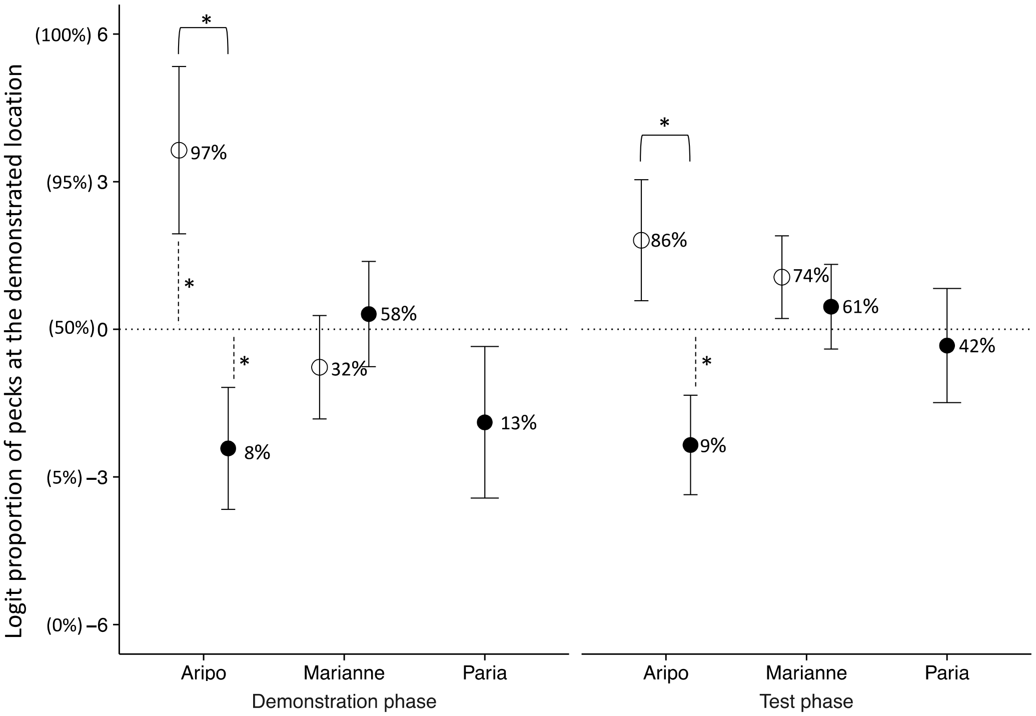

Figure 2.2: Estimates +/- SE (on the logit scale) of the proportion of pecks performed at the demonstrated feeder by five populations of fish in the three rivers for the demonstration and test phases. The back transformed scale is provided in parenthesis, as well as back transformed estimates for each group. The dashed line at zero represents our chance expectation of 50% on the original scale. A difference from chance (50%) is indicated by a star and a dotted line, while a difference between populations is indicated by an * above the compared groups. Only populations from the Aripo river differed from each other and from chance.

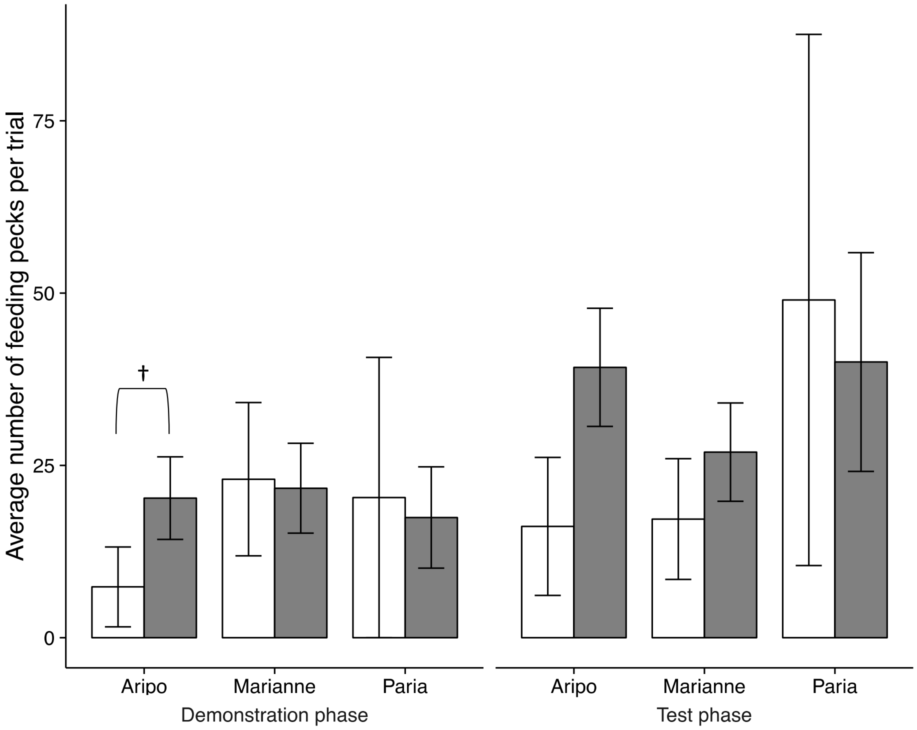

Figure 2.3: The effect of having a demonstration on the mean number of feeding pecks +/- SE on fish from the three rivers, during the demonstration and test phases. Exposure to demonstrators increased overall foraging rate in the Aripo river, although not significantly so. No similar effect was observed in the Marianne or Paria river. Open bars: control subjects; filled bars: subjects exposed to demonstrations. † represents 0.05 < p-values < 0.1.

LINKING STATEMENT TO CHAPTER 3

Chapter 2 provided evidence that social information use and social learning tendencies vary between populations of wild guppies. While such drastic variation in a widely-used study system for social information use and social learning is intriguing, my results cannot speak to the causal factors underlying these differences. However, in the river Aripo, they correlated as expected with population specific ecological conditions and behaviours. While habitats are often characterized based on the presence versus absence of predators of adult guppies, the social environment has also been proposed as an influence on social abilities. Phenotypic traits, such as social information use and social learning tendencies, could be fixed by selective pressure, or be adjusted to local conditions during early life through developmental plasticity, or be responsive to current conditions through plasticity. Since early life conditions have a unique opportunity to affect the development of systems, I investigated in chapter 3 the contribution of early life experiences to social information use and social learning tendencies. To do this, I tested guppies reared in the laboratory under a 2x2 design varying the social environment and predation cues using the same social information use and social learning experimental design as in Chapter 2. Those fish were also used to investigate other behavioural and physiological effects, as described in Chapters 4 and 5.

CHAPTER 3: HIGH SOCIAL DENSITY DURING DEVELOPMENT DECREASES THE USE OF SOCIAL INFORMATION ABOUT FOOD RESOURCES IN FEMALE GUPPIES

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To be submitted to *Behavioural Processes*

ABSTRACT

To gather information about the environment, animals can sample resources personally, or rely on information coming from other individuals. The costs and benefits of using personal and social information depends largely on local conditions, but can also be affected by individual characteristics, such as phenotype, and sex. Early-life experience of predation and social density have large effects on adult behaviour, and plausibly on the reliance on social information. Social density may decrease the propensity to copy other individuals through an increase in experienced competition, whereas predation risk may increase the propensity to copy others by increasing of the costs of exploration. It is still unclear whether these conditions have long-lasting effects on social information use and social learning propensities. We investigated the effect of early life manipulations of perceived predation risk and social density on social information use and social learning propensities. We reared juvenile guppies of both sexes in one of four conditions, manipulating social density and predation threat, and tested subjects' propensities to employ social information use and social learning when adult and after a period of common garden rearing. The proportion of pecks directed and the time spent at a feeding location demonstrated by conspecifics was recorded when demonstrators were present (testing "social information use"), and after demonstrators were removed (testing "social learning"). Supporting the idea that early-life competition affected feeding behaviour, females reared at high densities made more feeding pecks than fish reared at standard densities. We found that only females reared at standard housing densities used social information about food resources, although both sexes spent most of their time at the location demonstrated by other individuals. We found no evidence of social learning in our fish. Our results suggest that early life experience of conspecific density may affect the value of social information, and that sexes differ in the type of information they use.

INTRODUCTION

Animals assemble in groups for multiple reasons, for example to avoid predation or increase reproductive opportunities (Krause & Ruxton 2002), and by doing so benefit from an increased access to information produced by other individuals interacting with resources, i.e. 'social information' (Danchin et al. 2004). Animals may retain this information for later use, termed ‘social learning’ (Heyes, 1994). Like any information about resources, the value of social information depends on the situation (Giraldeau, Valone, & Templeton, 2002; Laland, 2004; Stephens, 1989). Individual level characteristics, such as individual learning ability (Katsnelson et al. 2011), tendency to take risks (Kurvers et al. 2010; Trompf & Brown 2014), or metabolic rate (Mathot et al. 2009) are known to correlate with the propensity to use social information. There is mounting evidence that the propensity to rely on social information is shaped by characteristics such as the social environment (Chapman, Morrell, Benton, & Krause, 2008; Lindeyer, Meaney, & Reader, 2013), direct recent or early-life experience of the costs and benefits of social information (Leris & Reader 2016; Dawson et al. 2013) or through experience of pre- or post-natal stress (Boogert et al. 2013; Farine et al. 2015). We also know that wild populations experiencing different local conditions differ in their use of social information and social learning (Dolman et al. 1996; Chouinard-Thuly & Reader, Chapter 2). Early life conditions (Bateson 1979; Carlson 2017) may be particularly important in shaping such propensities, as they influence the development of many physiological and neurological systems, such as the stress axis, that may play a role in regulating social information use and social learning propensities.

Predation pressure influences the behaviour of animals, and is likely to also have direct and indirect impacts on social learning and social information use propensities (Rieucau & Giraldeau 2011). As well as learning about predators themselves or learning proper anti-predator behaviours (Griffin 2004) direct benefits of copying others under predation threat include a reduction of the need for risky personal exploration, and the possibility of locating safe foraging patches. We thus expect an increase of reliance on social information in environments with predation. Consistent with this ‘costly information’ hypothesis (Boyd & Richerson 1985), minnows (*Phoxinus phoxinus*) spend more time in a foraging patch where conspecifics were previously seen when simulated predation risk is high (Webster & Laland 2008). Indirectly, predation may influence grouping tendencies and other behaviours (Bell & Sih 2007; Harris et al. 2010; Carlson 2017), which may impact social information use and social learning propensities (Trompf & Brown 2014; Kurvers et al. 2010; Rosa et al. 2012). Although we have evidence that direct manipulation of the value of social information can change propensities to utilize it (e.g. Dawson et al. 2013), whether predation risk in early life, through direct or indirect effects, has long-lasting effects on social information use and social learning is still unclear.

The development of social information use and social learning propensities may also be affected by the social environment. As some resources might become depleted if overused, it would be beneficial not to copy in high competition or high density environments (Rendell et al. 2011; Seppanen, et al. 2007). Supporting this, the type of intraspecific competition in populations of doves (*Zenaida aurita*) correlates with their social learning preferences (Dolman et al. 1996). Social density has been shown to shape shoaling tendencies in guppies (*Poecilia reticulata*) which in turns affects their propensity to rely on social information (Chapman, Ward, & Krause, 2008). In parallel, research demonstrates that the complexity of early life social environment induces long-lasting changes in the brain that may also affect social information use and social learning. Isolating rats during early life induces long-lasting changes in the brain that affect social behaviours (Weiss et al. 2004). The type of maternal care received by young rats, with multiple effects on brain development (Liu et al. 2000) also shapes their propensity to socially learn as adults (Lindeyer et al. 2013a; Melo et al. 2006; Lévy et al. 2003). In addition, the composition of the early social environment of cichlids (*Neolamprologus pulcher*) affects their social competence, or their ability to use social information optimally (Taborsky et al. 2012) through physiological changes (Nyman et al. 2018). Thus conspecific density in early life may be an important impact on social information use.

In a laboratory experiment, we investigated the effect of early experience of predation and social density on the social information use and social learning propensities of adult guppies. Guppies are a small freshwater fish originating from Trinidad, where natural between-population differences in local conditions influence behaviour, life-history (Magurran 2005), and most pertinently social information use and social learning propensities (Chapter 2). We also investigated whether there are sex differences in social information use and social learning in guppies. Guppies exhibit strong sex differences in behaviour (Magurran 2005; Leris 2016), life history (Magurran & Macias Garcia 2000), and in learning (Lucon-Xiccato & Bisazza 2017), and undergo different selection pressures in the wild (Magurran & Nowak 1991a; Mckellar & Hendry 2011). However, most research on guppy social learning has focused on females. Thus it is unknown whether the sexes differ in social information use and social learning propensities, although there is evidence that information flows more rapidly through female than in male groups

(Reader & Laland, 2000). Due to mating cost asymmetries, and life-history, males devote little time to foraging or shoaling, and thus we expect to see social information use and social learning in a foraging context mainly in females. However, if the costs of using social information is low, males could potentially benefit from a 'scrounging strategy' whereby patches are discovered by following others, with low personal effort.

METHODS

ANIMAL SUBJECTS AND HOUSIN

Our fish were part of an experiment on the effects of developmental conditions on adult phenotype (Leris 2016; Chouinard-Thuly et al. 2018; this thesis Chapters 4, 5; Reddon et al. in 2018) and were tested at about 50 days on other behavioural responses (Leris 2016). We reared guppy fry in one of four developmental conditions, varying exposure to predation cues and social density. The parental fish were at least third generation of a mixed lineage of guppies sampled from wild populations originating from the Lower Aripo and Lower Quare rivers in Trinidad, areas with significant predation risk from large fish predators such as the pike cichlid (*Crenicichla* sp.; Magurran 2005). Each day, we collected fry guppies from breeding tanks consisting of only pregnant females. We pooled the fry and separated them at random between the four developmental conditions until we had three replicate rearing aquaria per developmental condition (12 aquaria total). Throughout, fish were housed in the laboratory at McGill university at 26±1ºC on a 12 hour light cycle (lights on at 07:00 h). We fed fish twice a day on weekdays until 85 days, and once a day on weekends and after day 85 at random times between 1000h and 1700h, commercial tropical fish flakes (TetraMin, Tetra, Germany) and re-hydrated, decapsulated brine shrimp eggs (Brine Shrimp Direct Inc., Utah, USA).

DEVELOPMENTAL MANIPULATIONS

We manipulated social density and exposure to predation cues. We created a "standard" social density by placing ~10 fry, and a "high" social density by placing ~30 fry, in each 18 l aquarium until day 50, at which we transferred them in new tanks in common garden conditions (~10 fish per 18 l aquarium). We exposed fry for the first 45 days to visual and olfactory cues of a stimulus fish, either a natural guppy predator pike cichlid (*Crenicichla* sp.) for the predation cues treatment, or of a non-predatory sucker-mouth catfish (*Pterygoplichthys* sp.) for the non-predation cues treatment, on weekdays at a random time between 1000h and 1600h, separated by at least 30 minutes from feeding. We used three different pike cichlids and three different catfish as the stimulus fish. We fed the stimulus cichlids bloodworms and the catfish algae wafers (Hikari, Hayward, California, USA) once a day during the visual cues presentation. Four times a week, we also paired the visual and olfactory cues with alarm substance, a chemical released from the skin of injured conspecifics (Pfeiffer 1977), for the predation cues conditions, or distilled water for the non-predation condition.

To create the visual cues, we removed an opaque partition placed between rearing aquaria and the stimulus fish housing tank. We did not note the behaviour of the predator during such exposure, though we included guppy housing tank in the statistical analyses. For the olfactory cues of the stimulus fish, we collected holding water from the housing aquaria of four pike cichlids fed with euthanized guppies, and of four catfish fed with spinach (Brown, Paige, & Godin, 2000). We fed each individually housed cichlid one euthanized guppy per day for four days as there is evidence that prey fish learn about the relative threat of a predator when conspecifics' alarm substance is present in predator faeces (Brown, Chivers, & Smith, 1995), but we switched to feeding the cichlids bloodworms after the collection was finished to minimize the number of euthanized guppies. We harvested the alarm substance from euthanized guppies following Brown & Godin (1999), briefly, by homogenizing skin and muscle tissues from 36 euthanized male and 25 euthanized female guppies. The solution was filtered with filter wool and diluted to 0.1 cm^2 of tissue per ml with ddH_20. We added 5ml of each cue on the days where it was scheduled.

SOCIAL INFORMATION USE AND SOCIAL LEARNING TEST

For these tests, fish were ~250 days of age. We covered the bottom of a 115 l tank with beige gravel, and filled it to 19 cm with conditioned water. Inside the tank, we placed a white corrugated plastic box (40 by 40 cm) with a waterproof camera (1080p at 30fps, GoPro3 Black Edition, San Mateo, California) mounted on one side, facing the side where we attached a feeding wall during the demonstration and test phases. The testing protocol and apparatus inside the aquaria were identical to those used Chapter 2, except that we only used yellow feeding columns as colour previously had no detectable effect in the Chapter 2 study, and placed groups of two, instead of four, same sex fish as observers to increase our effective sample size. We created the feeding walls by drawing the feeding columns and the black circles pattern with a black permanent marker on a thin transparent plastic sheet (figure 1). We poured gelatin (KNOX, Treehouse Foods, New York State, USA) mixed with yellow food colouring (Club House, McCormik Canada, London Ontario, Canada), and sprinkled with flakes (Tetramin, Tetra, Germany) on the feeding columns and left to solidify for at least 20 minutes. We then proceeded to conduct the test as in Chapter 2, using pairs of the same sex. We used two trials a day as controls, the first of the day and another randomly chosen, in which there were no demonstration. We only used females in the control trials, as those fish served as demonstrators for the following trials. Females feed more reliably thus providing consistent demonstrations, and are attractive to both males and females as opposed to males who might be avoided by females (Darden & Croft 2008). We thus used female demonstrators only. Demonstrators were observed to successfully feed every trial.

Observers blind to the subjects' developmental conditions watched the videos and recorded the number of feeding pecks (L.C.T.; Dussault & Kramer, 1981) and the time spent (L.X.) at each of the feeding locations. Fish were recorded as at the feeding locations when they were within 15 cm of the front wall, with fish recorded as at the left or right feeder when they were in the left or right half of the tank, respectively. In total, we ran 81 trials (i.e. 81 pairs of subjects), of which 23 were controls and 58 had a demonstration. Of the trials with a demonstration, 18 trials tested female pairs and 40 trials tested male pairs. We had less female pairs with a demonstration as we used 23 female pairs for the control trials. We tested 28 pairs of fish reared with no predation cues, 30 pairs reared with predation cues, 44 pairs reared in high density, and 14 reared in low density. Since we reared three tanks per condition, there were approximately triple the number of subjects reared at high density than standard density.

STATISTICAL ANALYSES

To investigate whether early life experience and sex influenced where fish pecked and spent their time, we used generalized linear mixed-effect models (GLMMs). The response variable “pecks at the demonstrated location” was treated as a binomial distribution, where we counted pecks produced at the demonstrated location as “successes” and pecks produced at the alternative location as “misses”, allowing the model to account for total number of pecks, used as a proxy for motivation of the fish. The model output provides the proportion of pecks at the demonstrated location. Trials in which fish did not feed (41 trials in the demonstration phase and 26 in the test) were given zero weight in the model investigating proportion of pecks. We also investigated the response variable “time spent at the demonstrated location”, similarly treated as a binomial distribution. Using a binomial distribution allowed us to account for total time spent near the feeders. A second spent at the demonstrated location, was considered as a “success” and at the alternative location a “miss”. The output of the model is the proportion of time spent at the demonstrated location. All fish spent time near the feeders thus no fish were excluded from this analysis.

Because of the small sample size, we could not analyse the interaction of early life experience of predation and density in the same model, and thus investigated separately the effect of either early life experience of “social density” (with levels “standard social density” and “high social density”) or “predation cues” (with levels “no-predation” and “predation”), with the factors “sex” (with levels “females” and “males”) their interaction during the demonstration phase or during the test phase. Our models included the random effect “housing tank” to account for tank effects, and an observation-level random effect to correct for overdispersion (Harrison 2015). We verified if pecks at the demonstrated location were different than a random use of the feeders by removing the intercept, thus forcing the model to compare each group’s estimates to zero on the latent scale, or 50% (our ‘random’ expectation) on the original scale, instead of comparing each group to the reference level (intercept). The approach described above was also used for the analysis of time spent at the demonstrated location.

We also investigated whether a demonstration and the effect of early life experience of density and predation influenced the total feeding pecks performed at both locations, to assess whether seeing demonstrators feed prompted the tested fish to feed more. The response variable “total pecks” was treated as a Poisson distribution, as it is discrete, positive, and bounded by zero. We used the same approach as above, and ran models with the main effects “demonstration” (with levels “control” and “with demonstration”), and either “density” or “predation cues” and their interactions for the demonstration phase and the test phase. Our models included the random effects “housing tank”, and an observation-level random effect. For these models, pairs making zero pecks were included in the analyses. We could not investigate the effect of a demonstration on males as only females were used in our controls.

All statistical analyses were performed using R v3.2.2 (R Core Team 2015) with the packages lme4 (Bates et al. 2015) to fit the models, and the package ggplot2 (Wickham 2016) to produce the graphical representations.

RESULTS

Demonstration Phase: Social Information Use

We found evidence for social information use, but not in all experimental conditions. In terms of feeding pecks, females reared in standard social density directed 99% of their pecks at the demonstrated location, significantly more than expected by chance (*P* = 0.042; table 3.1; figure 3.2), while in all other experimental treatments, although sometimes directing a substantial proportion of pecks at the demonstrated location, subjects did not differ significantly from chance (table 3.1, figure 3.2). In terms of time spent, all groups had a significant preference for the demonstrated location, except for females with early life experience of high social density and females with early life experience of predation cues (table 3.2, figure 3.3). We did not find any difference between sexes, or early life experiences on the proportion of pecks at the demonstrated location (table S3.1) or in the time spent at the demonstrated feeder (table S3.2).

Exposure to demonstrators did not significantly increase the total number of feeding pecks made (*P*s > 0.4, table 3.3; note only females were analysed). Females with early life experience of high density pecked more than females reared at standard density (*P* = 0.0018, table 3.3), with no significant interaction between density and demonstration (*P* = 0.47). We did not find any significant effect of early experience of predation on total pecks produced (table 3.3).

Test Phase: Social Learning

We found no evidence for social learning, with no significant preferences for or against the previously demonstrated feeder in terms of the proportion of feeding pecks (table 3.3, figure 3.2 or time spent (table 3.3, figure 3.3). There were no significant differences between sexes and early life experiences on the proportion of pecks directed at the previously demonstrated location (table 3.1; figure 3.2) or on the time spent at the previously demonstrated location (table S3.3).

Exposure to demonstrators did not significantly increase the total number of feeding pecks made (*P*s > 0.15, table 3.3; note only females were analysed). Females with early life experience of high density pecked more than females reared at standard density (*P* = 0.0035, table 3.3), with no significant interaction between density and demonstration (*P* = 0.92). We did not find any significant effect of early experience of predation on total pecks produced (table 3.3).

DISCUSSION

We found that both male and female guppies exposed to a variety of early-life conditions preferred to spend time near the location where conspecific demonstrators were foraging, but only females reared at standard housing densities during early life preferentially directed feeding pecks at this location. We found no evidence of social learning, as no fish retained a preference to direct pecks or spend time at the feeder conspecifics had previously fed at during the test phase, when conspecific demonstrators were absent.

During the demonstration phase, both males and females spent more time than expected by chance where female conspecifics were foraging versus an equally rewarded feeder empty of conspecifics.

However, only females directed more feeding pecks than expected by chance at this demonstrated location. This finding is consistent with different grouping motivations in females and males: females approach same-sex conspecifics to forage, or as an anti-predator measure that facilities foraging, while males approach opposite-sex conspecifics to mate, or as an anti-predator measure but without any effect on foraging. Previous research in guppies has shown a large sex difference in the trade-off between mating and feeding activities, where males spend more time searching for mates and less foraging (Griffiths 1996), and similar sex differences have been described in other species in which males exhibit a fast life-history (Hendrichs et al. 1991; van Noordwijk & de Jong 1986). Investment in reproductive activity comes at a cost, and sexes who adopt a fast life history, like male guppies, tend to reduce energy allocated to other activities (Scharf et al. 2013). In domestic chicks (*Gallus gallus)* females are more motivated by social reinforcement than males (Vallortigara et al. 1990). This stresses that sex differences in cognitive or behavioural tasks may sometimes be explained by motivational differences, and that looking at measures utilizing different motivators may explain between-sex variation (Lucon-Xiccato & Dadda 2016). Our result stresses that investigation of both sexes is crucial for the understanding of sexually dimorphic species.

Females with early life experience of standard housing density preferred to peck and spend time at the demonstrated feeder, whereas females reared in high density did not show evidence of a feeder preference. Although the impact of demonstrated location differed considerably between females reared at standard and high density, it was not statistically different, compromising firm conclusions on the role of rearing density. We note that our study had a relatively low sample size, and variation between pairs was considerable, meaning that statistical power was limited. High rearing densities could decrease the propensity to rely on social information in favour of personal information, through a higher experienced competition for food, or via a decreased reliability of social information in conditions of low resource predictability (Boogert et al. 2013). Although our fish were fed *ad libitum* it is possible that fish at high social densities perceive high competition for food. Notably, fish reared at high density pecked more than fish reared at standard density, even when demonstrators were absent, supporting the idea that high density fish have been influenced by feeding competition. Our results are in line with previous research demonstrating that fish reared in low density utilized social information whereas fish reared in high social densities did not, possibly due to a change in shoaling tendencies (Chapman et al., 2008), although this does not explain why we did not see a similar effect in males. One possibility is that social density during early life impacts the sexes differently, reflective of the different demands and pressure both sexes are subjected to (Kotrschal et al. 2012). In our previous social learning experiment with wild guppies, we found that female fish from the population displaying low shoaling propensities avoided the demonstrated location, preferentially feeding at an alternative location (Chapter 2), which may be an extreme form of the phenomenon seen here by females with early life experience of high social density who do not spend more time with demonstrators. Our findings provide additional evidence against the hypothesis that dwelling in large social groups will always increase reliance on social information (Reader & Lefebvre 2001; Street et al. 2017).

Contrary to our expectations, we did not detect an effect of early life experience of predation cues on information use or social learning about foraging locations. Although this could be due to weak effects of early life experiences, we have evidence that predation cues were salient enough to induce a difference in brain mass of males (Reddon et al. 2018), and in cortisol release of females when combined with a high social density (Chapter 4). Our experiment was designed to specifically test whether early life experiences would shape social information use and social learning propensities of adults in a foraging context. Our findings contrast our field experiment (Chapter 2) and other experiments (Webster & Laland 2008; Mathot & Giraldeau 2008), although in all three of these studies where perceived predation threat was present through the experiment, in contrast to the present study. It is possible that the effects of early-life exposure to predators is only detectable when fish are exposed to a mild stress, such as in the field conditions in Chapter 2. Predation is a strong stressor, with considerable influence on the development of the stress axis (Creel et al. 2013), which is a likely candidate for regulation of social information use and social learning strategies (Boogert et al. 2013; Farine et al. 2015). As we did not find a strong effect of predation alone on the stress axis of our fish (Chapter 4) more research is needed to confirm the link between the stress axis and social information use and social learning propensities.

While the presence of other individuals affected our behavioural measures, we had no evidence for social learning: the identity of the demonstrated feeder had no detectable effect on the subsequent preference for that feeder when demonstrators were absent. Social learning of foraging locations has been found using a setup almost identical to the present apparatus (Chapter 2) as well as in similar foraging tests (Reader, Kendal, & Laland, 2003) in wild-type guppies in the field. Social learning of foraging locations has been found in the laboratory in domestic (Leris & Reader 2016; Swaney et al. 2001; Kendal et al. 2004), feral (Trompf & Brown 2014), and wild-derived guppies (Chapman et al. 2008; Hasenjager & Dugatkin 2017). In our experiment, fish had access to food at both feeders during the test phase that measured social learning, and it is possible that they rapidly detect and utilize this alternative food source, abolishing any social effects on choice. However, previous work (Chapter 2, Reader, Kendal, & Laland, 2003) has used a similar design and yet still found evidence for social learning. Perhaps laboratory conditions reduced any retained reliance on social information, as it provides stable and unlimited feeding opportunities.

While social information use and social learning have received considerable research interest, relatively little is known about the underlying biological mechanisms of these processes (Heyes, 2016). Evidence is accumulating that developmental conditions shape the propensities to use social information and learn socially, either through the learning of the value of social cues (Leadbeater & Chittka 2009), or potentially through effects on a correlated phenotype, such as sociality, which might affect the opportunity to gather information (Mesoudi et al. 2016; Chapman et al. 2008). Our results suggest that some conditions, such as social environment, may have long-lasting effects on social information use, whereas other, like predation, might have stronger effects on the current value of social information. Our results support the idea that social information use is developmentally plastic, and can be shaped by early environment.

ETHICS

The study was approved by McGill University and the Canadian Council on Animal Care under protocol 2012-7133/2015-7708, and conformed to ABS/ASAB ethical guidelines. We did not carry out any fieldwork. All subjects recovered quickly from the procedures and were returned to housing aquaria after the experiment. We sacrificed 16 guppies to feed the cichlids during the collection of predator odour cues (cichlids were otherwise fed bloodworms) and 61 guppies to produce the damage induced alarm substance. Prior to being fed to the cichlids or dissected for alarm cue preparation, the guppies were euthanized by immersion in ice water (Blessing et al.

2010). They were then consumed within seconds by cichlids or swiftly decapitated for alarm cue preparation.

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TABLES

Table 3.1: Comparison against chance expectation (50%) to test whether fish feeding peck locations were influenced by demonstration. Models were fitted without intercept. Effect of density (top) or predation (bottom) and sex on the proportion of pecks directed at the demonstrated location for the demonstration (left) or test (right) phase. The estimates are presented on the logit scale. The GLMM included also the random effect of housing tank, and a correction for overdispersion. Significant p-values (P<0.05) are presented in bold.

	Demonstration phase				**Test phase**			
parameter	**estimate**	**s.e.**	***z*-value**	***p*-value**	**estimate**	**s.e.**	***z*-value**	***p*-value**
Density								
Females Std. density	7.26	3.57	2.04	**0.042**	-0.33	1.15	0.29	0.77
Females High. Density	-0.024	0.90	0.008	0.99	1.36	1.42	0.96	0.34
Males Std. density	3.00	3.79	0.79	0.43	-0.21	1.22	0.17	0.86
Males High density	-0.22	2.25	0.098	0.92	0.29	0.70	0.17	0.86
Predation								
Females No predation	1.50	3.43	0.44	0.66	-0.68	1.20	0.57	0.57
Females Predation	4.98	3.18	1.57	0.12	1.49	1.27	1.17	0.24
Males No predation	2.29	4.06	0.56	0.57	-0.034	0.80	0.043	0.97
Males Predation	1.33	2.80	0.48	0.64	0.39	0.87	0.46	0.65

Table 3.2: Comparison against chance expectation (50%) to test whether fish locations were influenced by demonstration. Models were fitted without intercept. Effect of density (top) or predation (bottom) and sex on the proportion of time spent at the demonstrated location for the demonstration (left) or test (right) phase. The estimates are presented on the logit scale. The GLMM included also the random effects of housing tank, and a correction for overdispersion. Significant p-values (P<0.05) are presented in bold.

	Demonstration phase				**Test phase**			
parameter	**estimate**	**s.e.**	***z*-value**	***p*-value**	**estimate**	**s.e.**	***z*-value**	***p*-value**
Density								
Females Std. density	1.39	0.50	2.78	**0.0054**	-0.22	0.38	0.58	0.56
Females High Density	0.39	0.36	1.07	0.28	0.00031	0.27	0.0010	0.99
Males Std. density	1.30	0.44	2.97	**0.0029**	-0.61	0.35	1.74	0.082
Males High density	1.41	0.21	6.59	**<0.0001**	-0.26	0.17	1.52	0.13
Predation								
Females No predation	1.15	0.42	2.70	**0.0069**	0.28	0.31	0.93	0.36
Females Predation	0.34	0.40	0.85	0.39	-0.40	0.30	1.32	0.19
Males No predation	1.47	0.26	5.57	**<0.0001**	-0.35	0.21	1.69	0.092
Males Predation	1.31	0.28	4.63	**<0.0001**	-0.30	0.22	-1.39	0.16

Table 3.3: Total counts. Effect of having a demonstration and density or predation on total number of pecks of female pairs. The estimates are presented on the log scale for the demonstration phase (left) and the test phase (right). Our reference levels were no demonstration for the demonstration factor, and standard social density for the social density treatment or control cues for the predation treatment. The GLMM included also the random effects of housing tank, and a correction for overdispersion. Significant p-values (P<0.05) are presented in bold.

	Demonstration phase				**Test phase**			
parameter	**estimate**	**s.e.**	***z*-value**	***p*-value**	**estimate**	**s.e.**	***z*-value**	***p*-value**
Density								
Intercept	-3.47	1.40	2.48	**0.013**	0.60	0.65	0.93	0.35
Demonstration (demonstration)	0.88	1.56	0.56	0.57	-1.37	0.98	1.40	0.16
Density (high)	6.07	1.95	3.12	**0.0018**	3.16	1.08	2.92	**0.0035**
Demonstration * density	-1.85	2.58	0.71	0.47	0.16	1.57	0.10	0.92
Predation								
Intercept	-3.71	2.28	-1.63	0.10	0.84	0.92	0.91	0.36
Demonstration (demonstration)	1.73	2.46	0.71	0.48	-0.53	1.22	0.43	0.67
Predation cues (predation)	1.72	2.25	0.76	0.44	1.07	1.22	0.88	0.38
Demonstration * predation cues	-2.10	3.49	-0.60	0.55	-1.31	1.69	0.78	0.44

Table S3.1: Effect of density (top) or predation (bottom) and sex on the proportion of pecks directed at the demonstrated location for the demonstration (left) or test (right) phase. The estimates are presented on the logit scale. Our reference levels were females, standard social density for the social density treatment, and no predation for the predator cues treatment. The GLMM included also the random effects of housing tank, and a correction for overdispersion. Significant p-values (P<0.05) are presented in bold.

	Demonstration phase				**Test phase**			
parameter	**estimate**	**s.e.**	***z*-value**	***p*-value**	**estimate**	**s.e.**	***z*-value**	***p*-value**
Density								
Intercept	7.26	3.57	2.04	**0.042**	-0.33	1.15	0.29	0.77
Density (high)	-7.29	4.90	1.49	0.14	1.69	1.82	0.93	0.35
Sex (males)	-4.26	3.38	1.26	0.21	0.12	1.43	0.084	0.93
Sex * density	4.06	4.65	0.87	0.38	-1.19	2.03	0.59	0.56
Predation								
Intercept	1.50	3.43	0.44	0.66	-0.68	1.20	0.57	0.57
Predation cues (predation)	3.48	4.66	0.75	0.46	2.17	1.73	1.26	0.21
Sex (males)	0.79	4.75	0.17	0.87	0.65	1.31	0.50	0.62
Sex * predation cues	-4.44	5.04	0.88	0.38	-1.74	1.90	0.92	0.36

Table S3.2: Effect of density (top) or predation (bottom) and sex on the time spent at the demonstrated location for the demonstration (left) or test (right) phase. The estimates are presented on the logit scale. Our reference levels were females for the factor sex, and standard social density for the social density treatment and no predation cues for the predation treatment. The GLMM included also the random effects of housing tank, and a correction for overdispersion. Significant p-values (P<0.05) are presented in bold.

	Demonstration phase				**Test phase**			
parameter	**estimate**	**s.e.**	***z*-value**	***p*-value**	**estimate**	**s.e.**	***z*-value**	***p*-value**
Density								
Intercept	1.39	0.50	2.78	**0.0054**	--0.22	0.38	-0.58	0.56
Density (high)	-1.00	0.62	1.61	0.11	0.22	0.45	0.48	0.63
Sex (males)	-0.095	0.63	0.15	0.88	-0.39	0.47	0.83	0.41
Sex * density	1.12	0.74	1.51	0.13	0.13	0.53	0.24	0.81
Predation								
Intercept	1.15	0.42	2.70	**0.0069**	0.28	0.31	0.93	0.36
Predation cues (predation)	-0.81	0.58	-1.38	0.17	-0.68	0.42	1.61	0.21
Sex (males)	0.32	0.48	0.67	0.50	-0.63	0.34	1.88	0.061
Sex * predation cues	0.65	0.67	0.98	0.33	0.72	0.46	1.56	0.12

FIGURES

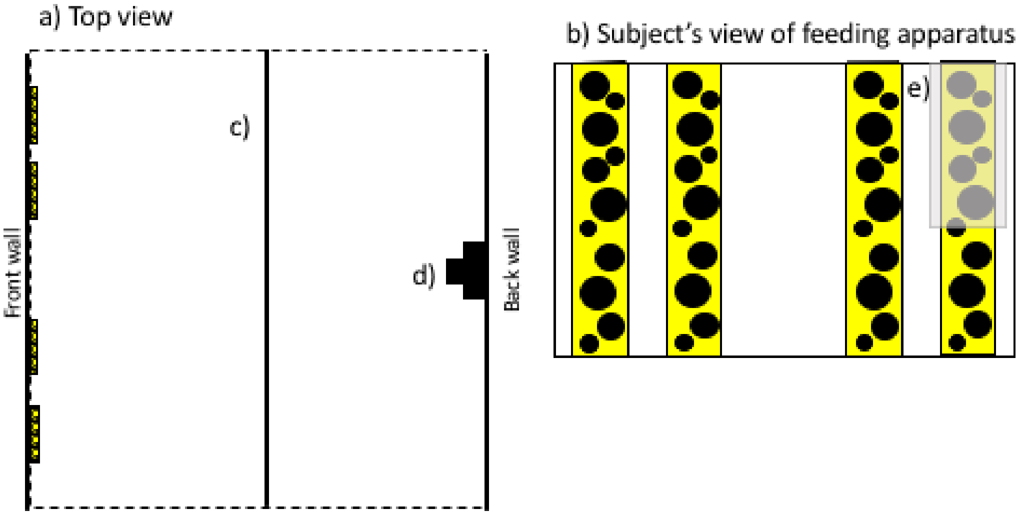

Figure 3.1: (a) top view of the testing enclosure and (b) subjects' view of feeding apparatus (b). the walls were made of white corrugated plastic. The removable opaque partition (c) was inserted in the center. The feeding apparatus on the front wall was made up of two sets of two 5 cm wide columns, separated by 3 cm. We defined "feeding location" as each set of two columns (the two locations were separated by 10 cm). The feeders were marked with 11 filled black circles ranging from 1.3 cm to 2.5 cm in diameter. The camera (d) was placed on the back wall. The demonstrator's box (e) was made of transparent perforated plastic and could be placed in front of one of the four feeding columns.

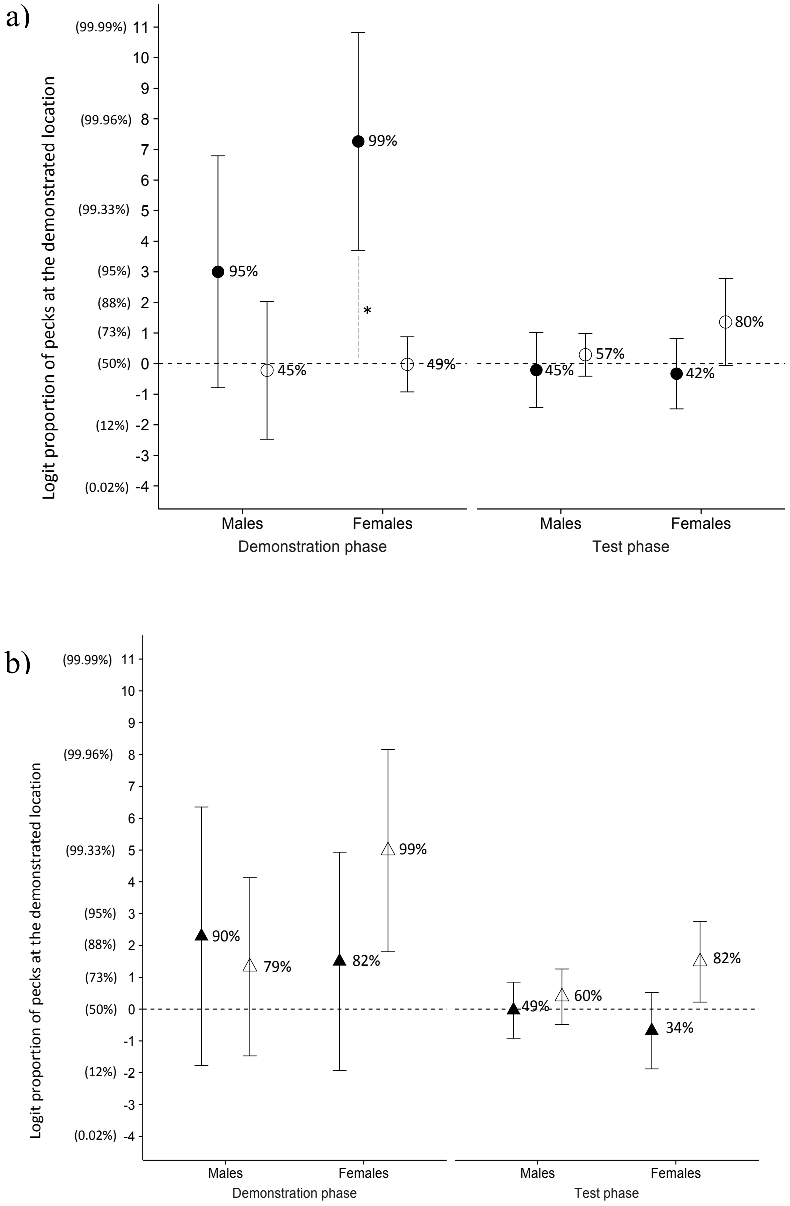

Figure 3.2: Effect of a) density and b) predation on proportion of pecks at the demonstrated location. Estimates +/- SE (on the logit scale) of the proportion of pecks performed at the demonstrated feeder by the fish during the demonstration and test phases of males and females. The dashed line at zero represents our chance expectation of 50% on the original scale. A difference from chance (50%) is indicated by a star next to the proportions (back transformed estimates). Filled symbols represent early life experience of standard social density or no predation cues, while empty symbols represent high social density or predation cues.

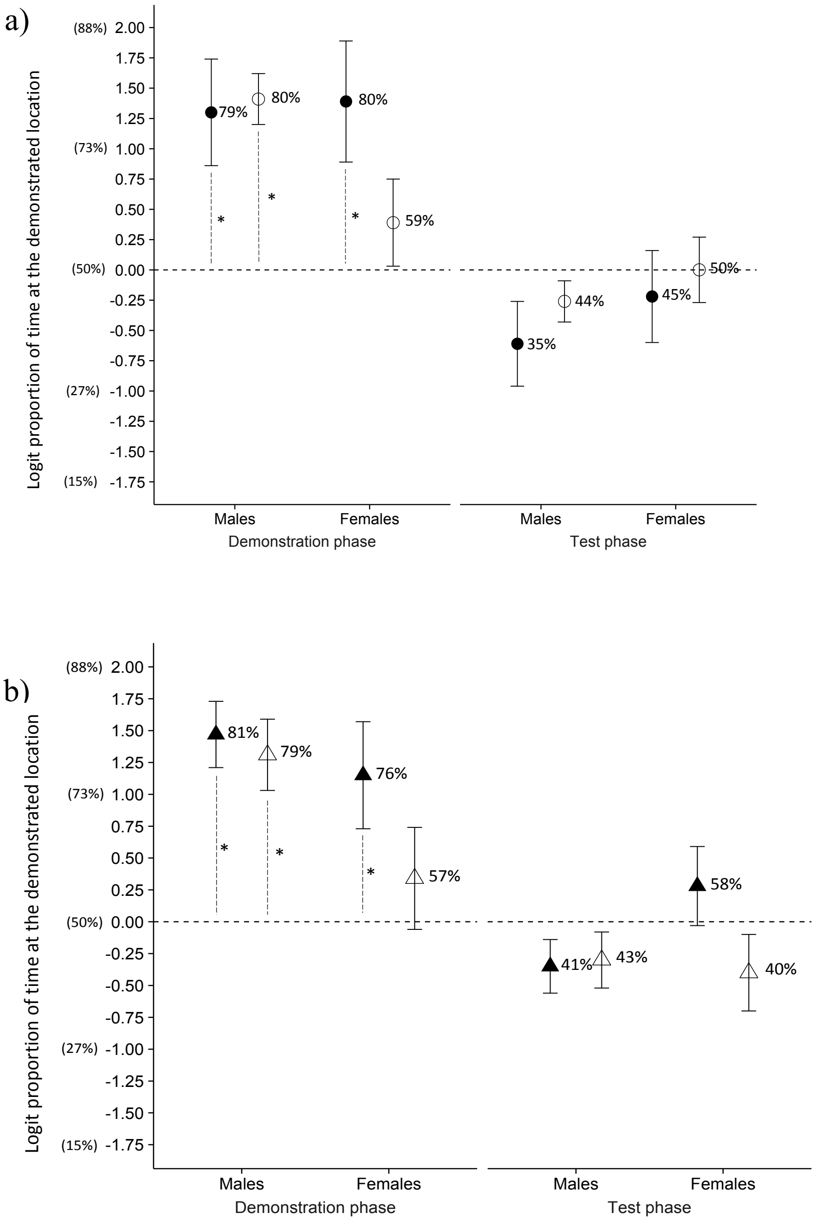

Figure 3.3: Effect of a) density and b) predation on proportion time spent at the demonstrated location. Estimates +/- SE (on the logit scale) of the proportion of time spent at the demonstrated feeder by the fish during the demonstration and test phases of males and females. The dashed line at zero represents our chance expectation of 50% on the original scale. A difference from chance (50%) is indicated by a star next to the proportions (back transformed estimates). Filled symbols represent early life experience of high social density or no predation cues, while open symbols represent standard social density or predation cues.

LINKING STATEMENT TO CHAPTER 4

In Chapter 3, my results demonstrated that early life conditions, particularly social density, can shape social information use propensities, and that sexes differed in which kind of information they attend to and how early life conditions affected them. The Pace-Of-Life Syndrome hypothesis suggests that there should be correlation between behavioural traits, either because they are more advantageous when displayed together or because they are ultimately controlled by similar mechanisms. Notably, the stress response is a likely candidate to be a core part of this syndrome. I thus investigated in Chapter 4 the effect of developmental conditions on the release of the glucocorticoid hormone cortisol in response to a minor stressor.

CHAPTER 4: DEVELOPMENTAL PLASTICITY OF THE STRESS RESPONSE IN FEMALE BUT NOT IN MALE GUPPIES

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ABSTRACT

To survive, animals must respond appropriately to stress. Stress responses are costly, so early-life experiences with potential stressors could adaptively tailor adult stress responses to local conditions. However, how multiple stressors influence the development of the stress response remains unclear, as is the role of sex. Trinidadian guppies (*Poecilia reticulata*) are small fish with extensive life-history differences between the sexes and population variation in predation pressure and social density. We investigated how sex and early-life experience influence hormonal stress responses by manipulating conspecific density and perceived predation risk during development. In adults, we sampled cortisol twice to measure initial release and change over time in response to a recurring stressor. The sexes differed considerably in their physiological stress response. Males released more cortisol for their body mass than females and did not reduce cortisol release over time. By contrast, all females, except those reared at high density together with predation cues, reduced cortisol release over time. Cortisol responses of males were thus less dynamic in response to current circumstances and early-life experiences than females, consistent with life-history differences between the sexes. Our study underscores the importance of early-life experiences, interacting ecological factors and sex differences in the organization of the stress response.

INTRODUCTION

Individuals experience a variety of stressors, and appropriate, proportionate responses to these stressors are important for individual fitness. Stress responses carry numerous costs, such as energy mobilization or lost opportunities to forage or mate, and therefore are expected to be finely tuned to ambient environmental risk levels to avoid both unnecessary stress responses and failure to respond to a legitimate threat (McEwen & Wingfield 2003; Romero et al. 2009; Boulton et al. 2015; Wendelaar Bonga 1997; Pigliucci 2001). However, temporal and spatial variation in risk complicates such fine-tuning. When the environment experienced in early-life reliably predicts risk later in life, developmentally plastic organisms can effectively use early-life cues to "adaptively programme" adult stress responses, thus matching their responses to the local environment (Reeder & Kramer 2005; Love & Williams 2008; Bateson 1979; West-Eberhard 1989). For example, the experience of predation or stressful early-life conditions adaptively programmes individuals to function in a similar environment (Ferrari et al. 2015; Chaby et al. 2015). Alternatively, exposure to stressors during early life may have long-term detrimental effects, either because of a mismatch in ambient risk between early and later life, or because of pathological or collateral effects of early stress (Murgatroyd et al. 2009; Meaney et al. 1993). As responses to stressors are mediated by shared endocrine mechanisms, early-life exposure to particular stressors probably impacts responses to multiple stressors later in life (Boulton et al. 2015; Hau et al. 2016).

While many studies have examined developmental effects on stress responses, these have typically manipulated only one environmental factor at a time (Jonsson & Jonsson 2014), even though the effects of simultaneous stressors on a developing animal may be additive, multiplicative, synergistic or antagonistic (Depasquale et al. 2016). Predation and social environment are two stressors that are relevant to the ecology of many animals, and that probably have interacting effects. When taken in isolation, exposure to predators or repeated adult aggression tends to increase the ability to deal with future stressors, potentially for multiple generations (Love & Williams 2008; Sheriff et al. 2015). Physiologically, this may be mediated by a high potential range of hormonal reaction ("reactive scope", Romero et al. 2009) in stressful environments, allowing fine-tuned energy mobilization. Similarly, taken in isolation, conspecific density can also be instrumental in shaping the stress response (Hau et al. 2016; Jonsson & Jonsson 2014; Creel et al. 2013). High social density can be stressful especially when resources are limited (e.g. Ramsay et al. 2006), such that high conspecific density can increase the recovery time required following a stressful event (e.g. Pottinger & Pickering 1992). Low conspecific density or social isolation can also be a stressor in group-living species (Buckingham et al. 2007). Given the role of social grouping in anti-predator responses in many prey species (Krause & Ruxton 2002), interaction between predation pressure and the social environment during early life may generate particularly large effects on stress–response phenotypes.

Sexes often differ considerably in their susceptibility to stressors. For example, males and females in the same predation environment may nonetheless be under different predation risk as a consequence of sexual size dimorphism or sex differences in ornamentation, colour or behaviour (Magurran 2005). Furthermore, males and females of the same species may have different life histories and energetic demands, altering their risk-taking strategies and thus their stress responses (Reeder & Kramer 2005). Hormonal and behavioural systems are expected to coevolve with differences in life histories, potentially due to physiological constraints, adaptation or genetic correlations among traits (Réale et al. 2010; Sinervo & Svensson 2002). As a result, we expect sex differences in the stress response.

We investigated the role of developmental experience and sex on the hormonal stress response of adult Trinidadian guppies, *Poecilia reticulata,* by repeatedly measuring water-borne cortisol in fish experimentally reared under different early-life conditions and then placed in identical housing conditions. Specifically, we investigated three interrelated hypotheses: that early-life conditions would shape the hormonal stress response; that different conditions would interact in this process; and that the two sexes would respond differently. Trinidadian guppies are a small tropical live-bearing fish found in habitats of varying predator pressure and social density, with considerable sex differences in morphology, parental investment and life history (Magurran 2005; Reznick 1996). We predicted, according to the reactive scope model (Romero et al. 2009), that experience of predation cues early in life would alter the stress response, and specifically that predator-experienced fish would show a strong initial response to a stressor, but also rapid habituation to this stressor. We also predicted that the social environment would modify the effect of experiencing predation cues, with high rearing densities amplifying the effect of predation cues. Male guppies are typically smaller, more colourful, bolder and faster maturing than females (Magurran 2005; Griffiths 1996), leading to our prediction that males would respond initially less intensely, and habituate more rapidly to stressors than females (Réale et al. 2010; Koolhaas et al. 1999). To study stress responses, we employed a widely used method of inducing mild stress by capturing and confining individuals in a small container (Eaton et al. 2015), which also allowed us to collect water-borne cortisol. Cortisol was used as a measure of the physiological mechanisms that govern the stress response. To investigate the speed of habituation to this stressor, individuals were exposed to a second confinement immediately following the first one. Furthermore, given that guppies typically live in groups, we investigated whether social isolation affected cortisol release by manipulating visual exposure to conspecifics during the second confinement period.

MATERIALS AND METHODS

ANIMAL SUBJECTS AND REARING PROCEDURES

Fish were laboratory-reared descendants of a mixed lineage of wild-caught guppies from high-predation populations in the Aripo and the Quare Rivers in Trinidad (for housing and feeding procedures; see the electronic supplementary material). We placed pregnant females together in female-only tanks and collected newborn fry each day. To ensure siblings were mixed across replicates, we pooled fry from all breeding tanks before we randomly assigned each fry to one of four developmental conditions and placed them in their designated rearing aquaria. We repeated this until we had three replicate rearing aquaria per developmental condition (12 in total). We reared juvenile fish under either a simulated predation condition or a no-predation condition combined with either a high (approx. 30 fish per aquarium) or standard (approx. 10 fish per aquarium) housing density, creating four distinct developmental conditions in a factorial design. During weekdays of the first 45 days of rearing, at a random time between 10.00 h and 17.00 h, we exposed fish in the predator condition to visual and olfactory cues of a wild-caught guppy predator, a pike cichlid (*Crenicichla* sp.), until they had received 31 exposures to those cues. To create temporal variation in cue exposure, we paired exposure to the predator with alarm substance (i.e. the odour of injured conspecifics) on 4 of those 5 days until they had received 25 exposures to those cues (see the electronic supplementary material for details on the preparation of cues). Most fish species, including guppies, produce typical anti-predator behaviours such as freezing or fleeing when exposed to the odour of injured conspecifics (Brown et al. 2010; G. Brown & Godin 1999). Using the same schedule, we exposed fish in the no-predation condition using the visual and olfactory cues of a non-predatory sucker-mouth catfish (*Pterygoplichthys* sp.) and paired with distilled water rather than the odour of injured conspecifics. To present the visual cues, we removed an opaque partition between the guppies' rearing aquaria and the stimulus fish in an adjacent aquarium for 5 min.

After 50 days, all fish were transferred into common garden conditions of approximately 10 fish per 18 l aquaria (standard housing density in our laboratory) without any further exposure to heterospecific cues until they were approximately 200 days old, at which point we conducted the cortisol collection. From the total pool of fish, we randomly selected 101 fish for testing, 25 exposed to no-predation cues and high density, 26 to no-predation cues and standard density, 26 to predation cues and high density, and 24 to predation cues and standard density. Aquaria and water samples were coded to ensure that the experimenters conducting collection and extraction of cortisol were blind to the treatments. On the day of the cortisol collection, all fish were fed at 09.00 h to avoid variation in hunger levels and any anticipatory effects of feeding on cortisol release.

HORMONE COLLECTION PROCEDURES

We gently captured fish using a dip net and placed them individually in 400 ml glass beakers containing 200 ml of aged and oxygenated municipal tap water heated to 27 ± 1°C. To avoid contamination, we cleaned the beakers with ethanol and rinsed them with distilled water, experimenters wore clean examination gloves for each manipulation and water was aged in a covered tank. We collected the holding water after two consecutive hour-long collection periods, held at the same time each day for our different replicates to account for diurnal variation in cortisol release. Holding the fish in small beakers and collecting the water afterwards provide a tractable way to repeatedly assess relative cortisol levels in fish too small for repeated blood sampling. The hormones diffusing in the water from the gills provide a reliable estimation of circulating levels (Ellis et al. 2004; Archard et al. 2012; Fischer et al. 2014), but the most conservative way to interpret the hormone concentrations is as a relative value among individuals and conditions.

For collection 1 (at 11.00 h), we placed the beakers containing the fish in a water bath of the same temperature and arranged the beakers in clusters of at least three of mixed sex, so that each fish could see at least two familiar conspecifics (i.e. fish from the same tank; (Magurran et al. 1994). After 1 h, we collected and immediately froze the water, and fish were placed into a new clean beaker with a fresh 200 ml of water for cortisol collection 2. During collection 2 (beginning at 12.00 h), half of the fish were randomly assigned to the "social isolation" treatment in which plastic barriers were inserted between the adjacent beakers, so that each fish in the social isolation treatment was visually isolated from all conspecifics. Grouping is a typical response to stressful situations in guppies (e.g. Magurran & Pitcher 1987), and thus, visual contact with familiar conspecifics may have an anxiolytic effect (Al-Imari & Gerlai 2008). We predicted that social isolation would increase stress and would produce different levels of cortisol depending on the developmental conditions the fish experienced early in life (Archard et al. 2012; Chapman et al. 2008). The other half of the subjects were exposed to the same social treatment as in collection 1 (i.e. at least two familiar mixed-sex social conspecifics were visible in adjacent beakers). After an hour, we collected and immediately froze the water from collection 2. We then anaesthetized the fish using 60 ppm Eugenol, weighed them to the nearest mg using an analytical laboratory balance (Mettler Toledo ME104E) and measured their standard length. All fish were returned to their housing aquaria after they recovered from anaesthesia.

HORMONE EXTRACTION

Frozen water samples were shipped overnight to the University of Alabama, where cortisol was extracted using reversed-phase chromatography and assayed with enzyme immunoassay (EIA). Hormone was extracted from the water samples by gently drawing the samples through Waters Sep-Pak C18 columns using a vacuum. We then eluted the free fraction of the hormone (i.e. the fraction not conjugated to glucuronides or sulfates) by passing ethyl acetate through the columns. After evaporating the ethyl acetate under nitrogen, the hormone was resuspended in EIA buffer. The dilution at which to assay the resuspended hormones was determined for each sex to ensure that the sample concentrations would fall on the linear phase of the standard curve. We determined, after conducting serial dilutions of a pooled sample for each sex, that a 1 : 8 dilution was optimal for males, and a 1 : 16 dilution was optimal for females.

All samples were run in duplicate on six 96-well plates. The 1 : 8 diluted male pool was included in duplicate at the beginning and end of each plate to determine the intra- and inter-assay coefficients of variation (CVs). Intra-assay CVs were 2.87%, 3.98%, 1.93%, 4.80%, 2.70% and 3.89% for the six plates. The inter-assay CV was 7.58%. Cayman Chemicals, Inc. protocols were followed strictly for all assays. Additional procedural details are given in the electronic supplementary material.

STATISTICAL ANALYSES

We calculated the cortisol release rate in ng/h^{-1}. We used the cortisol measure (in ng/h) from collection 2 divided by the cortisol measure from collection 1 for each fish as our measure of the speed of habituation to the collection procedure. This ratio represents the change in cortisol release across the collections, with the division eliminating body mass and partially accounting for individual differences in baseline cortisol released.

To analyse the influence of the experimental manipulations on cortisol release and the speed of habituation, we ran generalized linear mixed-effects models (GLMMs) fitted by maximum likelihood with a gamma error distribution. We used the gamma family with an "inverse" link because the response variables were continuous but bounded by zero (GLMM, glmer function from lme4 package in R v.3.2.2). We ran two models, the first one looking at cortisol release (ng/h) during collection 1 including body mass as a covariate and the second looking at the ratio of cortisol release across the two collections. We also ran a model looking at sex differences in the ratio of cortisol release, including only sex as a predictor. Some of the sample containers cracked during shipping. We therefore reanalysed the data eliminating any sample that had lost more than 25% in volume (10 samples for collection 1 and 12 for collection 2), and the results were qualitatively unchanged. We thus present results for the entire dataset, in which we adjusted the extracted hormone in any samples with lost volume to a standard 200 ml volume.

The final models tested for the main effects of predation, density, sex and the two- and three-way interactions. Housing aquarium was included as a random factor to account for any between-aquarium variance. For the model examining cortisol ratio across the collections, we also included the treatment of collection 2 (social or isolation) as a main effect, as well as its two- and three-way interactions, but not the four-way interaction.

RESULTS

During the first collection, males from all rearing treatments released 1.6 times as much cortisol for their body mass than females (GLMM "sex" $p = 0.0026$; table 4.1, figure 4.1) but rearing treatment had no significant effect on cortisol release in either sex (GLMM "predation" $p = 0.59$, “predation : sex” $p = 0.22$, “density” $p = 0.55$, “density : sex” $p = 0.57$; table 4.1, figure 4.1). As body mass may be confounded with pregnancy stage in females, we ran the same model correcting cortisol with standard length instead of mass, and the results were qualitatively unchanged. A common practice in the quantification of fish hormones is to use a body mass-corrected measure by dividing release rate by body mass to obtain a rate of release in ng/g/h (Archard et al. 2012), rather than including body mass as a covariate. We obtained similar results when accounting for body mass in this alternative manner (electronic supplementary material table S4.2).

Over the two collections, females decreased their cortisol release significantly more than males (GLMM “sex” $p < 0.001$; electronic supplementary material table S4.1). While males showed little change in cortisol release (mean ratio ± s.e.m. = 1.02 ± 0.092), females showed a significant decrease in cortisol release (mean ratio ± s.e.m. = 0.63 ± 0.097). In the full model, all two- and three-way interactions between sex, density and predation were significant (GLMM “predation:sex” $p = 0.03$, “density : sex” $p = 0.042$, “predation : density : sex” $p = 0.017$; table 4.2), providing evidence that the developmental conditions affected males and females differently (figure 4.2). Further separating the analysis on the basis of sex (table 4.3) revealed that developmental conditions significantly affected female guppies. For females reared in high social density (the reference level in the model), exposure to predation cues during development dampened the decrease in cortisol between collections (GLMM “predation” $p = 0.03$; figure 4.2, table 4.3), and this effect tended to disappear when females were reared in standard social density (GLMM “predation : density” $p = 0.057$; figure 4.2, table 4.3). Developmental conditions had no significant effect on the change in cortisol release in males (table 4.3). Cortisol release during collection 1 and collection 2 were strongly correlated ($r = 0.69$) within fish, supporting the reliability of our procedures and generally, repeatability of the fish.

Female fish had a mean mass of 0.65 g (s.d. = 0.20) and a mean standard length of 28.8 mm (s.d. = 2.88), and were significantly heavier (linear model $(\mathrm{LM})_{\mathrm{mass}}$ ”sex” $p < 0.001$) and longer ($\mathrm{LM}_{\mathrm{length}}$”sex” $p < 0.001$) than males, which had a mean mass of 0.10 g (s.d. = 0.02 g) and a mean standard length of 16.2 mm (s.d. = 0.74). Standard density females were 0.13 g (20%) lighter and 2.1 mm (7%) shorter than high-density females, but these differences were not significant

(LM_{mass} ”density” $p = 0.076$, “density : predation” $p = 0.19$; $\text{LM}_{\text{length}}$ ”density” $p = 0.06$, “density : predation” $p = 0.37$). Developmental condition had no effect on male body mass (LM_{mass} $p > 0.14$), but within the no-predation treatments, standard density males were 0.8 mm (5%) shorter than high-density males ($\text{LM}_{\text{length}}$ ”density” $p = 0.014$, “density : predation” $p = 0.038$).

The main effect of “social isolation” during the second collection and its interactions with all other factors were not significant (GLMM $p > 0.4$; table 4.2), and therefore had no detectable effect on the speed of habituation to the stress of the collection procedure.

DISCUSSION

Our study demonstrates the importance of sex and early-life experiences on adult cortisol release, which mediates the stress response in guppies. Males exhibited high cortisol release rates (for their body mass), and maintained these rates over the two collection periods of the experiment. In comparison, females exhibited lower initial cortisol release rates, and these rates decreased over the two experimental collections, suggesting they habituated to the procedure. Moreover, the speed of habituation was affected by rearing conditions in females but not males. Adult females reared at high density and with predator cues showed no evidence for habituation, whereas females reared in all other conditions showed a dramatic decrease in cortisol release over the two collection periods. Combined, our results suggest that the physiological stress responses of males and females are under different selection pressures, possibly due to different life histories, and thus exhibit different sensitivity to local conditions.

Contrary to our predictions and to other research on animals, particularly rodents (Panagiotakopoulos & Neigh 2014), males released more cortisol for their body mass than females. Research in closely related fish (*Brachyrhaphis episcopi*) found male and female cortisol release rates were similar (Archard et al. 2012). We propose two hypotheses for the observed sex differences in cortisol response. First, under the “reactive-males hypothesis”, males are more sensitive to the stressor (i.e. the capture and confinement involved in the experimental procedure) than females, and thus show higher initial cortisol release and slower habituation to the procedure than females. Males are more susceptible to predation owing in part to their greater conspicuousness (Magurran 2005), and as a result may be more reactive to stress than females, leading to their relatively high and continued levels of cortisol release. Second, under the "unresponsive males" hypothesis, males may exhibit a higher baseline circulating level of cortisol than females, resulting in a small scope for responsiveness and thus little change in cortisol levels in response to a stressor. We argue that our results are consistent with the unresponsive males hypothesis, because stress-induced high levels of cortisol typically correlate with behavioural responses such as freezing and reduced activity, which is inconsistent with the behavioural patterns typically observed in male guppies (Clinchy et al. 2013).

If male guppies are unresponsive to stressors, it implies that males carry a high baseline level of cortisol. Baseline cortisol supports essential processes such as locomotion, homeostasis, immune responses and investment in reproduction (Hau et al. 2016; Barton 2002; Mommsen et al. 1999). Compared to females, male guppies tend to be bolder, take more risks and display a "fast" life history with quick maturity and early death (Magurran 2005; Harris et al. 2010). Owing to physiological constraints or correlated selection, individuals with a fast life history, like male guppies, are predicted to also display a low reactivity to stressors (Ricklefs & Wikelski 2002). In Swedish warblers (*Phylloscopus trochilus*), northern populations are constrained by a reduced reproductive period and display a faster life history and lower reactivity to stressors than southern populations (Silverin et al. 1997), paralleling our observations of male and female guppies. Chronically elevated cortisol supports high energy investment in these activities, but also results in a small range of reaction before reaching detrimental levels. In other words, individuals with high baseline levels will quickly exceed the hormonal limit if they are also highly reactive (Romero et al. 2009). Consequently, a high baseline cortisol level should be combined with low reactivity, consistent with our results showing little change in male cortisol across collections. In contrast to males for whom mating is the only reproductive investment, female guppies have high obligate parental investment in the form of live-bearing (Magurran 2005). This could potentially explain why females maintain a high reactive scope, allowing quick response to stressors and potentially maximizing fitness for slow life strategies. Thus females are potentially under greater selective pressure than males to exhibit plasticity in their stress response (Nonacs & Blumstein 2010), an idea supported by our finding that only females' stress response was affected by our developmental conditions.

Whereas females from most groups showed a decline in cortisol release between the two collection periods, an indicator of habituation to the procedure, females raised in the combination of predation cues and high social density showed little decline in cortisol levels, suggesting that social conditions and predation threat interact to shape stress response phenotypes. Previous research investigating the effect of predation cues on stress responses found that individuals with experience of high predation tended to show reduced stress responses (Fischer et al. 2014; Brown et al. 2005). One possible explanation for this difference is that the relationship between predation cues and stress response is nonlinear, and an interaction with a high social density modulates the effect of predation cues. Perhaps, high social density made the predation cues more salient during development, because more fish are likely to spot and react to the predator (i.e. "many-eyes effect"; Lima 1995). Stress responses may be "contagious" among members of a social group in that they propagate and are amplified among group members, causing groups of animals to react more strongly to stressors than the same individuals when tested alone (Giacomini et al. 2015). Social contagion of stress may have been more dramatic under the high-density housing conditions during development, causing the stress of the predator to have a greater effect on females in this treatment group. Chronic physical challenges such as competition for food or restricted food intake can also trigger stress responses (although fish were fed ad libitum in our study), and foraging is often impaired under the presence of predators (Clinchy et al. 2013), which could exacerbate this effect. Therefore, high social density could amplify the effect of predation cues or vice versa, and create higher levels of stress than in any of the other developmental conditions.

Contrary to predictions, social isolation during collection 2 did not have detectable effects on cortisol release. Perhaps visual exposure to conspecifics in our set-up was insufficient to evoke a social response, although adjacent fish in the social treatment were observed to interact. The stress of the confinement procedure may have masked any effect of social isolation. In our experiment, we measured water-borne cortisol levels twice but only an hour apart, thus our second measure does not represent a fully habituated baseline level of cortisol release. Instead, the change between the two collections provides a measure of the speed of habituation, and thus it is possible that this habituation process is masking the effect of the social treatment on collection 2.

While our study demonstrates that the physiological stress response varies between sexes, and is shaped by developmental conditions, whether the observed phenotypes are adaptive, or a maladaptive result of physiological constraint produced by repeated stress remains to be determined. Habituation to stress might be a poor response in certain environments and hence our females might be demonstrating a phenotype suited to the conditions they experienced early in life. Alternatively, as a larger group could dilute the chances of being depredated (Hamilton 1971), a prolonged stress response might be a suboptimal phenotype produced by developmental constraints created by recurring high levels of stress during early life (Wingfield 2005). Future experiments manipulating social stress will be required to disentangle the possible functional consequences of the differences in stress habituation we observed in females from different developmental conditions. Sex differences in guppies offer a salient example of dissimilar life strategies, however, we expect the same predictions to hold when looking at continuous variation of life histories among individuals. Our results emphasize that looking at both sexes is imperative, and combining multiple developmental treatments to look for interactions between factors is required to understand the implications of developmental plasticity.

ETHICS

The study was approved by McGill University and the Canadian Council on Animal Care under protocol 2012- 7133/2015-7708, and conformed to ABS/ASAB ethical guidelines. We did not carry out any fieldwork. All subjects recovered quickly from the procedures and were returned to housing aquaria after the experiment. We sacrificed 16 guppies to feed the cichlids during the collection of predator odour cues (cichlids were otherwise fed bloodworms) and 61 guppies to produce the damage induced alarm substance. Prior to being fed to the cichlids or dissected for alarm cue preparation, the guppies were euthanized by immersion in ice water (Blessing et al. 2010). They were then consumed within seconds by cichlids or swiftly decapitated for alarm cue preparation.

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TABLES

Table 4.1: Estimates and standard error of fixed parameters and their interactions for the GLMM with response variable cortisol release per hour (ng/h) during collection 1. (Estimates are given on the scale of the "inverse" link (1/x), and negative estimate values thus represent an increase in cortisol release. The model estimates represent the difference between the level of a factor (identified in parenthesis) with the reference levels. As our factors each contain two levels, the estimates represent the difference between the two groups. The reference levels were no-predator cues for predation, high density and females. Housing group was included as random effect in the model, and body mass as a covariate. Significant p-values ($p<0.05$) are shown in italics.)

parameter	**estimate**	**s.e.**	***t*-value**	***p*-value**
intercept	0.088	0.016	5.43	*<0.0001*
predation (predation)	−0.008	0.016	0.54	0.59
density (standard)	−0.009	0.016	0.60	0.55
sex (males)	0.14	0.045	3.01	*0.0026*
mass (g)	−0.052	0.029	1.79	0.073
predation × density	0.025	0.023	1.09	0.28
predation × sex	−0.062	0.05	1.22	0.22
density × sex	−0.033	0.057	0.57	0.57
predation × density × sex	0.098	0.080	1.23	0.22

Table 4.2: Estimates and standard error of fixed parameters and their interactions for the GLMM with response variable cortisol ratio between the hour-long collection periods (cortisol release during collection 2 divided by cortisol release during collection 1). (Estimates are given on the scale of the "inverse" link ($1/x$), and negative estimate values represent an increase in cortisol concentration. The model estimates represent the difference between the level of a factor (identified in parenthesis) with the reference levels. As our factors each contain two levels, the estimates represent the difference between the two groups. The reference levels were no-predator cues for predation, high density, females and "social" for social treatment. Housing group was included as random effect in the model. Significant p values ($p < 0.05$) are shown in italics.)

parameter	**estimate**	**s.e.**	***t*-value**	***p*-value**
intercept	2.93	0.65	4.54	*<0.001*
predation (predation)	−1.64	0.72	2.26	*0.02*
density (standard)	−1.18	0.72	1.62	0.10
sex (males)	−1.75	0.61	2.85	*0.004*
social treatment (isolation)	−0.40	0.61	0.14	0.54
predation × density	2.15	0.85	2.52	*0.01*
predation × sex	1.61	0.73	2.36	*0.03*
density × sex	1.36	0.76	2.03	*0.04*
predation × social treatment	0.12	0.68	0.18	0.86
density × social treatment	0.54	0.69	0.78	0.44
sex × social treatment	0.17	0.69	0.25	0.80
predation × density × sex	−1.68	0.70	2.40	*0.02*
predation × density × social treatment	−0.37	0.73	0.58	0.53
predation × sex × social treatment	0.02	0.69	0.02	0.98
density × sex × social treatment	−0.55	0.67	0.83	0.41

Table 4.3: Estimates and standard error of fixed parameters and their interactions for the GLMM with response variable cortisol ratio between the hour-long collection periods (cortisol release during collection 2 divided by cortisol release during collection 1) separated by sex. (Estimates are given on the scale of the "inverse" link (1/*x*), and negative estimate values represent an increase in cortisol concentration. The model estimates represent the difference between the level of a factor (identified in parenthesis) with the reference levels. As our factors each contain two levels, the estimates represent the difference between the two groups. The reference levels were no-predator cues for predation and high density. Housing group was included as random effect in the model. *p*-values below or approaching 0.05 are shown in italics.)

parameter	**estimate**	**s.e.**	***t*-value**	***p*-value**
females				
intercept	2.70	0.58	4.64	*<0.001*
predation (predation)	−1.55	0.74	2.08	*0.03*
density (standard)	−0.84	0.75	1.10	0.27
predation × density	1.95	1.02	1.90	*0.057*
males				
intercept	1.04	0.26	3.95	*<0.001*
predation (predation)	−0.003	0.37	0.009	0.99
density (standard)	0.16	0.38	0.41	0.68
predation × density	−0.002	0.55	0.004	0.99

FIGURES

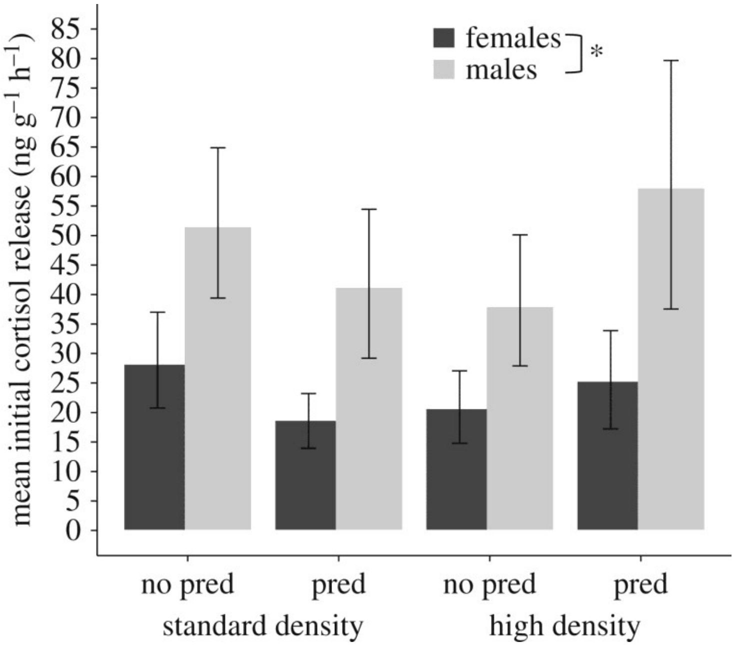

Figure 4.1: Cortisol released during collection period 1. For ease of exposition, data are plotted per gram of body mass (in ng g^{-1} h^{-1}). The *x*-axis shows the developmental manipulation of predation cues (predation versus no-predation) and housing density (high versus standard), and bar shading sex (black: females; grey: males). Means ± 95% confidence interval (CI). The asterisk indicates a significant difference of $p < 0.05$ (electronic supplementary material table S2 provides analyses of cortisol release as ng g^{-1} h^{-1}; the main text analyses include body mass as a covariate in the statistical model).

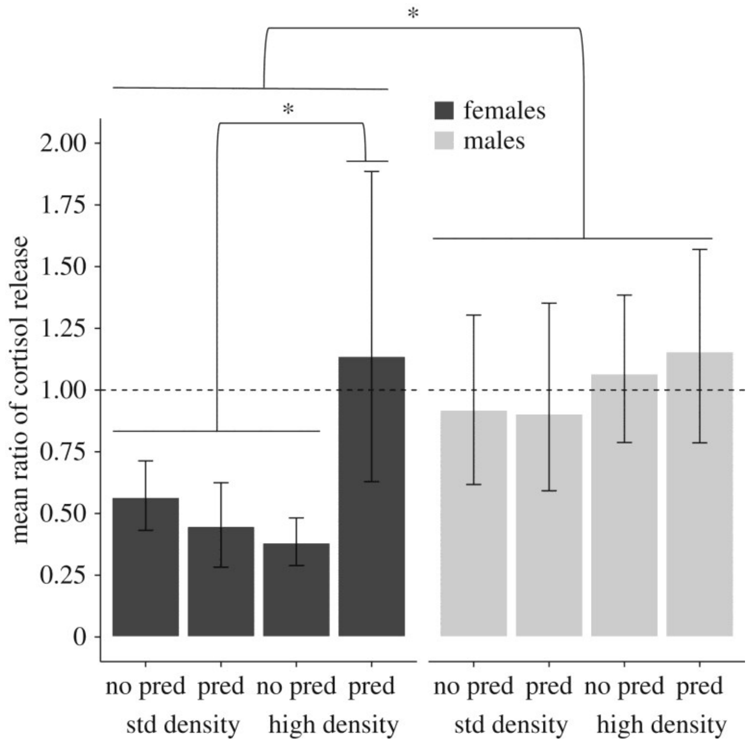

Figure 4.2: Ratio of cortisol between the 2 h long collection periods (cortisol in collection 2 divided by cortisol in collection 1). Values less than 1 (dotted line) indicate a decrease in cortisol release, values around 1 indicate no change and above 1 indicate an increase in cortisol release in the second collection period. The *x*-axis shows the developmental manipulation of predation cues (predation versus no-predation) and housing density (high versus standard), and bar shading sex (black: females; grey: males). Means ± 95% CI. The asterisks indicate significant differences of $p < 0.05$.

APPENDIX 1: SUPPLEMENTARY MATERIAL FOR CHAPTER 4

METHODS

Feeding and housing

Guppies were housed at 27±1ºC and fed commercial tropical fish flakes (TetraMin, Tetra, Germany) and re-hydrated, decapsulated brine shrimp eggs (Brine Shrimp Direct Inc., Utah, USA). Fish were fed *ad libitum* twice a day at random times between 1000h and 1700h, during weekdays and once a day on weekends until age 85d. The random times were generated with a pseudo-random number generating function in AutoIt v3, but set to be at least 30 minutes apart from the developmental cue exposure. After day 85 and until the cortisol collection, fish were fed daily and supplemented with decapsulated brine shrimp eggs on Mondays and Fridays.

Cues used in the experimental conditions

Harvesting of the alarm substance followed Brown & Godin (1999). We homogenized skin and muscle tissues from 36 male and 25 female euthanized guppies, filtered the solution with filter wool and diluted it with ddH_20 until we obtained a concentration of 0.1 cm^2 of tissue per ml. We obtained the olfactory cues of the predator and control fish following (Brown et al. 2000). We collected holding water from pike cichlids (*Crenicichla* sp.), and sucker-mouth catfish (*Pterygoplichthys* sp.) after being fed with euthanized guppies and blanched spinach leaves, respectively, for four consecutive days. After this holding water was collected, we fed the cichlids bloodworms and the catfish algae wafers (Hikari, Hayward, California, USA). This feeding procedure minimized the number of guppies used in cue preparation (16 total) and ensured catfish ate an entirely plant diet while olfactory cues were collected.

Hormone collection

After each hour-long collection period, we collected the holding water by pouring the contents of the glass beaker through a clean dip net. We then placed the fish in a clean beaker with 200 ml of fresh water. We split each 200 ml water sample into two 100 ml sealed containers to minimize the risk of sample loss during shipping, and immediately froze it to -20°C. We transferred the samples to a -80°C freezer 48h before shipping.

Hormone extraction

Samples were transferred to -20ºC upon arrival at the University of Alabama and were moved for thawing to 4ºC one day prior to extraction. The two 100 ml samples per individual for a given collection phase were combined and filtered through Whatman Grade 1 filter paper (single use) fitted to a glass funnel that was pre-cleaned with ethanol and distilled water prior to filtering each sample. Samples were filtered into 250 ml beakers that also were pre-cleaned with ethanol and distilled water. Exact sample volume was assessed via graduated cylinder.

Reversed-phase chromatography was conducted using Waters Sep-Pak C18 columns fitted to a 24-port vacuum manifold and primed with 2 x 2 ml methanol followed by 2 x 2 ml distilled water; on the second pass of distilled water, a small volume was retained to keep the column moist. Tygon® tubing (Saint Gobain formulation 2275, which eliminates adsorption and leaching) was fitted to the C18 column and the other side of the tubing was inserted into the filtered guppy water sample. The vacuum was engaged and the water samples were drawn through the corresponding C18 columns slowly (drip by drip). Following full extraction, 2 ml of distilled water was passed over the C18 columns to remove residual salts.

To elute the free fraction of the hormone (i.e., the fraction not conjugated to glucuronides or sulphates), 2 x 2 ml HPLC grade ethyl acetate was vacuumed through the columns into labelled 13 x 100 mm borosilicate vials. The ethyl acetate was evaporated in a manifold under a gentle stream of nitrogen (~7 bar) in a 37ºC water bath, leaving a hormone residue. This residue was resuspended immediately in 600µl of 5% ethanol:95% enzyme-immunoassay (EIA) buffer (i.e., 30µl ethanol, 1 minute vortex, 570µl EIA buffer, 20 min vortex); EIA buffer was provided with the Cayman Chemicals, Inc. (Ann Arbor, USA) kits and prepared according to manufacturer's instructions. Resuspended samples were stored at 4ºC while the assays were conducted.

To determine the dilution at which to assay the resuspended guppy hormones so that the sample concentrations would fall on the linear phase of the standard curve, a pool was generated for each sex. For males, 30µl was taken from each of 45 resuspended samples to produce a 1.35 ml pool; for females, 30µl was taken from each of 56 resuspended samples to produce a 1.68 ml pool. A serial dilution was conducted for each with a beginning 1:1 volume of 400µl, and a final dilution of 1:128. It was determined that a 1:8 dilution was best for males, and a 1:16 dilution was best for females; this was accomplished by taking 50µl of the original resuspension for each animal and mixing it with 350µl or 750µl of EIA buffer for males and females, respectively.

The serial dilution also allowed us to assess parallelism between the kit standard curve and the guppy serial dilution curve. These curves were parallel for both males (slope comparison test [Zar 1996 p. 355]: $t_{12} = 0.02$, P=0.98) and females ($t_{12} = 0.07$, P=0.94).

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Zar J.H., 1996. *Biostatistical analysis*. 3rd Edition. Upper Saddle River, New Jersey: Prentice Hall.

Table S4.1: Estimates and standard error of fixed parameter "sex" for the GLMM with response variable cortisol ratio between the hour-long collection periods (i.e. cortisol during collection 2 divided by cortisol during collection 1). Estimates are given on the scale of the "inverse" link (1/x), and negative estimate values represent an increase in cortisol concentration. The model estimates represent the difference between the level of a factor (identified in parenthesis) with the reference levels. As the factor contains two levels, the estimate of the factor represents the difference between the two groups. The reference level was females. Housing group was included as random effect in the model. Significant p values ($p < 0.05$) are shown in bold.

parameter	**estimate**	**s.e.**	***t*-value**	***p*-value**
(Intercept)	1.75	0.21	8.48	<0.0001
Sex (males)	-0.50	0.15	-3.36	<0.001

Table S4.2: Estimates and standard error of fixed parameters and their interactions for the GLMM with response variable cortisol concentration per gram of body mass per hour (ng/g/h) during phase 1. Estimates are given on the scale of the "inverse" link (1/x), and negative estimate values thus represent an increase in cortisol concentration. The model estimates represent the difference between the level of a factor (identified in parenthesis) with the reference levels. As our factors each contain two levels, the estimates represent the difference between the two groups. The reference levels were no-predator cues for predation, high density, and females. Housing group was included as random effect in the model. Significant p values ($p < 0.05$) are shown in bold.

parameter	**estimate**	**s.e.**	***t*-value**	***p*-value**
Intercept	0.47	0.08	5.64	<0.0001
Predation (predation)	-0.07	0.10	0.68	0.50
Density (standard)	-0.09	0.10	0.92	0.36
Sex (males)	-0.21	0.09	2.25	0.024
Predation * density	0.19	0.15	1.20	0.23
Predation * sex	0.002	0.12	0.02	0.99
Density * sex	0.01	0.11	0.11	0.91
Predation * density * sex	-0.07	0.18	0.38	0.71

LINKING STATEMENT TO CHAPTER 5

In Chapter 4, I demonstrated that male and female guppies have very different waterborne cortisol profiles in response to acute stress. Males responses align with the non-reactive hypothesis, whereas females would be reactive but with a quick habituation. However, females reared with both predation cues and under high social densities did not show signs of habituation. Cortisol profile correlated with behavioural responses to stressors, and is thus hypothesized to be the proximate mechanism underlying behavioural responses to stressors. It is thus important to also investigate how early life experiences affect responses that change how individuals respond to ecologically relevant stressors. I investigated in Chapter 5 how developmental experiences influence the responses to a non-predatory and a predatory fish.

CHAPTER 5: LIFETIME EFFECTS OF EARLY LIFE PREDATOR CUES AND SOCIAL ENVIRONMENT ON GUPPY PREDATOR APPROACH

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ABSTRACT

For prey species, efficient anti-predator behaviours are crucial for survival. As anti-predator behaviours carry costs, they are expected to develop with sensitivity to local conditions. Learning from previous non-lethal experiences with predators can play a large role in the development of efficient predator detection, recognition, assessment, and avoidance. Since grouping and other social behaviours are common anti-predator responses, the social environment and predator threat are often intertwined. Yet, it is unclear how early life experiences of predation and social environment influence adult anti-predator behaviours, particularly within each sex. In Trinidadian guppies *Poecilia reticulata*, females bear the largest reproductive costs, and are thus expected to be more sensitive to local conditions. This experiment investigated the effect of sex, and early life experiences of simulated predation risk and social density on predator responses of pairs of adult *P. reticulata.* The approach distance of adult fish to a visually exposed non-predatory sucker-mouth catfish *Pterygoplichthy* sp. and then to a predatory pike cichlid *Crenicichla* sp. was recorded, as well as the time pairs spent together. As expected from life-history differences between the sexes, females shoaled more, and stayed further away from both stimulus fish than males. Early life experience of predation prompted fish of both sex to stay further away from a predatory than a non-predatory fish, but did not influence how much time either sex spent together. Early life experience of high rearing density increased shoaling in males, but not females. Early life experience of high density influenced the approach distance to the predator versus a non-predator in different ways in each sex. These results confirm that males and females differ in predator responses and shoaling tendencies, and suggests that early life experiences play a large role in shaping approach distances to a predatory fish, but only weakly influence shoaling responses.

INTRODUCTION

Balancing anti-predator behaviour with other activities is a vitally important trade-off for many prey species (Lima & Dill 1990). While genetic predispositions underlie some anti-predator behaviour, such as the recognition of visual features of predators (Blumstein et al. 2000) or the use of social cues like alarm calls (Sherman 1977) or alarm substance (Wisenden 2000), learning plays a key role in the development of predator detection, recognition, assessment, and avoidance (Wund et al. 2015; Magurran 1990; Kelley & Magurran 2003). Even prey without recent evolutionary experience of predation can effectively learn anti-predator behaviours (Griffin 2004), although life experience is most efficient when fine-tuning genetic predispositions (Kelley & Magurran 2003). Successful anti-predator behaviours are crucial for survival but often come at a cost to other activities, and thus should be expressed with sensitivity to the risk in the environment, but also the level of risk that each predator poses (Giaquinto & Volpato, 2001; Magurran, Seghers, Carvalho, & Shaw, 1993). Experience of predation can result in long term changes in anti-predator behaviours (Brown & Smith 1998), however, as predation risk often varies through time, being able to adjust behaviour to current conditions is often expected to be beneficial. Indeed, there is some evidence that individuals can adjust their response to the relative risk in the current environment, such as common minnows *Phoxinus phoxinus* (Linnaeus 1758) who adjust the strength of their behavioural response based on the most recent risk perceived rather than an average of past experiences (Ferrari & Chivers 2006). As early life exposure to predation can cause irreversible changes in life history (Ball & Baker 2014), brain development (Reddon et al. 2018), and shape correlations between personality traits (Bell & Sih, 2007), the length of time that early life experiences influence predator responses is still to be determined in many species, as are sex differences in these early-life effects.

Through experience of predation, prey develop anti-predator behaviours such as shoaling, freezing behaviour, and predator inspection behaviours that increase chances of survival (Fuiman & Magurran 1994). Schooling and shoaling in fish reduces risk (Pitcher & Parrish 1993). For example, individual banded killifish *Fundulus diaphanus* (Lesueur 1817) are less at risk of being predated on when in a large shoal (Morgan & Godin 1985) and shoaling response is strengthened by previous experience of predation in many species of fish (Dugatkin & Godin 1992). Freezing behaviour reduces detection possibility and number of attacks (Chelini et al. 2009), particularly when predators hunt by detecting movement (Fuiman & Magurran 1994) and is increased by experience of predation (Brown & Smith 1998). Experience of predation also shapes predator inspection behaviour. Inspections should be conducted with great care, but can serve to deter predators from an attack (Dugatkin & Godin 1992) and provide information about the level of threat posed by the intruder (Licht 1989) and the need to escalate to more costly anti-predator responses. Since escaping monopolizes time and energy, keeping at a distance might be the optimal response. Approaching predators can be dangerous, and learning to avoid particularly dangerous areas, such as the head region, is crucial and shaped by experience (Magurran & Seghers, 1990). Different anti-predator behaviours might co-vary, for example because they are mediated by the same mechanisms, and/or are most beneficial when displayed in concert (Bell & Sih, 2007; Smith & Blumstein, 2010).

In social fish, responses to predators often involve conspecifics, either through shoaling responses or through collective predator inspection. In turn, the social environment also influences how and with whom social anti-predator behaviour patterns are conducted (Dugatkin & Alfieri 1991). The density of the social group in early life influences shoaling tendencies in many fish species, which may also shape shoaling responses in an anti-predator context (Olla et al. 1998). High social densities also can increase fish aggressiveness, particularly if driving competition for food. Highly aggressive fish may be less likely to shoal, but also may devote less time to predator vigilance, or be more willing to take risk during predator inspections (Olla et al. 1998; Ryer & Olla 1995). As the early social environment has large effects on the development of fish behaviour (Jonsson & Jonsson 2014), it likely also affects how adults respond to predation threats later in life.

This experiment investigated the influence of sex and early life experience of predation and conspecific density on the responses to potential predators in Trinidadian guppies *Poecilia reticulata* Peters 1859. *Poecilia reticulata* fry of both sexes were reared in unique combinations of predation cues (present or absent) and social density (high or standard) in a 2 x 2 factorial design. The shoaling and approach response of these adults to a non-predatory and a predatory fish was recorded. Because of the asymmetry in the costs of reproduction and high sexual dimorphism, male *P. reticulata* generally adopt more risky behaviours than females, apparent in their response to predators (Magurran, 2005). Thus, females are expected to shoal more and stay further away from a predator than males, regardless of early life conditions. In their natural habitat, *P. reticulata* also experience different ecological conditions, and fish from high predation habitats shoal more and inspect predators more often but stay further away from predators (Magurran & Seghers, 1990). If early life experiences are sufficient to trigger the expression of appropriate anti-predator behaviours, manipulating early life experience of simulated predation risk should produce similar phenotypes to *P. reticulata* from high predation environments in the wild (i.e., high shoaling and staying distant from a predator). Moreover, if early life experience of predation cues can trigger a generalized neophobic response to novel potential threats (Brown et al. 2013), fish with early life experience of predation cues may also display predator responses when exposed to a novel non-predatory fish, compared to fish without early life experience of predation cues. Early life experience of high density environments where resources are limited can prompt competitive interactions between adults (Magurran & Seghers 1990; Olla et al. 1998; Herczeg et al. 2016; Fischer et al. 2017), potentially resulting in similar behavioural phenotype to low predation environments (i.e. low shoaling, approaching predators more closely).

METHODS

ANIMAL SUBJECTS

The subjects were the same fish tested in Chapters 3 and 4 and were also tested at about 50 days (Leris 2016). Early-life manipulations and rearing conditions are described in Chapter 3. Fish were tested at ~250 days of age.

PREDATOR RESPONSE TEST

The predator response test took place in one of two identical 115 l testing aquaria (90 cm x 46 cm x 33 cm) filled with 19 cm of conditioned water, and lined with beige gravel. Black lines drawn on a plastic sheet placed on top of the gravel allowed the visual division of the aquaria into 18 zones of 5 cm, each approximately 2 guppy body lengths (figure 5.1). The aquaria were fitted with removable opaque partitions (A, B, C, D). Partitions A and B could be inserted between zones 5 and 6 (figure 5.1) and served to create a central section of the aquaria. Partitions C and D served to visually isolate 18 l tanks that were placed adjacent to the testing aquaria, and in which resided either the non-predatory or the predatory stimulus fish. There were two identical testing arenas, each using different stimulus fish, so that subjects were not exposed to the same individual stimulus fish they had experienced during early-life (for example, during *Crenicichla* exposure, they were exposed to a different *Crenicichla* individual to the one they had experienced in early life). This was done to reduce any potential effect of the individual identity of the stimulus fish on subject behaviour. A GoPro camera (1080p at 30 fps, GoPro 3 Black Edition, San Mateo, California) was placed above the tank to record subjects' positions. All *P. reticulata* from the same housing tank were tested on the same day.

Same sex pairs were tested on their response to a visually exposed stimulus fish. Subjects were tested in pairs to avoid any isolation stress and to reflect the fact that guppies typically group in the wild (Magurran 2005), while keeping the sample size required reasonable. The *Pterygoplichthys* (henceforth 'non-predator') was used as a non-predatory but similar-sized control fish. Subjects were captured from their housing tank with a transparent plastic cup, and placed into a transparent cylinder of 10 cm diameter in the middle of the testing aquarium. The cylinder was gently lifted to release the fish into the experimental aquaria. A test started with a 3 minute 'exploration phase' where fish could explore the entire tank, with partitions A and B lifted. This initial exploration phase was followed by a 5 minute pause, where fish were left to swim in the central area with partitions A and B lowered. Then, fish were replaced in the centre with the cylinder and the partitions C and A were lifted, revealing the adjacent non-predator. The fish were then released for the 'non-predatory stimulus fish phase' and their behaviour was recorded for 10 minutes. After ten minutes, fish were returned to the middle section (with partition A and B inserted) for 5 minutes. Then, partitions B and D were lifted, revealing the adjacent *Crenicichla* (henceforth 'predator'), and fish were released for the 'predatory stimulus fish phase' and their behaviour was recorded for 10 minutes. We anticipated that the predatory stimulus fish would be a greater stressor than the non-predatory stimulus fish. The non-predatory stimulus fish was thus always presented before the predatory stimulus fish to keep the order of testing constant while reducing the probability of carryover stress from the visual presentation of the predatory stimulus fish.

Behaviour was recorded using a focal interval sampling method (Altmann 2009; Rose et al. 2016; Dawkins 2007). Every 10 s while a stimulus fish was exposed, the subjects' positions were recorded, for a total of 60 observations per phase. The measures analyzed were approach distance, the distance from the stimulus fish, and whether paired individuals were shoaling together, i.e. in close proximity (within two body lengths; Chapman et al. 2008). Approach distance was estimated with two measures, first by taking approach distance of the fish that was the closest to the exposed fish, and second, by taking the mean of the approach distance of the subject pair from the stimulus fish. We did not count predator inspection events (sensu Magurran & Seghers 1994). Overall, 41 pairs of females, and 39 pairs of males were tested; 13 female and 8 male pairs were exposed to standard density housing during early life, 25 female and 33 male pairs exposed to high density housing during early life, 19 female and 23 male pairs exposed to no predation cues during early life, and 20 female and 18 male pairs exposed to predation cues during early life.

STATISTICAL ANALYSIS

The measures were analyzed using mixed effects linear models (GLMM and LMM), accounting for the fact that repeated measures were made on subject pairs, modelled with the packages lme4 (Bates et al. 2015), and lmerTest (Kuznetsova et al. 2015) in R v3.2.2 (R Core Team 2015). Graphs were created using the package ggplot2 (Wickham 2016).

Approach distance was analyzed using a normal distribution, after a square root transformation to normalize the errors. The proportion of time spent together was analysed with a binomial distribution. Analyses were conducted in three steps. First, in separate models for each sex, the effect of the mean distance to the predator or non-predator exposed on time spent together was examined. This was performed because the behavioural measures might be correlated, for example if fish tend to group together when they are near the exposed stimulus fish. The models contained the response variable ‘time spent together,’ and the fixed effect ‘subject in zone 1’, which is whether a fish was in the zone closest to the exposed stimulus fish. Second, sex differences in mean distance and time spent together were examined, with a model containing the fixed effects “sex”, “stimulus fish exposed” and their interaction. Third, the effect of early life experiences of “social density”, and “predation cues” treatments were examined. As the sample size was insufficient (less than five of each sex for the combinations with low density) to investigate the combined effects of the two early life manipulations together, the effect of each was analyzed separately. The overall models thus contained the fixed effects “sex”, “stimulus fish exposed” and either early life experience of “predation cue” or “social density”, with all interactions. All models also included the random effects “time” and “pair identity” to account for repeated measures, as well as “tank” to account for housing tank effects. When the response variable was binomial, the models also included an observation-level random effect to correct for overdispersion when needed (Harrison 2015). We expected sex differences based on the high sexual dimorphism in this species (Magurran, 2005) and previous experiments (Chapter 4; Leris 2016; Kelley & Magurran 2003). Thus, whenever the two or three way interactions between the early life experience conditions factor (either early life experience of “social density” or “predation cues”) and “sex”, were significant, the analysis was split further by sex. Post-hoc analyses using mixed effects models with a subset of the data were ran as needed when interactions were significant. The tables present the fitted models thus the estimates presented on each row represent the difference from the intercept, calculated for the reference levels, caused by changing the level of that factor. Thus, when an interaction is significant, the effect of a binomial factor should be interpreted as its effect at the reference levels for the other factors.

RESULTS

In females, who shoaled together on average 67% of the time, approach zone did not significantly affect time together ($P > 0.1$; table 5.1), with no significant effect of whether the predator or non-predator was exposed ($P > 0.1$; interaction $P > 0.1$; table 5.1). In males, who shoaled together on average 55% of the time, fish spent more time together when the predator was exposed versus the non-predator ($P < 0.001$; table 5.1) and when in the closest zone to the stimulus fish ($P < 0.001$; table 5.1). In males, there was a also significant interaction effect between the type of stimulus fish and being close to the stimulus fish on time spent together ($P < 0.001$; table 5.1). All fish approached the predator and non-predator at least once, entering the closest zone to it. In females, at least one fish was in the closest zone 32% of the time the predator was exposed, and 37% when the non-predator was exposed, while for male pairs these figures were 54% and 60%, respectively.

SEX DIFFERENCES IN RESPONSES

When we pooled all subjects, both sexes stayed further away from the predator than the non-predator regardless of sex ($P < 0.001$; table 5.2), and females stayed significantly further away from the stimuli fish than males (*P* < 0.001; table 5.2). There was no significant interaction between stimulus fish type and sex on approach distance (interaction *P* > 0.05). These mean approach distance results were similar when the distance of the closest fish to the stimulus fish was analysed (table 5.2). Female pairs spent significantly more time together than male pairs when the non-predator was exposed (*P* < 0.005; table 5.2). There was no significant effect of the stimulus fish type in females on time spent together (*P* > 0.05). There was a significant interaction between sex and stimulus fish (*P* < 0.05). Post-hoc analyses revealed that males spent more time together when the predator was exposed compared to the non-predator (*post-hoc P* < 0.01). While these analyses indicate overall differences between sexes, there were considerable differences depending on the early-life treatment, as analysed below.

EFFECTS OF EARLY LIFE EXPERIENCE OF PREDATION CUES

There were significant interactions between the effects of early-life experience of predation cues and sex, and between the effects of early life experience of predation cues, sex, and fish exposed on the mean approach distance to the exposed stimulus fish (table S5.1). Analysis of the effect of early life experience of predation on mean approach distance was thus separated by sex (table 5.3). Female pairs with early life experience of predation cues stayed further away from the predator compared to the non-predator (*post-hoc P* < 0.0001; interaction *P* < 0.005), while this was not the case in female pairs with no early life experience of predation (*P* > 0.05; table 5.3; figure 5.2a). The results were different when analysing the approach distance of the closest fish: in females, the closest fish stayed further from the predator than the non-predator *(P* < 0.01*),* with no interaction between early life experience and stimulus fish type (interaction *P* > 0.1).

In male pairs, there was no significant effect of early life experience of predation cues when the non-predatory fish was exposed (table 5.3; *P* > 01) and there was no effect of the stimulus fish exposed on fish with no early life experience of predation cues (*P* > 0.05). There was a significant interaction between stimulus fish exposed and early life experience of predation cues (*P* < 0.0001), and males with early life experience of predation cues stayed significantly further away when the predator was exposed than when the non-predator was (*post-hoc P* < 0.001). When analysing the distance of the closest fish, results were the same as for the mean distance of male pairs (table 5.3).

There were no significant effects or interactions of early-life experience with predation cues on time spent together (table S5.1; figure 5.2b) although there was again a significant interaction effect between sex and stimulus fish exposed ($P < 0.01$) as presented in the *sex differences in responses* section (above).

EFFECTS OF EARLY LIFE SOCIAL DENSITY

There were significant interactions between the effect of early life experiences of social density, sex, and fish exposed on the mean approach distance and on the proportion of time spent together (table S5.2). The analysis of the effect of early-life rearing density on approach distance and time spent together were thus separated by sex. Female pairs with early life experience of high social density stayed further away from the predator than from the non-predator ($P < 0.0001$; table 5.4; figure 5.3a). There was a significant interaction between early life experiences of social density and stimulus fish ($P <0.0001$), with female pairs with early experience of standard social density staying further away from the non-predator than the predator (*post-hoc* $P < 0.001$). The same result was found when analysing the distance of the closest fish. Early life experiences of social density did not affect the proportion of time females spent together (table 5.4; figure 5.3b).

Within males, only pairs with early life experience of standard social density stayed further away from the predator than from the non-predator (*post-hoc* $P < 0.0001$; interaction $P < 0.0001$; table 5.4; figure 5.3a). This effect was also found when analysing the distance of the closest fish. However, the closest fish distance was smaller for male pairs with early life experience of standard social density than of high social density when exposed to the non-predator ($P < 0.05$). There was a significant interaction between stimulus fish and density ($P < 0.0001$), as for males with early experience of standard social density, the closest fish approached the predator closer than the non-predator (*post-hoc* $P < 0.0001$). Males with early life experience of standard social density spent less time together than males with early life experience of high social density ($P < 0.01$; table 5.4; figure 5.3b), and there was a significant interaction between early life experience and stimulus fish exposed ($P < 0.001$). Male pairs with early life experience of standard social density spent more time together when exposed to the predatory than the non-predatory fish (*post-hoc* $P < 0.001$).

DISCUSSION

In this experiment, *Poecilia reticulata* females spent more time together and stayed further away from the stimulus fish than did males. Early life experience with predators prompted both males and females to distinguish between a predatory and non-predatory stimulus fish: pairs of both sexes stayed further from the predator than non-predator, while no such discrimination was observed in fish with no early life experience of predator cues. Early life rearing density also had effects on response to the predator and non-predator, but the effects were different in the two sexes. In females, fish reared at high density in early life stayed further from the predator than the non-predator, while the opposite pattern was observed in fish reared at standard density. In males, fish reared at high density in early life did not show strong differences in responses between the predator and non-predator, while fish reared at standard density stayed further from the predator than the non-predator and spent more time together when exposed to the predator. Males also spent more time together if they had been reared at high versus standard density.

These results demonstrate that *Poecilia reticulata* of both sexes could distinguish between the predatory and the non-predatory fish when they had early life experience with predation cues. These findings are similar to the "cautious" behaviour observed in *P. reticulata* from high predation environments or recently exposed to high predation cues (Dugatkin & Godin 1992; Krause & Liesenjohann 2012), and to effects of early exposure to predation cues on subsequent responses (Herczeg et al. 2016). There is evidence that there are distinguishing features of predatory fish, like the distance between the eyes and the size of their mouth (Karplus & Algom 1981), as well as the specific movement patterns (Brown & Warburton 1997). The fact that fish stayed further away from the predatory than the non-predatory fish based on their previous experience suggests that predator responses are at least partly dependent on previous experience (Ferrari et al. 2005; Brown & Godin 1999). It is likely that fish learn from association between the early-life cues of the alarm substance and/or predator faeces, which many species of fish are predisposed to respond to (Brown & Godin 1999), with the visual features of the predator, producing a long-lasting response well into adulthood. Alternatively, it could be that recurrent exposure to predator features (visual or olfactory) during early life, as in this experiment, sensitised fish to those features potentially through the activation of the stress axis (Adamo & McKee 2017).

Regardless of the mechanisms, those results show that previous experience with cues of a predator, even restricted to early life, is enough to induce behavioural changes in adults in response to that predator.

An alternative explanation for the observed differences in response to the predator and non-predator is that they are an artifact of the order of presentation. We always presented the non-predator before the predator, and thus responses to the predator could be due to it being the second-presented fish rather than a predator per se. If this explanation is correct, early-life predator cues increase sensitization to repeated exposures to large fish. We feel this explanation it is unlikely, however. For example, guppies from high predation sites habituate more, not less, rapidly to repeated stressors when compared to guppies from low predation sites (Fischer et al. 2014, see also Chapter 4), which would lead to the prediction that repeated exposures to large fish would cause habituation, and that this would be strongest in predator-exposed fish. This is not what we found.

Female *P. reticulata* stayed together more and further away from any exposed fish than males. Consistent with the results presented here, Magurran & Macias Garcia (2000) found that female guppies, compared to males, performed more predator inspections, stayed further away from predators on average, and devoted more time to anti-predator responses such as shoaling, while our previous work has found that males were physiologically less reactive to stressors (Chapter 4). These results are consistent with a risk-averse strategy in females compared to males. Although males approached the exposed fish more closely than did females, both sexes approached both the predator and non-predator, consistent with them exhibiting predator inspection, rather than remaining as far away as possible. Predator inspection is risky and can increase mortality (Dugatkin & Godin 1992), but can provide valuable information about the behaviour of the predator and the need for escape (Magurran & Macias Garcia, 2000). Staying further away is particularly beneficial for female *P. reticulata* (Magurran & Nowak 1991), which are larger and thus more profitable prey than males when caught, and have a high reproductive investment through livebearing (Magurran & Nowak 1991; Clutton-Brock 1991). Given the difference in reproductive investment between males and females, it has been hypothesized that females should invest more than males in defence against and learning about predators (Kelley & Magurran 2003).

The findings of the present study are broadly consistent with this view, with enhanced antipredator responses in females, although we did not see evidence that females learned more about predators in early life than males.

Experience with predation was expected to increase shoaling during the test, but found no evidence for this. Increasing shoaling tendencies when facing a predator is an efficient strategy to reduce risk (Magurran & Pitcher, 1987), and population differences in *P. reticulata* shoaling correlate with increased predation risk in the wild (Magurran & Seghers, 1990). Since shoaling tendencies in females were also not significantly affected by early life rearing density, contrary to previous work (Chapman et al. 2008; Olla et al. 1998), or by current exposure to a predator versus non-predator, it is possible that female shoaling tendencies are already at a plateau, masking the effects of early life or current conditions, that shoaling tendencies in females represent genetic predispositions with little environmental influence, or that our test was not sensitive enough to detect effects on shoaling. Previous work has found changes in female *P. reticulata* shoaling in response to both current and past predation threat (Brown et al. 2009; Kelley & Magurran 2003). In contrast to females, males (although only those reared at standard density) shoaled more when exposed to a predator versus a non-predator, akin to previous findings (Swaney et al. 2015).

Males reared in high density spent more time together than when reared in standard density. This result contrasts with work finding that high conspecific density reduces shoaling tendencies in *P. reticulata* (Chapman et al. 2008) and in other species (Olla et al. 1998), arguably due to competition for resources. Our finding might be explained by the fact that fish were fed *ad libitum*, so that there was little competition for food. Alternatively, it is possible that the decrease in housing density that high-density reared fish experienced (all fish were transferred to standard density housing after early life, and thus only high-density fish experienced a density change) caused an increase in shoaling tendencies. Rearing density also had varied effects on predator mean approach distance: females reared at high density and males reared at standard density stayed more distant from the predator than the non-predator, whereas females reared at standard density approached the predator more closely than the non-predator. Again, it is possible that the behaviour in females guppies reared in high density is due to a change in density rather than density itself (Kotrschal &

Taborsky 2010). However, rearing density conditions affected the sexes differently, potentially as the result of sex differences in competition for food and mates.

It was not possible to investigate the combined effects of predation and rearing density in early life, due to a limited sample size. However, social environment is expected to affect how predation impacts behavioural development. First, when many individuals are present in a group, it is more likely that the predators will be spotted through a many-eyes effect (Lima 1995), and the response of many individuals might multiply the perceived threat through social contagion (Giacomini et al. 2015). Second, a high social density might result in increased competition for resources. This competition might influence the trade-offs between responding to predators and foraging, changing how individuals react when exposed to a predator (Clinchy et al. 2004; Sogard 1997). This experiment cannot speak to whether developmental conditions have additive or multiplicative effects, and future studies could usefully address this. Our previous work has demonstrated that in female *P. reticulata*, the early life combination of high density and predation have multiplicative effects, and produce longer lasting reactions to stressors within an individual's lifetime (Chapter 4). If physiological and behavioural responses align, as is expected (Archard et al. 2012), females with this combination of experiences would be expected to stay most distant from predators and to show the highest shoaling tendencies.

The results of the current study also highlight that for the sex with the highest reproductive investment (here, females), shoaling is probably a crucial behavioural response regardless of conditions, highlighting the multiple benefits provided by grouping tendencies (Krause & Ruxton 2002). Even when restricted to early life, experience with a predatory fish induced within lifetime long-lasting behavioural changes when fish were exposed to a predator versus a harmless but similarly sized fish, suggesting that experiential effects are a crucial part of developing predator recognition in *P. reticulata*, as is the case in many fish (Kelley & Magurran 2003b). It is possible that the changes in predator recognition observed in the present study might be generalised to other predator species with similar visual features or olfactory cues. In *P. phoxinus,* acquired recognition of olfactory cues from a predator through pairing with alarm substance carries over to other closely related predators (Ferrari et al. 2007), but not distant ones. Investigating more closely whether fish can distinguish between predatory and non-predatory species, or if the effect of experience is restricted to similar species is important to understand when, how and why prey species generalize predator responses, an important part of dealing with novel threats such as invasive predators.

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TABLES

Table 5.1: Effect of being close to the exposed predator or non-predator on time subjects spent together. Estimates and standard errors of fixed parameters for the binomial generalized linear mixed effect model (GLMM) with response variable time spent together for females (top) and males (bottom). Fixed factors included whether fish were in the zone closest to the exposed fish, and stimulus fish exposed. Reference levels were all zones away from the exposed fish, and the non-predatory *Pterygoplichthys* for stimulus fish. The model estimates represent the difference between the level indicated in parenthesis and reference levels. The models also included the random effects pair ID, trial number, and housing tank. Significant p-values (<0.05) are indicated in bold.

	Time together			
parameter	**estimate**	**s.e.**	***z*-value**	***p*-value**
FEMALES				
Intercept	0.86	0.21	4.017	**<0.0001**
Stimulus fish (predator)	-0.057	0.088	-0.65	0.51
Zone (close)	0.14	0.11	1.27	0.20
Stimulus fish * zone	0.031	0.16	0.19	0.85
MALES				
Intercept	-0.17	0.19	-0.87	0.38
Stimulus fish (predator)	0.36	0.10	3.53	**<0.0001**
Zone (close)	0.41	0.10	4.095	**<0.0001**
Stimulus fish * zone	-0.28	0.14	-2.085	**<0.0001**

Table 5.2: Effect of sex and stimulus fish exposed on mean approach distance (top), approach distance of the closest fish (middle), and time spent together (bottom). Estimates and standard errors of fixed parameters for the linear mixed effects model (LMM) with response variable average distance of pairs or of the closest fish and the generalized mixed effects model (GLMM) with the response variable time spent together. Fixed factors included sex of the pair, and stimulus fish exposed. Reference levels were females for sex, and the non-predatory *Pterygoplichthys* for stimulus fish. The model estimates represent the difference between the level indicated in parenthesis and reference levels. The models also included the random effects pair ID, trial number, and housing tank. Significant p-values (<0.05) are indicated in bold.

parameter	**estimate**	**s.e.**	**d.f.**	***t*-value**	***p*-value**
Mean approach distance of pairs					
Intercept	2.30	0.11	53	21.53	**<0.0001**
Sex (males)	-0.37	0.10	68	3.64	**<0.0001**
Stimulus fish (predator)	0.093	0.022	9590	4.20	**<0.0001**
Sex * stimulus fish	-0.0044	0.031	9580	-0.14	0.89
Approach distance of closest fish					
Intercept	2.15	0.11	56	20.38	**<0.0001**
Sex (males)	-0.45	0.10	68	-4.48	**<0.0001**
Stimulus fish (predator)	0.12	0.023	9590	5.38	**<0.0001**
Sex * stimulus fish	0.0076	0.031	9580	0.25	0.81
Time spent together				***z*-value**	
Intercept	0.85	0.19		4.48	**<0.0001**
Sex (males)	-0.77	0.26		2.98	**0.0029**
Stimulus fish (predator)	-0.055	0.069		0.80	0.43
Sex * stimulus fish	0.23	0.093		2.49	**0.013**

Table 5.3: Effect of early experience of predation on mean approach distance (top) and approach distance of the closest fish (bottom). Estimates and standard errors of fixed parameters for the LMM for females (left) and males (right). Fixed factor included experience of predation, and stimulus fish exposed. Reference levels were no predation cues for predation experience, and the non-predatory *Pterygoplichthys* for stimulus fish. The model estimates represent the difference between the level indicated in parenthesis and reference levels. The models also included the random effects pair ID, trial number, and housing tank. Significant p-values (<0.05) are indicated in bold.

	FEMALES					**MALES**				
parameter	**estimate**	**s.e.**	**d.f.**	***t*-value**	***p*-value**	**estimate**	**s.e.**	**d.f.**	***t*-value**	***p*-value**
Mean approach distance of pairs										
Intercept	2.23	0.15	22	14.73	**<0.0001**	1.98	0.11	66	17.9	**<0.0001**
Stimulus fish (predator)	0.025	0.032	4600	0.79	0.43	-0.041	0.027	4901	-1.53	0.13
Predation cues (predation)	0.16	0.21	20	0.75	0.46	-0.25	0.15	43	-1.69	0.098
Stimulus fish * predation cues	0.14	0.046	4610	3.081	**0.0021**	0.30	0.041	4900	7.19	**<0.0001**
Approach distance of closest fish										
Intercept	2.02	0.15	22	13.31	**<0.0001**	1.75	0.10	75	17.22	**<0.0001**
Stimulus fish (predator)	0.096	0.033	4603	2.92	**0.0035**	0.029	0.027	4911	1.06	0.29
Predation cues (predation)	0.26	0.21	20	1.23	0.23	-0.25	0.13	44	-1.90	0.064
Stimulus fish * predation cues	0.053	0.047	4608	1.12	0.26	0.23	0.041	4909	5.49	**<0.0001**

Table 5.4: Effect of early experience of social density on mean approach distance (top), approach distance of the closest fish (middle), and time spent together (bottom). Estimates and standard errors of fixed parameters for the LMM, and the GLMM with response variable time spent together for females (left) and males (right). Fixed factor included rearing social density, and stimulus fish exposed. Reference levels were high for social density, and the non-predatory *Pterygoplichthys* for stimulus fish. The model estimates represent the difference between the level indicated in parenthesis and reference levels. The models also included the random effects pair ID, trial number, and housing tank. Significant p-values (<0.05) are indicated in bold.

	Females					**Males**				
parameter	**estimate**	**s.e.**	**d.f.**	***t*-value**	***p*-value**	**estimate**	**s.e.**	**d.f.**	***t*-value**	***p*-value**
Mean approach distance of pairs										
Intercept	2.32	0.13	27	17.64	**<0.0001**	1.89	0.10	23	18.89	**<0.0001**
Stimulus fish (predator)	0.22	0.028	4614	7.72	**<0.0001**	0.00099	0.023	4910	0.043	0.97
Density (standard)	-0.067	0.23	20	-0.28	0.78	-0.092	0.19	14	-0.49	0.63
Stimulus fish * density	-0.35	0.047	4604	-7.44	**<0.0001**	0.39	0.049	4910	7.91	**<0.0001**
Approach distance of closest fish										
Intercept	2.17	0.13	27	16.13	**<0.0001**	1.72	0.092	83	18.74	**<0.0001**
Stimulus fish (predator)	0.24	0.029	461	8.28	**<0.0001**	0.0091	0.023	4910	0.40	0.69
Density (standard)	-0.086	0.24	20	-0.36	0.723	-0.36	0.16	14	-2.26	**<0.05**
Stimulus fish * density	-0.34	0.049	460	-6.92	**<0.0001**	0.53	0.049	4906	10.90	**<0.0001**
Time spent together				***z*-value**					***z*-value**	
Intercept	0.93	0.25		3.77	**<0.001**	0.36	0.19		1.91	0.057
Stimulus fish (predator)	0.035	0.089		0.39	0.69	0.063	0.070		0.89	0.37
Density (standard)	-0.089	0.43		0.21	0.84	-1.31	0.41		3.22	**<0.01**
Stimulus fish * density	-0.23	0.14		1.61	0.11	0.52	0.15		3.41	**<0.001**

Table S5.1: Effect of early experience of predation. Estimates and standard errors of fixed parameters for the LMM with response variable average distance (top) and the GLMM with response variable time spent together (bottom). Fixed factors included sex of the pairs, experience of predation, and stimulus fish exposed. Reference levels were females for sex, no predation cues for predation experience, and the non-predatory *Pterygoplichthys* for stimulus fish. The model estimates represent the difference between the level indicated in parenthesis and reference levels. The models also included the random effects pair ID, trial number, and housing tank. Significant p-values (<0.05) are indicated in bold.

parameter	**estimate**	**s.e.**	**d.f.**	***t*-value**	***p*-value**
Mean approach distance of pairs					
Intercept	2.22	0.14	46	15.71	**<0.0001**
Sex (males)	-0.18	0.14	68	1.32	0.19
Stimulus fish (predator)	0.025	0.032	9578	0.81	0.432
Predation cues (predation)	0.16	0.19	36	0.85	0.40
Sex * stimulus fish	-0.064	0.043	9574	1.55	0.12
Sex *predation cues	-0.42	0.20	66	2.085	**<0.05**
Stimulus fish *predation cues	0.14	0.044	9588	3.18	**<0.01**
Sex * stimulus fish * predation cues	0.15	0.061	9578	2.52	**<0.05**
Time spent together				z-value	
Intercept	0.76	0.25		2.97	**<0.01**
Sex (males)	-0.54	0.33		1.61	0.11
Stimulus fish (predator)	-0.033	0.095		0.35	0.72
Predation cues (predation)	0.19	0.36		0.53	0.60
Sex * stimulus fish	0.34	0.13		2.71	**<0.01**
Sex *predation cues	-0.53	0.49		1.08	0.28
Stimulus fish *predation cues	-0.044	0.14		0.32	0.75
Sex * stimulus fish * predation cues	-0.25	0.19		1.36	0.17

Table S5.2: Effect of early experience of social density. Estimates and standard errors of fixed parameters for the LMM with response variable average distance (top), and the GLMM with response variable time spent together (bottom). Fixed factor included sex of the pairs, rearing social density, and stimulus fish exposed. Reference levels were females for sex, high for social density, and the non-predatory *Pterygoplichthys* for stimulus fish. The model estimates represent the difference between the level indicated in parenthesis and reference levels. The models also included the random effects pair ID, trial number, and housing tank. Significant p-values (<0.05) are indicated in bold.

parameter	**estimate**	**s.e.**	**d.f.**	***t*-value**	***p*-value**
Mean approach distance					
Intercept	2.32	0.13	48	18.48	**<0.0001**
Sex (males)	-0.38	0.11	64	3.34	**<0.01**
Stimulus fish (predator)	0.22	0.027	9601	7.92	**<0.0001**
Social density (standard)	-0.073	0.22	31	0.33	0.74
Sex * stimulus fish	-0.21	0.036	9588	5.89	**<0.0001**
Sex * social density	0.093	0.23	68	0.41	0.68
Stimulus fish *social density	-0.35	0.046	9578	7.64	**<0.0001**
Sex * stimulus fish * social density	0.74	0.068	9570	10.78	**<0.0001**
Time spent together				z-value	
Intercept	0.85	0.21		4.06	**<0.0001**
Sex (males)	-0.49	0.28		1.76	0.078
Stimulus fish (predator)	0.036	0.089		0.40	0.69
Social density (standard)	-0.077	0.36		0.21	0.83
Sex * stimulus fish	0.030	0.11		0.27	0.79
Sex * social density	-1.24	0.54		2.29	**<0.05**
Stimulus fish *social density	-0.23	0.14		1.61	0.11
Sex * stimulus fish * social density	0.75	0.21		3.59	**<0.001**

FIGURES

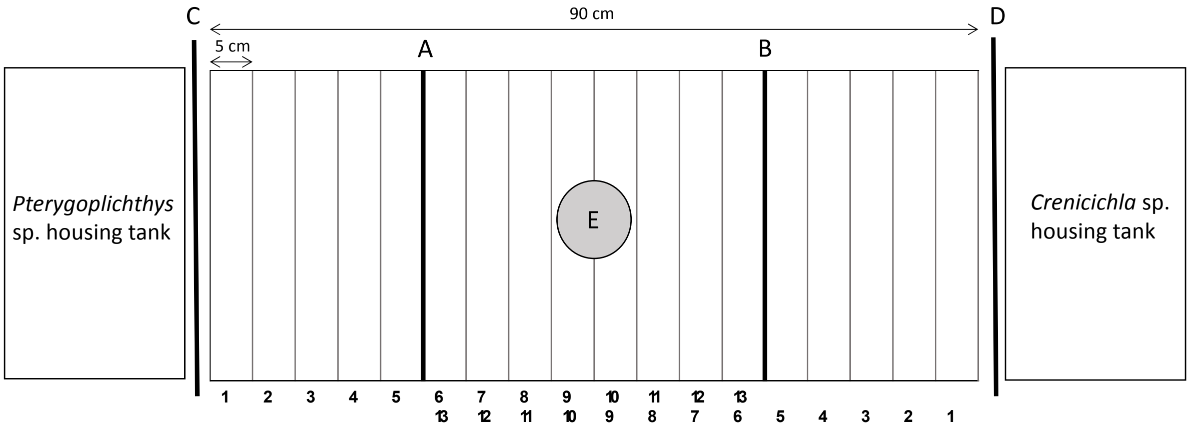

Figure 5.1: Testing tank and stimulus fish housing tanks. The partitions A and C were removed to expose the non-predatory *Pterygoplichthys* sp. fish, while partitions B and D were removed to expose the predatory *Crenicichla* sp. fish. Cylinder E was used to release subject *P. reticulata* pairs; thus fish were 9.5 zones (47.5 cm) from the stimulus fish at release. The zones were numbered from the exposed fish to the remaining partition, for a total of 13 zones of 5 cm during each trial.

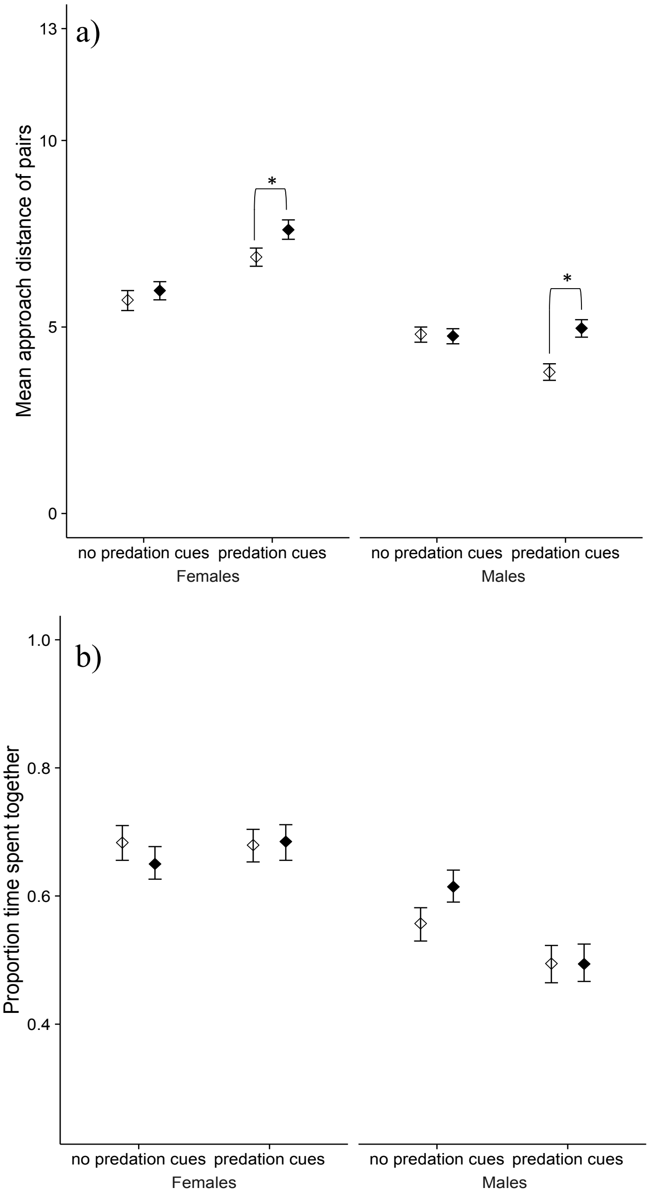

Figure 5.2: Effect of early experience of predation during development on a) the mean approach distance and b) time spent together of pairs of female and pairs of male *P. reticulata* to a non-predatory *Pterygoplichthys* sp. fish ('non-predator'; open points) or a predatory *Crenicichla* sp. fish ('predator'; filled points), exposed sequentially in two tests. Approach distance is measured in 5-cm zones from the stimulus fish, and time spent together is whether subjects were within two body lengths of each other. Tests began with subjects 9.5 zones from the stimulus fish. Any asterisks indicate significant differences of p<0.05 in response to the predator versus non-predator, based on fitted models (table 5.3) or *post-hoc* analyses.

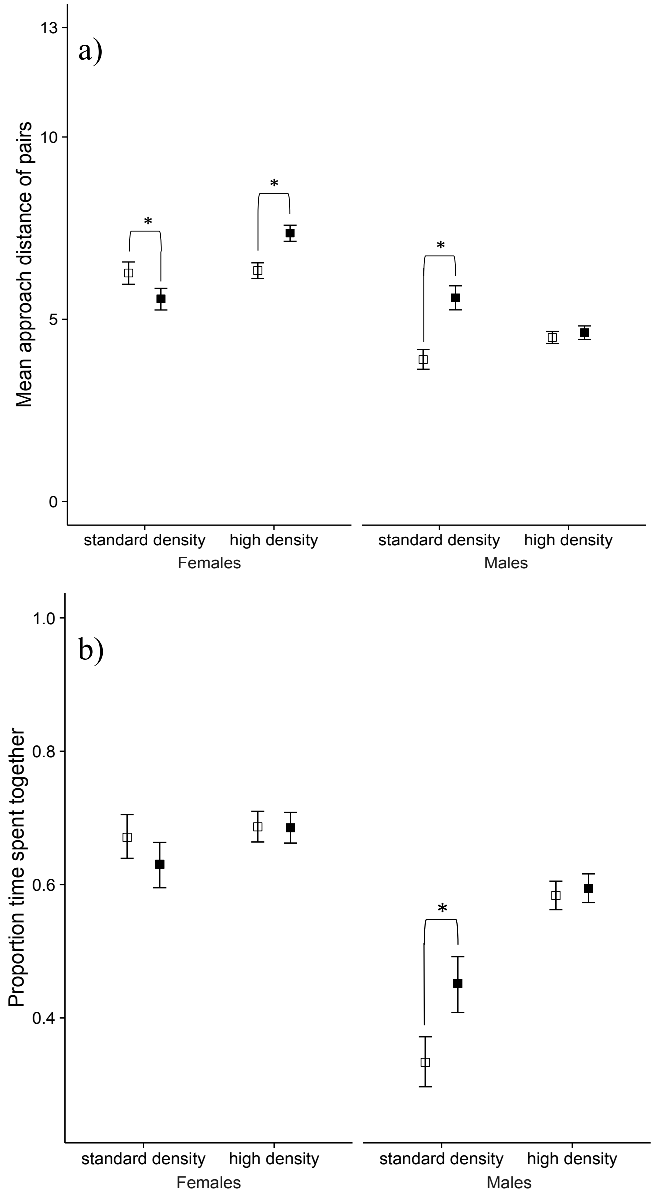

Figure 5.3: Effect of early experience of social density during development on a) the mean approach distance and b) time spent together of pairs of female and pairs of male *P. reticulata* to a non-predatory *Pterygoplichthys* sp. fish ('non-predator'; open points) or a predatory *Crenicichla* sp. fish ('predator'; filled points), exposed sequentially in two tests. Approach distance is measured in 5-cm zones from the stimulus fish, and time spent together is whether subjects were within two body lengths of each other. Tests began with subjects 9.5 zones from the stimulus fish. Any asterisks indicate significant differences ($p<0.05$) in response to the predator versus non-predator, based on fitted models (table 5.4) or *post-hoc* analyses.

CHAPTER 6: GENERAL DISCUSSION

In this general discussion, I synthesize the chapters of this thesis with the existing literature and draw attention to the new questions my thesis brings, and how we might potentially address them. I do so by discussing firstly how social information and social learning propensities are shaped, secondly how developmental conditions affect adult social information use and stress phenotypes, and thirdly how sexes differ. I finish by drawing together the results of my chapters.

INFLUENCES ON SOCIAL INFORMATION USE AND SOCIAL LEARNING PROPENSITIES

In Chapter 2, I investigated whether differences in social information use and social learning existed between wild populations of Trinidadian guppies. This was based on the premise that under conditions of predation, where personal exploration of resources could be costly, copying others would be beneficial, whereas under conditions without predation and where fish display more intraspecific aggression, copying others would be costly. While I found support for these predictions in the Aripo river, fish from the Marianne river did not show evidence of social information use and social learning. The tested fish from the high-predation Lower Aripo population copied the demonstrated location, as predicted by the “costly information hypothesis” (Boyd & Richerson 1985, Laland, 2004). A similar effect has been demonstrated in minnows, who preferred to socially learn when immediate predation risk was high (Webster & Laland, 2008). Fish from the Upper Aripo population avoided where other individuals are seen feeding, a finding that could be explained by social cues reliably indicating the absence of food in the Upper Aripo environment and/or by high intraspecific aggression and low shoaling propensities increasing the costs of foraging with others and decreasing the likelihood that others are attended to. When social learning provides a large enough advantage, the development of specialized social learning abilities could emerge. For example, freshwater ninespine sticklebacks *Pungitius pungitius* are more at risk than threespine sticklebacks *Gasterosteus aculeatus* because of their lack of body armour, and only the ninespine sticklebacks display the ability to learn the relative profitability of foraging patches from the rate at which other individuals are feeding (Coolen et al. 2003; Chouinard-Thuly 2012). Developmental explanations for this difference have been excluded by rearing in common garden conditions (Webster, Chouinard-Thuly, Herczeg, Kitano, Riley, Rogers, Shapiro, Shikano & Laland, *unpublished data*), meaning that evolved differences explain this finding (although given that only two species are compared, it is impossible to determine whether predation risk is causal in driving the species difference). While findings from Chapter 2 showed population differences, it raised the question of whether evolutionary processes shape these differences between guppy populations, or if they are driven by within-lifetime experiential effects.

In Chapter 3, I thus addressed the hypothesis that early life experiences were key in shaping social information use and social learning propensities. The premise behind investigating early life experiences effect is that previous evidence suggests that 1) individuals can learn the values of social cues (Dawson et al. 2013; Leris & Reader 2016), 2) that social learning propensities co-vary with other phenotypic traits that are shaped by experience (Chapman et al., 2008; Kurvers et al., 2010; Rosa, Nguyen, & Dubois, 2012; Trompf & Brown, 2014), 3) that early life stress can affect social information use and social learning strategies (Farine et al. 2015; Boogert et al. 2013; Lindeyer et al. 2013), and 4) that early life experiences can affect the development of a broad suite of social behaviours (Fischer et al. 2015; Weiss et al. 2004; Sih 2011). Results of Chapter 3 provided some evidence that early life experience of the social environment influenced adult social information use strategies. Although we did not find significant differences between groups, only females with early life experience of high social density used social information about foraging locations, copying the choice of other individuals. Contrary to predictions, early-life predation did not affect adult social information use, although its effects were detectable on other behavioural traits (Chapter 5). It is possible that the perceived costs of personal exploration of the environment and thus its impacts on social information use are only sensitive to the current threat of predation. As predation threat varies in time, and since predation pressures on juveniles and adults can differ considerably, many species display a certain amount of plasticity in their antipredator behaviour to adjust to current risk (Brown et al. 2015; Ferrari et al. 2016). That social information use and social learning propensities are only influenced by current or recent threat would explain why we see differences between the field and laboratory studies (Chapters 2 and 3). In the field, predators are present in the locality, increasing the potential costs of obtaining personal information, but in the laboratory study subjects have not experienced predators in their adult life (Chapter 3). There is evidence in bumblebees that individuals can learn the local value of social information (Dawson et al. 2013), which will be affected by the social environment. Consistent with recent literature, our results suggest that the early social environment has noteworthy influence on adult phenotypes.

Taken together, the findings of Chapters 2 and 3 raise questions as to which neuroendocrine systems mediate social information use and social learning propensities, and the extent to which these systems are specialized to process social information. In rats, phenotypic differences induced by maternal style is a well-studied area that has been linked to social learning. For example, only rats that were reared by mothers exhibiting high grooming rates socially learned food preferences (Lindeyer et al. 2013). Lindeyer et al. suggest that low grooming maternal style could impact the development of social interactions, the hippocampus, or the stress axis, which are all known to be affected by maternal style (Liu et al. 2000; Mirescu et al. 2004). The stress axis profile could play a role in shaping how much risk individuals are willing to take and/or their social tendencies, and thus whether they would readily personally explore versus exploiting the discoveries of others (Tudorache et al. 2013). In Japanese quail pre-natal elevated levels of cortisol increase the propensity to copy others (Boogert et al. 2013). The same experiment however demonstrated that post-natal elevated stress had the opposite effect. Contrary to pre-natal stress, Boogert al. (2013) induced post-natal stress not by altering experienced cortisol levels, but by changing the reliability of food availability. This, in addition to the fact that, compared with social density stress, simulated predation did not have a long-lasting effect on social information use propensities, even though it shaped behavioural and hormonal responses to stressors (Chapters 4 and 5), highlights that stressors might have domain-specific effects. In the cricket *Gryllus texensis*, recent research shows that predation cues reduce egg laying, but that direct activation of the ‘fight-or-flight’ response affected body mass and life-span (Adamo & McKee 2017). The authors suggest that the exposure to predation cues activates to a lesser extent the stress axis, inducing different effects on life-history traits in the cricket. They argue that predation cues do not necessarily equal imminent death from a predation event, and thus call for different energy allocation strategies. The rearing social environment has known effects on the stress responses in other species of fish than guppies, particularly in the cooperatively breeding cichlid *Neolamprologus pulcher*, affecting their social competence (Nyman et al. 2018). It is possible that our predation cues, though having measurable effects on the brain (Reddon et al. 2018), did not have similar impacts to social stressors, either because of the context or strength to the stressor. So while the stress axis might mediate responses to social information, not all stressors necessarily have long-lasting effects, and their effect might depend on the strength of the activation, or engage different neuroendocrine, neurological or physiological systems (Sih 2011).

In my Chapter 2 and 3 experiments, observer fish had access to the food reward when other individuals were demonstrating it. This contrasts with the usually adopted social learning design where subjects are physically separated from the apparatus and demonstrators, which creates an observational social learning paradigm (Hoppitt & Laland 2013). We allowed fish to interact with the food reward to simulate ecologically relevant foraging conditions of guppies, which are a highly social species seldom foraging alone, even if groups do vary in size. Social fish spend a lot of time in close proximity, and information diffuses through groups (Laland & Williams, 1997; Magurran & Higham, 1988), and it is thus ecologically important to understand the processes at play in natural conditions. In Chapter 2, I demonstrated that if no demonstrator fish are present, fish do not learn a preference for the previously used patch, leaving no doubt as to the facilitating effect of other individuals on learning, which is the definition of social learning that I follow in my thesis (Hoppitt & Laland 2013). The social learning mechanism involved in my Chapter 2 design is possibly local enhancement, defined as when "a demonstrator attracts an observer to a specific location, which can lead to the observer learning about objects at that location" (Rendell, Fogarty, Hoppitt, et al. 2011). Our experiment produces the same outcome as observational social learning: individuals forage on the same resources that others had previously foraged on. However, it is possible that the neural mechanisms differ. In our example of social learning, the neural mechanisms involved are most likely the social decision-making network, which is composed of the social behaviour network and the reward system (O'Connell & Hofmann 2012). The sight of conspecifics can act as a positive reinforcer, as can the experience of eating food, and engage the brain's reward system (Al-Imari & Gerlai 2008), while grouping behaviour most likely involved the pre-optic area, part of the social behaviour network (Cabrera-Álvarez et al. 2017). In our case, using the non-observational learning paradigm, it is possible that these brain areas suffice to make the association between a location selected by shoaling and the food reward. Whether other cases of social learning require specialised mechanisms is a long-standing debate, though most evidence points to similar neural and psychological mechanisms (Lefebvre & Giraldeau 1996; Reader & Lefebvre 2001; Reader 2016; Chouinard-Thuly & Reader 2014; Heyes 2012; Leadbeater & Chittka 2007).

While questions of social information use or social learning often appeal to researchers on human behaviour due to its consequences for human culture (Mesoudi et al. 2016), I would like to draw attention to the idea that the transmission of information has important implications for ecological communities (Schmidt et al. 2010). Social information use and social learning by predators can affect the evolution of species in other trophic levels, for example by favouring prey clumping (Hamblin et al. 2010), or by allowing the evolution of aposematism through the social transmission of unpalatability (Thorogood et al. 2018). Just as the personality of predators can shape local communities (Start & Gilbert 2017), the dynamics of information transfer could impact lower trophic levels. As demonstrated in Chapters 2 and 3, local conditions can affect how information spreads, and thus how fish from different groups might use their environment differently. Notably, fish form the Upper Aripo population might avoid demonstrated food sources, potentially creating more diverse pressure on lower trophic levels. Social learning processes can be powerful, and the original solution to a food discovery might persist through conformity effects, where individuals have a tendency to disproportionately copy the option used by the majority, even if all options are equally rewarding (Aplin et al. 2015). Processes like these could affect how local populations interact with other species, and might contribute to explaining differences between communities.

THE EFFECTS OF EARLY EXPERIENCE ON ADULT STRESS PHENOTYPES

In Chapter 4, I investigated how early life experiences shaped the development of the stress axis. I provided evidence that the combination of early stressors of high social density and simulated predation risk affected changes in waterborne cortisol release during repeated exposure to a mild stressor in adults. Glucocorticoid hormones, cortisol in fish and most mammals, mediate a wide variety of responses, but are mostly responsible for the attribution of energy between those activities (McEwen & Wingfield 2003). This means that elevated levels will move energy and time attributed to foraging to survival activities, but chronically or extremely elevated levels lead to pathologies and death (Sapolsky et al. 2000). It is therefore imperative to adjust the reactivity of the stress axis to environmental conditions. A popular model is that individuals living in highly stressful environments should display a very reactive profile, characterized by low baseline levels but high acute levels, allowing a wide range of reactions before reaching the threshold, and a quick down-regulation of stress response (Romero et al. 2009). This model was supported in two previous studies in two poeciliid fish; it was demonstrated that both evolutionary and developmental exposure to predation individually decreased 'baseline' cortisol levels in guppies (Fischer et al., 2014), and that low predation populations of *Brachyhaphis episcopi* release increased levels of cortisol after exposure to a mild stressor (Archard et al. 2012). Our results would seem to contrast with these predictions and previous findings, as we did not find any strong overall individual effect of either early-life predation or social density developmental conditions on cortisol release, and because females from the developmental conditions assumed to be the most stressful did not differ from other females in their initial cortisol reaction to a stressor, and did not decrease in cortisol levels between the two phases with a mild stressor. The predictions related to predation also find limited support in the literature, where Robertson et al. (2011) found no differences between high and low predation populations of eastern fence lizards, and Clinchy et al. (2004) find higher levels of cortisol in low predation populations of song sparrows *Melospiza melodica*. Our measures give no indication of baseline levels, and we argue that it is very difficult to predict what would be an optimal profile of the stress axis in very stressful environments, because the changes are regulated not only by the release of hormones, but also by their transport, and the distribution and density of their receptors.

In Chapter 5, I investigated whether early life affected behavioural responses to the sight of a predator and a non-predatory large fish. I provided evidence that fish with previous experience with a predatory fish stayed further away from the predatory than from the non-predatory fish, a reaction termed 'cautious' by some authors (Magurran & Seghers, 1994). In my work, predation risk was simulated in early life by pairing alarm substance and predator odour cue to the visual cue of the predator. Learning is important in the acquisition of the recognition of predators: while the response to alarm substance seems conserved, recognition of specific predators is refined by experience (Brown, Chivers & Smith, 1997), though genetic predispositions might exist (Blumstein et al. 2000). Our results show that early life experiences can promote long lasting recognition of predators, and that fish can accurately visually distinguish learned features of threatening species. We could expect, in high risk environments, that individuals might adopt an overall risk averse strategy (or "neophobic response") to all large fish or novel objects, as has been shown in guppies, cichlids *Amatitlania nigrofasciata*, and wood frog tadpoles *Rana sylvatica* (Brown et al. 2013). It seems however that this neophobic strategy should be most beneficial when predation threat is somewhat variable in intensity (Brown et al. 2015) or when the predator's identity is uncertain (Ferrari et al. 2018). In the developmental conditions, though the predation cues were deliberately presented at random times and with variation in whether alarm cue or alarm cue and predator odour were presented, the risk (as indicated by the concentration of the alarm substance; Ferrari et al. 2016), and the identity of the predator were held constant. As there was little uncertainty in the predation risk, our finding that they respond selectively to the known predator, and not to the novel large fish is consistent with this recent literature. Unfortunately, our final sample size limited investigation of the combination of early life experience of predation and social density in this experiment. Our Chapter 4 and 5 results together demonstrate that sex differences in behavioural and physiological responses are similar, and within females we also observe consistent behavioural and physiological responses to early-life experience. High density or predation cues alone in early life prompted females to stay further away from the predatory than the non-predatory fish, indicative of a highly reactive stress response, while the combination of both decreased habituation as measured by cortisol release. While we did not observe an overall effect of the treatments on cortisol release, this could potentially be due to the fact that we used a very mild and recurrent stressor during cortisol collection instead of the exposure to predation threat itself as used in the predator approach experiment.

Chapters 3, 4 and 5 were part of a larger set of studies investigating the effect of early life experiences on multiple behavioural (exploration, boldness, and shoaling tendencies: Leris, 2016, predator approach: Chapter 5; social learning: chapter 3) and physiological measures (cortisol release: Chouinard-Thuly et al. 2018; brain size: Reddon et al. 2018). Overall, combining these findings, we found that early-life experience of predator cues (compared to no predation cues) 1) increased exploration, for fish reared in standard density, in both sexes, 2) decreased time spent with a shoal, in males, 3) produced long-lasting changes in response to a predator compared to a non-predator, in both sexes, and 4) increased relative brain mass, in males. A high compared to standard rearing density 1) increased the time spent shoaling, in males, 2) decreased the time spent at feeders, in both sexes, and 3) decreased the approach distance to a predatory fish compared to a non-predatory fish, in females. In combination, high rearing density with predation cues 1) increased boldness in both sexes, and 2) decreased habituation in females as measured by cortisol release. In summary, our combined results point to a competitive environment in fish reared in high density, resulting in adults that feed more and shoal less, while predation seems to allow fish to distinguish between a predatory and a non-predatory fish, but did not alone have strong effects on other behavioural traits. Early life environment is known to affect life histories, with predation or other stressors inducing a faster life history (Ball & Baker, 2014; Reznick et al., 2001), but it is difficult to predict how behaviours develop in response to early life environment, especially since the effect of predation can vary depending on the risk level, the number of encounters with predators and their hunting style (Nonacs & Blumstein 2010).

Recently, it has been observed in many animals that some behaviour patterns might be correlated and form a 'syndrome' (Sih et al. 2004). One hypothesis for the existence of such syndromes between traits is the 'constraint hypothesis', that states that when proximate mechanisms underlying traits are shared, selection on one trait could result in changes in another trait (Stamps 1991). Recent studies in sticklebacks have shown that activity, aggression, and boldness, appear correlated in some populations but are uncoupled in other, providing evidence that syndromes might occur only when selected for by local conditions (Bell 2005; Dingemanse et al. 2007). In wild populations of Trinidadian guppies, individuals from high predation environments typically have a faster life history than their low predation counterparts, but display more risk averse behavioural strategies (Magurran & Seghers 1991) and higher stress reactivity (Fischer et al., 2014). Our results align – with complications- with what is found in wild Trinidadian guppies, where individuals exposed to predation are risk-averse, as could be predicted from the aforementioned wild studies but a high social density induces risk taking strategies. It also seems that, as is increasingly supported, social stress (and low food availability) might play a larger role than anticipated in shaping life-histories and behaviours (Arendt & Reznick 2005). While the syndromes literature focuses mostly on selective pressure, some authors note that plasticity might also facilitate the co-development of traits in response to local conditions (Bell & Sih 2007). Our results clearly suggest that developmental plasticity might partially explain differences in the co-development of behavioural traits.

In Chapters 3, 4, and 5, I investigated sex differences in responses to developmental conditions, in the propensity to employ social information and social learning, and on physiological and behavioural responses to stress. I provided evidence that further supports the literature describing male guppies as risk takers, as they react little to mild stressors, spend less time foraging, and stay closer to potential predators (Harris et al. 2010; Magurran & Nowak, 1991a; Piyapong et al., 2009). In Chapter 3, I demonstrated that while males copied the foraging location of female demonstrators, they did not copy foraging options, perhaps being attracted by the presence of potential mates rather than foraging opportunities. Previous studies have provided some evidence that females employ social information more than males (Dugatkin & Godin, 1992; Reader & Laland, 2000), but my results hint at the fact that in males, the use of social information is context-dependent. My results also unequivocally demonstrate that in a sexually dimorphic species, the impact of local conditions is sex-specific, which stresses that research should ideally be carried out in both sexes if we are to understand the species as a whole. In guppies and many other species, males are the sex with low reproductive investment, while females invest more in parental care due to inherent costs of live-bearing. Since female reproductive success depends on their long-term survival and body condition, energy might be directed to favouring longevity, and thus the display of risk averse strategies, devoting more time to foraging and antipredator behaviours (Magurran & Macias Garcia, 2000). Thus, contrary to our results for within sex responses to local conditions, between sex differences show a correlation between fast life histories (males) with risky behaviours. How could different correlations between traits occur at those two levels of variation within the same species? It is possible that the differences we see between sexes is the result of evolutionary processes, while the within sex phenotypes might be more reflective of developmental processes in responses to local conditions, particularly if both sexes experience local conditions differently (Hämäläinen et al. 2018; Immonen et al. 2018).

A limitation with the interpretation of sex differences in guppies is that males and females do not completely overlap in their range of mass, making it challenging to distinguish between body mass and sex effects. While some sex specific characteristics, such as body growth or the development of bright colouration could arise from sex-specific processes like differences in hormone production during development, other phenotypic differences could be the result of mass differences itself, for example if they are linked to physical abilities or body condition (Mathot & Giraldeau 2010). In the future, one way to investigate this question is to experimentally manipulate hormone concentrations, either during growth or before testing. Such experiments have showed that in guppies, parasite resistance increases with a reduction of current levels of male hormones (Dargent et al. 2015), showing a clear effect of sex as opposed to body mass. Another option is to investigate sex differences in related species in which sexes mass overlap, potentially shedding light on the effects of sex versus body mass. In domestic guppies, where mass differences between the sexes are small, it is possible to investigate separately the effect of sex and mass on phenotypes. Such research shows that in the case of the discovery of novel food sources, sex and mass had opposing effects, with females and small fish being the most likely to succeed (Laland & Reader 1999). Understanding how sexes differ in phenotypic traits is important to understand the causes and consequences of such variation, but interpretation or generalisations with sex as the proposed causal variable should be done carefully, especially when the effects of body mass have not been mitigated.

GENERAL CONCLUSIONS

The general aim of my thesis was to investigate when differences in social information use and stress responses occur, and how early life contributes to shaping adult phenotypes. My thesis provided support for the hypotheses that 1) socio-ecological factors can shape social information use and social learning propensities, 2) that social information use and social learning are developmentally plastic, and 3) that sex and early life conditions shape the development of physiological and behavioural stress responses. While my results raise important questions pertaining to the mechanistic causes and consequences of developmental plasticity, they nevertheless highlight that social information use and social learning propensities are influenced by population level processes, and that how individuals choose to respond to information will be shaped by previous as well as current experiences. Moreover, I demonstrated that physiological and behavioural systems are influenced by early life experience of social density and predation cues conditions too. Differences in the effects of early life also indicates that some traits, like brain size and stress profile, may be developmentally plastic but do not retain much plasticity once developed as we see differences in adults even when conditions are the same, while anti-predator behaviours may remain plastic to allow adjustment to rapidly changing threat levels. I also strongly advocate a thorough investigation of both sexes in species with sexual dimorphism, in order to gather a full understanding of how individuals within a species react to changing conditions, and how changing conditions may have different impacts on individuals, or populations, within a species.

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