

EXAMINING COGNITION IN A WILD FISH SPECIES WITH
MULTIPLE MALE PHENOTYPES

M.Sc. Thesis – S.E. Marsh-Rollo; McMaster University - Biology

EXAMINING COGNITION IN A WILD FISH SPECIES WITH MULTIPLE MALE
PHENOTYPES

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A Thesis Submitted to the School of Graduate Studies In Partial Fulfilment of the
Requirements for the Degree Master of Science

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Lay Abstract

Our understanding of the underlying mechanisms and evolution of cognition in animals has been furthered by research on numeracy, social preferences, and personalities in fishes. Research on fish cognition has been conducted mainly on domesticated, lab-acclimated, or lab-based strains of freshwater fish species. My project explores cognition in the context of behavioural plasticity in a wild-living marine fish with three male alternative reproductive tactics: nesting, satellite, and sneaker males. In the field, I used well-established behavioural assays to examine differences in memory, individual recognition, exploration and basic numerical abilities across these three male types. This work contributes to the field of fish cognition by not only adding a wild-living species, but also in a species where we can compare cognition across multiple male phenotypes.

Abstract

Research on numeracy, social preferences, and personalities in fishes has contributed to our understanding of the underlying mechanisms and evolution of cognition in animals. Research on fish cognition has been mainly conducted on domesticated, lab-acclimated, or lab-based strains freshwater fish species and a handful of marine fish species. My project explores cognition, specifically memory and individual recognition, in a wild-living Mediterranean marine fish with male alternative reproductive tactics (ARTs): nesting, satellite, and sneaker males. ARTs in this species change over the lifetime of an individual male, rather than by being determined by a genetic polymorphism. Males change in physiology, gene expression, hormone levels and suites of behaviour as they transition through these phenotypes. During the reproductive season, I captured wild-living fish and ran lab-based forced-choice assays to assess their response to a novel object and conspecifics. In a forced-choice assay for familiar versus unfamiliar conspecific females, I found that, nesting males preferred to spend more time with familiar conspecifics but that satellite males made more side switches, a measure of assessment or exploratory behaviour, than nesting males. A forced-choice assay for a familiar versus unfamiliar object revealed no differences in preferences but focal females were significantly faster to explore than sneaker males. In a group size choice assay, all three male types and focal females preferred to spend time with larger groups of conspecific females. These results show that individual recognition and numerical abilities are present in this species. This work contributes to the field of fish cognition by

highlighting differences in cognition in a wild-living species with multiple male phenotypes.

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To my desk goldfish. You got fatter over the course of writing this thesis. I hope that you are happy you have trained me to feed you a bagillion times a day. We'll see how long it takes to extinguish this mal-adaptive behaviour which was initially consistently rewarded then only rewarded on a time schedule (a possible follow-up thesis?).

During the three months spent writing this MSc thesis I also: Installed a stove, installed a clothes dryer, installed a toilet, did major repairs on the front cement steps, dismantled, repaired and reinstalled a mailbox, supported the development and birth of twin grandchildren and listened to Hamilton on repeat for three months (thank you Lin-Manuel Miranda!).



Dedication

This thesis is dedicated to the children in my life who bring me such joy and give me purpose whether near or far, young or old. My beloved grandchildren Ryder, Chase, Bodhi, Soleia and Ellowyn. My adored nieces and nephews, both biological and honorary, Steve, Jeffrey, Aidan, Nellie, Tyler, Kenna, Ben, Wes, Anna and Sydney. My special young friend and sister from another mother, Nina. My treasured grown children and their very special partners Oriana and Vince, Tynan and Claire, and Jordan and Harlee-Jean.

“How paramount the future is to the present when one is surrounded by children.”

~ Charles Darwin

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Introduction

“One fish, two fish, red fish, blue fish
Black fish, blue fish, old fish, new fish
This one has a little star
This one has a little car.
Say! What a lot of fish there are.
Yes. Some are red.
And some are blue.
Some are old.
And some are new.
Some are sad.
And some are glad
And some are very, very bad!
Why are they sad, and glad and bad?
I do not know.
Go ask your dad.”

~ **Dr. Seuss, “One Fish, Two Fish, Red Fish, Blue Fish”**

Why the field of animal cognition is important and interesting:

Animals face challenges in their environment when the environment changes and they then need to respond adaptively to those changes. Some responses are innate or instinctual, for example, a bear that hibernates for the winter or monarch butterflies that migrate to a wintering ground they have never been to before. Other responses require organisms to process environmental cues before responding adaptively, such as wolves or lions that must learn how to hunt together in a pack. A cognitive process is how an organism collects information from its environment (i.e., through thought, perception, or learning, Shettleworth, 2001). Adjusting an innate or instinctual behaviour in response to information allows an animal to respond appropriately to its environment. Proper responses ensure an organism's survival and ability to reproduce which contributes to its overall fitness. For example, populations of North American white throated sparrows (*Zonotrichia albicollis*) born with an innate singing ability originally for a triplet ending song have had their song dialect shaped over time by learning from tutors at their wintering grounds (Otter et al., 2020).

Just as we study physiology, such as hormones and gene expression, to understand or correlate with an animal's behaviour, studying cognition is yet another means of understanding underlying causal mechanisms of behaviour. Understanding how animals process information and either act upon that info or store the info for future use gives us a framework for predicting or interpreting how animals interact with their environment or other individuals. This then also enables us to compare the differential responses between individuals within a population or across species and even give insights to the evolution

of cognitive processes (Dukas, 2004). Such insight has been applied to the comparison of cognition between apes and corvids. Research on these two groups has suggested they experienced convergent evolution of cognition and employ similar strategies such as manufacturing tools, complex foraging, and problem solving using social cues (Seed et al., 2009).

Recently a growing number of studies have highlighted the importance of studying cognition in the wild or in wild-living organisms (Rosati et al., 2022). Despite the long-term existence of the field of animal cognition, much of the work in this field has been conducted on lab-reared or captive-bred animals, often due to the difficulties or complexities associated with studying cognition in the wild (Morand-Ferron et al., 2016). However, studying cognition in the wild is important for a number of reasons. Lab-reared animals may be lacking in experiences related to foraging, predator avoidance, environmental complexity and naturalistic social experiences which may in turn affect how they respond in assays of cognition that are not representative of their wild-living counterparts (Pritchard et al., 2016; Salena et al., 2021). Reporting the empirical context under which cognition is measured or examined in animals and their social context (i.e. either lab-reared or wild-living) is critical and can allow us to more accurately interpret the observed behaviours (Horn et al., 2022). For example dogs who experience different social contexts (i.e. pets or free-ranging) only show a difference between the two groups of dogs approaching a friendly but unknown experimenter when placed in different environmental contexts (i.e. distraction free location versus a public dog park) (Lazzaroni et al., 2020).

Cognition is often studied across populations, related species or between sexes (Boogert et al., 2018, Lucon-Xiccato and Bisazza, 2017a). In some species, individuals adopt discrete alternative reproductive tactics (Oliveira et al., 2008). Studying cognition in animal species that have alternative reproductive tactics provides an opportunity to ask whether and if so how cognition differs between these tactics and what role cognition may play in the reproductive success and therefore the evolution of those tactics. ARTs are typically discrete phenotypes that coexist within a single population, each with their own distinct physiology, behaviour and morphology (Gross, 1996; Oliveira et al., 2008). Male ARTs in many species arise as two distinct phenotypes: a larger and dominant male who may have other behaviours such as parental care or nest building and a smaller male which attempts to sneak mating opportunities in the presence of a dominant male (Gross, 1996; Oliveira et al., 2008). Some species also exhibit more than two ARTs. For example, bluegill sunfish (*Lepomis macrochirus*) have a third tactic referred to as a satellite male – an intermediate-sized male who hovers near an active nest, is similar in size to females, and sneaks fertilizations (Gross 1982)). Studying cognition in species with ARTs allows us to examine the interplay between sexual selection, mate choice, behavioural plasticity, personality and social competence. For example, in captive rose bitterling fish (*Rhodeus ocellatus*), sneaker males showed a positive correlation between reproductive success and their spatial learning ability in a maze assay (Smith et al., 2015). Problem solving abilities in African striped mice (*Rhabdomys pumilio*) for two different tasks were correlated with differences in ARTs and age. Personality is also a factor; mice that are more bold, active and exploratory are better problem solvers, irrespective of their ART

type. (Rochais et al. 2021). Ultimately, the comparison of cognition among ARTs could be used as a way to integrate the fields of social neuroscience and cognitive ecology by studying how discrete alternative phenotypes within a species differ in cognition as a result of development and/or change over the lifetime of an individual (Wallace and Hofmann, 2021a).

Why study cognition in fish:

Of all animal groups for studying cognition, fish rise to the top for several reasons. First, tetrapods (amphibians, reptiles, birds, mammals) share a common ancestor with fish as these groups descended originally from the bony fish. This common ancestry contributes to similarities among the taxa, including brain structure and function (Bshary and Brown, 2014). Fishes have homologous brain regions to the social decision network as seen in mammals (Bshary et al., 2014) but fish have far more neural plasticity than other vertebrates, gaining and losing neural connections throughout life (Ebbesson and Braithwaite, 2012). Neurogenesis in adulthood is restricted to two brain regions in mammals, the lateral ventricle and hippocampus, but occurs in dozens of brain regions in fish (Zupanc et al., 2005). Unlike mammals that only have neuroepithelial stem cells during embryonic development before transferring to mostly using glial stem cells in post-natal development, fish use neuroepithelial stem cells for neuronal development throughout their life (Pushchina et al., 2020). Having increased neural plasticity can serve many functions. The brown ghost knifefish has indeterminate growth and has increased neuronal cell number in its central nervous system which enables increases in this fish's

electrical output as it grows (Sîrbulescu et al., 2017). Other examples of neural plasticity are displayed by subordinate cichlid fish who show rapid increases in neuronal proliferation when given the opportunity to increase their dominance status (*Astatotilapia burtoni*, Maruska et al., 2012) and in the brains of salmon which go through structural reorganization as they transition from the freshwater stream they were born in to living in the ocean (Ebbesson et al., 2003). Another key reason for studying cognition in fish is that, since their split from the tetrapods, fish have radiated to occupy into a broad diversity of environments and they now make up more than half of all the vertebrate species (Nelson et al., 2016). Given this diversity, researchers can more readily examine how a fishes' ecology varies or is associated with its cognitive processes and compare the evolution of cognition across multiple fish species (Bshary and Brown, 2014).

What we know:

The explosive increase in cognitive research in fish over the past three decades has allowed greater insight into their capabilities and facilitates comparison to the more extensively studied terrestrial vertebrates (Miller, 2017). Research has been conducted on a wide range of behaviours such as the adjustment of cleaning versus cheating behaviour when observed by their clients (cleaner wrasses, *Labroides dimidiatus*, Bshary and Grutter, 2006) or the ability of fish to infer overall rank simply by watching bouts between multiple males (cichlids, *Astatotilapia burtoni*, Grosenick et al., 2007). By adjusting their behaviour to environmental cues, cleaner wrasses reduce the likelihood that their client fish will choose another station and cichlid males can decide whether or

not to expend energy fighting a male they know against which they know they cannot win. Even the common goldfish (*Carassius auratus*) has been shown to have the ability to use cognitive spatial mapping skills using landmarks and their relationship to each other (i.e. allocentric cues) which could enable them to forage more efficiently or increase their successful escape from predators (López et al., 1999; Rodriguez et al., 1994).

In fact in some disciplines, fish have emerged as the species of choice or model species to study key cognitive processes. For example, research on numerical abilities, social and non-social behaviour, and personalities in model fish species has furthered the understanding of underlying mechanisms and evolution of cognition in animals (Miller, 2017). I describe each of these in more detail below (and provide an illustrative summary in Figure 1).

Numerical abilities

Recent research has revealed that, similar to both mammals and birds, diverse fish species also possess numerical abilities (Agrillo et al., 2017). When exposed to predator cues, fish species such as minnows and zebrafish show the ability to discriminate between, and have a preference for, larger groups of conspecifics (Hager et al., 1991; Pritchard et al., 2001). Preferring larger groups of conspecifics can be seen as an adaptive response that can lower an individual's risk of predation, also known as the Dilution effect (Foster & Treherne, 1981). Along with lowering predation risk, the ability to discern group size can also impact foraging decisions, and mating tactic (Agrillo et al., 2017). Male guppies show the ability for quantity discrimination and prefer groups with a

higher number of female conspecifics, which would presumably increase their mating opportunities (Lindström and Ranta, 1993).

Social cognition

A wider variety of research has been conducted on social cognition in fish such as assessing preferences for specific individuals over others. A study on sticklebacks (*Gasterosteus aculeatus*) demonstrated their ability for rapid temporal flexibility in preferences for individuals living in the same habitat (Ward et al., 2007). Another study demonstrated the capacity of a cichlid fish species to recognize a familiar individual in less than 0.5 seconds (Kohda et al., 2015). The ability for true recognition of an individual over simply phenotype matching is cognitively demanding and has been hypothesized to evolve in species with stable social groups (Ward et al., 2020). Discus fish (*Symphysopdon aequifasciatus*) were able to use facial patterns to recognize familiar tank mates they had lived with for three months (Satoh et al., 2016) while guppies were able to recognize, and showed a preference for, familiar individuals after associating with them for only 12 days (Griffiths and Magurran, 1997). Comparisons of closely related African cichlid species showed that the social living species preferred familiar conspecific individuals while solitary living cichlids preferred to spend time with unfamiliar conspecifics (Salena and Balshine, 2020). Alternatively other species like male zebrafish show no preference for familiar or unfamiliar male conspecifics but will prefer to shoal with a conspecific over an empty chamber (Blonder and Tarvin, 2022).

Non-social cognition

To date, much less work has been done exploring recognition of non-social stimuli in fish species. The novel object test or one-trial object recognition task was first developed for use in mice and rats for probing short-term and long-term memory and took advantage of the natural behaviour of rats and mice to preferentially inspect novel items (Ennaceur, 2010). Placed in the proper context and using appropriate stimuli use of this test has been proposed for other non-mammalian species as a measure of memory and exploratory behaviour (Blaser and Heyser, 2015). Male and female cichlids (*Astotilapia burtoni*) both prefer a novel over a familiar object and have no difference in their latency to first movement but females increase their preferences for the novel object over time while the males decrease their preference over time (Wallace and Hofmann, 2021b). In zebrafish (*Danio rerio*), both males and females prefer a familiar object (Gaspary et al., 2018; May et al., 2016). Five day old guppies spend more time next to a familiar object yet do not show strong preferences for familiar shoal mates at this age unless there is an indication of predation (Pettrazini et al., 2012). This test has also been used to test the impact of compounds that are known to impair memory formation (zebrafish, Stefanello et al., 2019) and to explore the impact of environmental enrichment on recognition (guppies, Gatto et al., 2022).

Personality and individual differences

A recent review (Lucon-Xiccato and Bisazza, 2017a), highlighted that fish express individual differences in cognition across multiple criteria, including personality, gender

differences and variation among populations. Personalities refer to the tendency of an individual to have consistent behavioural reactions in social settings or during certain situations (Reale et al., 2007; Sih et al., 2004). Guppies that had a shy personality were better at learning an avoidance task (Budaev and Zhuikov, 1998) but in associative tasks with a food reward, bolder individuals learned the task faster (guppies, Dugatkin and Alfieri, 2003; Trompf and Brown, 2014; poeciliid, *Brachyrhaphis episcopi*, Depasquale et al., 2014). When examining gender differences, several studies looking at spatial abilities demonstrated that males outperformed females (freshwater blenny, Fabre et al., 2014; guppies, Lucon-Xiccato and Bisazza, 2017b; rose bitterling, Smith et al., 2015) though female guppies consistently displayed greater cognitive flexibility than males (Lucon-Xiccato and Bisazza, 2014, 2017b). When assessing differences between populations, most studies chose populations from habitats that differed in one aspect (i.e., level of predation risk, environmental complexity). In an avoidance task, three-spined stickleback from high-predation sites were faster learners (Huntingford and Wright, 1992) whereas river stickleback from low-predation sites were faster learners in a spatial task with a food reward (Brydges et al., 2008). However, sometimes individuals are behaviourally plastic and may not appear to have one consistent coping style or personality type. Future studies on individual cognitive differences could benefit by incorporating more information about individuals and linking it with their cognitive performance and reproductive success (Thornton and Lukas, 2012).

What we don't know:

We now have extensive research documenting the cognitive abilities of fish. Far less research has attempted to explain why these differences arise, the underlying mechanisms, or the importance of differences in cognition within a particular species. The majority of research on fish cognition has also been conducted on domesticated or lab-based strains of a few freshwater fish species and few studies have used wild living fish (Bshary and Triki, 2022; Salena et al., 2021; Ward et al., 2007). Lab-reared animals generally experience far less exposure to variation in stimuli during development and experience an overall safer environment. We would therefore expect differences in cognition between lab-reared and wild-living animals, as more exposure to variation often translates into richer brain development (Pollen et al., 2007). A highly enriched developmental environment can lead to greater potential complexity or variation in cognition and this level of complexity is difficult to replicate or reproduce in a captive-reared or lab setting (Simpson and Kelly, 2011; Smith et al., 2017). Studies on lab-reared animals may therefore underestimate or otherwise falsely characterize cognitive performance.

Though ARTs have been identified across a large number of fish families and appear to quite common (Taborsky, 2008), only a handful of studies have examined cognition in fish species with ARTs and only very recently. The round goby, a well-known invasive fish species, has two male reproductive phenotypes, a male that guards nest and a slightly smaller male that sneaks (Marentette et al., 2009). An assay measuring

social preference in wild-caught round goby found that, while both types of males preferred groups of conspecifics over an empty chamber, guarder males were more aggressive towards those conspecifics (Synyshyn et al., 2021). In lab-reared rose bitterling, a fresh-water fish species with males that can switch between two tactics, males that assumed a sneaker role showed a positive correlation between reproductive success and learning accuracy in a maze (Smith et al., 2015). In the high-backed pygmy swordtail (*Xiphophorus multilineatus*), males have two fixed polymorphisms or ARTs, with the larger courter male having a fixed behavioural strategy and the slimmer sneaker male, based on whether competitors are present, switching between both sneaking and courting tactics (Zimmerer and Kallman, 1989). Sneaker males were better learners in a classical conditioning assessment but female offspring of those sneaker males had the lowest probability of being a learner (Griebing et al., 2020). Early research therefore suggests that alternative male phenotypes may differ in learning, but further research is warranted, especially on additional components of cognition, such as memory and individual recognition (see also illustrative summary in Figure 1).

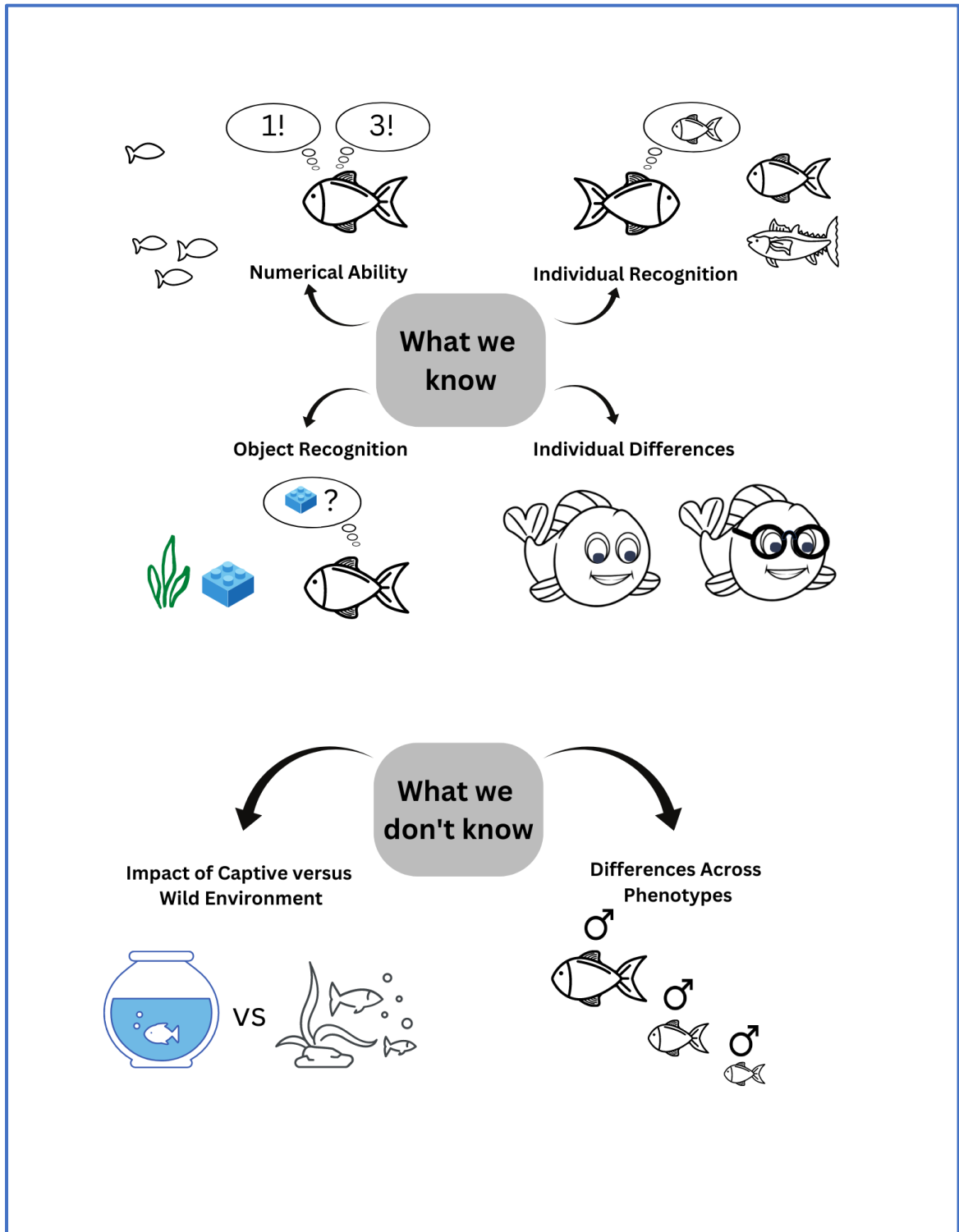


Figure 1. Summary of current concepts covered in the field of fish cognition and areas that are still lacking empirical work or consideration in interpreting empirical work.

Our model system:

The ocellated wrasse (*Symphodus ocellatus*) is endemic to the Mediterranean Sea, found at 1-30 m in depth and has a reproductive season that lasts from April-June. Males exhibit three alternative phenotypes that differ in their mating behavior and reproductive success (Figure 2) (Alonzo et al., 2000, Lejeune, 1985; Taborsky et al., 1987). These Alternative Mating Tactics (ARTs) are associated with two distinct life history pathways, determined by early differences in growth rather than resulting from a simple genetic polymorphism (Alonzo et al., 2000). One-year-old males can become sneaker males that hover near active nests waiting for opportunities to sneak fertilizations. Two-year-old nesting males build and maintain nests made of algae, court females and fan developing eggs for several days before they hatch. Intermediate-sized satellite males, are either one or two years old and form short-term cooperative relationships with un-related nesting males (Stiver and Alonzo, 2013). Satellite males assist the nesting male they are associated with by chasing away sneakers, and bringing in females, but also sneak fertilizations when they have the opportunity (Stiver and Alonzo, 2013; Taborsky et al., 1987). By being allowed closer to the nest, satellite males experience shorter delays in sneak-spawning than sneakers (Kustra et al., in preparation). The changes in male phenotype come with a variety of hormonal shifts, such as higher levels of androgens and lower levels of circulating cortisol in nesting and satellite males (Nugent et al., 2016), and behavioral shifts such as the addition of paternal care for nesting males and more complex social interactions between nesting and satellite males (Stiver and Alonzo, 2013; Taborsky et al., 1987). Females visit and spawn in multiple nests, provide no care and can

revisit the same nest several days later (Stiver et al., 2018). A key criterion that females use to choose a nest to spawn in is whether that nest has successfully attracted other spawning females rather than basing her choice on the quality of the nesting male or his nest (Alonzo, 2008). Highly successful nests attract more sneakers, resulting in high sperm competition (Alonzo and Warner, 2000), but females can also bias paternity to the nesting male via their ovarian fluid (i.e. cryptic female choice) (Alonzo et al., 2016).

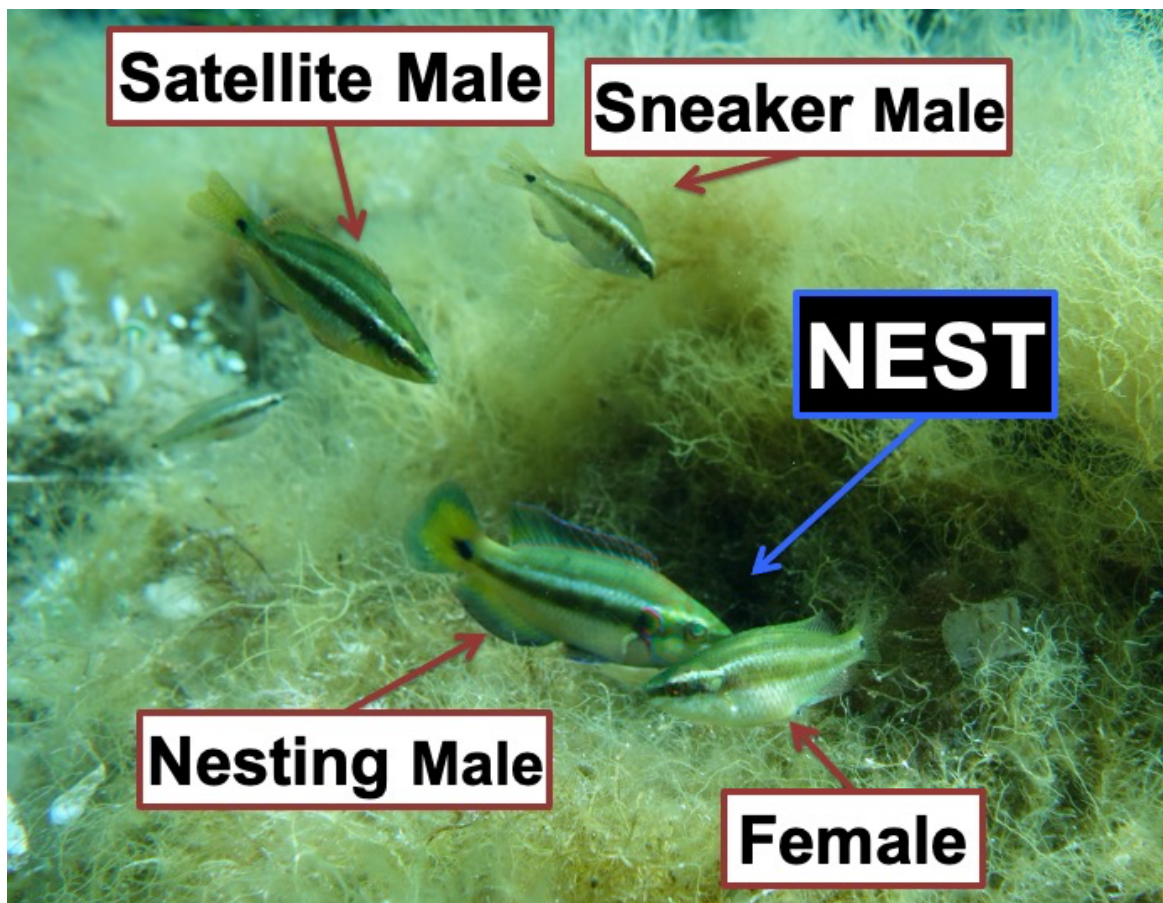


Figure 2. An active nest of the ocellated wrasse (*Symphodus ocellatus*). A nesting male at his algal nest with a female. A satellite male and sneaker male are also nearby waiting for an opportunity to sneak spawn. (Photo credit: S. Marsh-Rollo)

Study Aim:

The aim of this research was to ask whether alternative male phenotypes differ in memory, recognition, exploration and numerical abilities. Previous research on the three male types of the ocellated wrasse have shown differences in sperm production (Warner and Lejeune, 1985), sperm characteristics (Alonzo et al., 2021), gene expression and circulating hormones (Nugent et al., 2016). Each male phenotype also has a different suite of behaviours or behavioural repertoire (Taborsky et al., 1987) and nesting males and satellite males form short-term cooperative relationships and engage in complex social behaviours (Stiver and Alonzo, 2013). Standard behavioural assays (scototaxis, sociability and detour task) revealed that there is far greater cognitive-behavioural differences among the three male types than between the males and females (Cummings et al., 2022). Given the differences and plasticity in physiology, gene expression, behaviours and cognitive-behaviour we wanted to further explore differences in cognition and behavioural flexibility or plasticity in the ocellated wrasse. To do this I caught wild-living ocellated wrasse and ran them through a series of three discrimination assays using a forced-choice paradigm. I used a novel object recognition assay to assess memory and neophobia/neophilia (Blaser and Haser, 2015). I used a social assay with conspecifics to assess recognition and any direction of preference, and a group size assay to assess numerical abilities. Time spent on either side was used to assess preferences (i.e. for novel versus familiar objects and/or conspecific females), while the number of side switches and time to first movement was used to assess activity and boldness/anxiety (Mamuneas et al., 2015; Titulaer et al., 2012). While I did not test specific a priori

predictions regarding differences in these measures between phenotypes, I expected to observe similarities in these measures between satellite males and females, as they seem to be the most flexible in behaviour during the spawning season. Satellite males must be behaviourally flexible to appropriately and rapidly manage relationships with nesting males while still mating successfully, and females are constantly assessing the social landscape at nests before deciding to spawn (Alonzo, 2022).

Materials and methods

Animals and housing conditions

Field work was conducted from May to June (2018, 2019) at the Station de Recherches Sous-marines et Océanographiques de Calvi (STARESO) located in in the Baie de Revellata, Mediterranean Sea. Actively spawning nests of *S. ocellatus* were observed via SCUBA for 10 minutes. Using small hand-nets, we caught the observed nesting male and satellite male, as well as a female and sneaker male who were actively involved in reproduction and social interactions at the focal nest. Fish were slowly brought to the surface then acclimated in the lab in a forced-choice assay tank for two hours before the assays began.

Conspecific Female Shoal Group Tank:

During the field season, I maintained an aquarium (480 litres) of conspecific females in the lab, which were used as the stimulus fish in the familiar/unfamiliar conspecific and group-size assays described below. The ocellated wrasse is an omnivore that feeds off the algal substrate and in the water column. To feed the shoal group, freshly collected algae was exchanged daily in a tank with a saltwater flow-through system. These females were used as size-matched conspecific “stimuli fish” for the assays involving female conspecifics. I kept four size classes of females and gave them a small mark of elastomer (Northwest Marine Technology, Inc.) for continuous identification of each size class. The four size classes used were: 45, 48, 50, and 55 mm (± 2 mm).

Experiment: Introduction phase and discrimination assays

Forced-Choice Assay Tanks:

Focal fish were first acclimated and then observed in rigid plastic tanks in which the sides were lined with blue felt and the bottom with blue contact paper (Figure 3). Stimuli zones at either end were blocked off using clear acrylic dividers. The bottom of the tank was marked with a choice zone next to each stimuli zone. Light blue opaque dividers separated the stimuli zones from the rest of the tank in between trials. These tanks were filled to 15 cm depth with fresh salt water (piped in from the bay where these fish were observed and collected) at the beginning of a set of trials. In order to reduce other visual stimuli, the experimental tanks were surrounded by black fabric. Each trial was recorded from above using GoPro Hero 5 cameras.

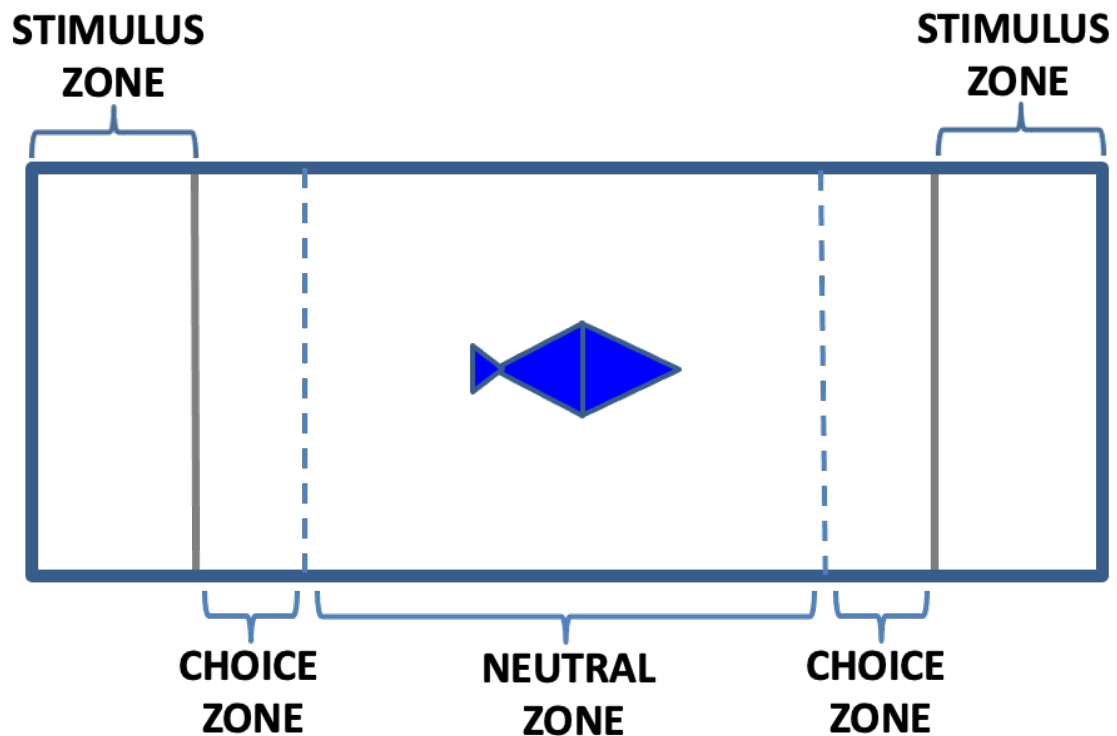


Figure 3. Top-down illustration of the forced-choice assay tank (50.5 cm L x 30 cm W x 24cm H). Stimuli zones at each end were 7 cm in width. Choice zones were 4 cm in width and are shown marked off next to each end where the stimuli were placed during the introduction phases and discrimination assays.

Introduction phases:

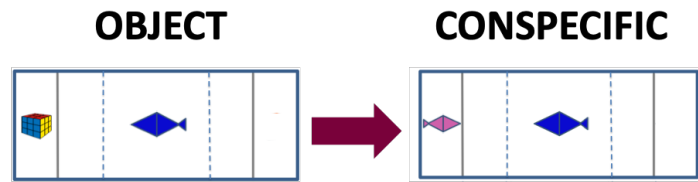
In order to create both a familiar object and conspecific female, we ran two introduction phases. After the two-hour acclimation period, we first exposed focal fish to an initially novel object and then to a conspecific female. We refer to these exposures as the two introduction phases. Focal fish were first exposed to a novel object (Rubik's cube) for 10 minutes that either had solid-coloured sides in a square shape or multiple-coloured sides in a skewed shape (see Figure 4a). We then removed the object and waited two minutes, after which we exposed the focal fish to a conspecific female for 10 minutes

(Figure 4a). Side placement was randomized. Blue opaque barriers occluded stimuli zones in between introductions and were removed at the beginning of each of the two introduction phases. After the second introduction there was an inter-trial interval of 30 minutes before the discrimination assays began.

Discrimination assays:

After the 30-minute inter-trial interval, we ran focal fish through a series of forced-choice assays (which we refer to as the discrimination assays). We ran a sequence of three forced-choice discrimination assays, always in the following order: 1) familiar versus unfamiliar object 2) familiar versus unfamiliar conspecific size-matched females 3) one versus three unfamiliar, conspecific females (Figure 4b). Each discrimination assay lasted for 10 minutes and was recorded from above by a GoPro Hero 5 camera. After each assay, opaque barriers were re-inserted, stimuli were changed, after which we waited two minutes before beginning the next discrimination assay.

a. INTRODUCTION PHASE:



b. DISCRIMINATION ASSAYS:



Figure 4. The forced-choice assay tanks were recorded from above for 10 minutes during **a.** each introduction phase (e.g. object and female conspecific) and **b.** each forced-choice discrimination assay (e.g. object, conspecific, one versus three conspecifics).

Behavioural scoring of videos

Undergraduate students scored the conspecific introduction phase and each of the three discrimination assays using CowLog v3 (<https://cowlog.org/>). Scorers were blind to the identity of the four types of focal fish (e.g. nesting male, satellite male, sneaker male and female) and, in the discrimination assays, to the identity of the familiar object or conspecific. The focal fish was scored as being in the choice zone when their head and gills crossed the line of the choice zone. All discrimination assays were also scored for activity of focal fish and, when conspecifics were used as stimuli, activity of stimuli fish. Scoring logs were summarized using R, from which I calculated the time spent in each choice zone (preference/discrimination), the number of times the focal fish switched sides (side switches), and the time to it took the focal fish to make the first movement after the

trial began (latency). Video scoring started as soon as the opaque barriers were fully removed and then continued for a total of ten minutes.

Ethics Statement

The procedures used in this study were reviewed and approved by UCSC IACUC Protocol Number Alons2017. All procedures adhered to the Animal Behavior Society of Americas ethical guidelines for research on vertebrates.

Statistical analyses

Analyses were performed using StatPlus 8.0.1.0 and all tests were two-tailed and used a significance cut-off value of $p=0.05$. As my data did not meet the assumptions of normality (as determined by Shapiro-Wilk tests), equivalent non-parametric tests were used to assess preferences within each phenotype and compare differences among alternative phenotypes in these preferences and other behavioral variables.

We ran a total of 171 trials for the two introduction phases and the object and conspecific discrimination assay. Of these, I excluded three failed trials (e.g. because focal fish or stimuli fish escaped through barrier) and four trials because they were an accidental second trial of the same focal fish. We ran a total of 129 trials for the group size discrimination assay. Of these, I excluded two trials because they were an accidental second trial of the same focal fish. When assessing preferences in the conspecific introduction phase and three discrimination assays, I excluded trials where the focal fish failed to sample both sides during the 10-minute period. This is standard for forced-

choice assays of this kind because it ensures that the focal animal sampled both sides in order prior to establishing a preference between the sides. The rate of participation by the focal fish, defined as sampling both sides, varied between the four types and across the conspecific introduction phase and three assays. To assess whether fish exhibited a preference in the conspecific introduction phase and each discrimination assay, I calculated a preference score (e.g. time spent next to the familiar object – time spent next to the unfamiliar object). I then used Wilcoxon signed rank tests to ask whether each of the four individual phenotypes (e.g. females, sneakers, satellite males or nesting males) exhibited a significant preference (e.g. a preference score that was on average significantly different than zero). I used a Kruskal-Wallis one-way analysis of variance test to ask whether there were significant differences in preference scores among the four alternative phenotypes (i.e. females, sneakers, satellites, and nesting males).

When assessing differences in number of side switches and latency to explore in the three discrimination assays among the four types, I conducted the described above on all of the “good” trials for each assay (i.e. unlike for the preference analyses, for these analyses we did not exclude the assays in which the focal fish did not sample both sides). I did this because we were examining differences in activity as a measure of exploratory behaviour and/or boldness which (unlike preference) can be assessed even when fish do not sample both sides (Mamuneas et al., 2015; Titulaer et al., 2012). I also conducted a second set of analyses that excluded trials in where the focal fish failed to sample both sides during the 10-minute period to ensure that my results were robust and comparable to the preference analyses described above. In both sets of analyses, I used Kruskal-Wallis

one-way analysis of variance tests to assess whether there were significant differences in the number of side switches and latency to explore between the four types in each of the three discrimination assays.

Results

Participation

The rate of participation by the focal fish, defined as sampling both sides, varied between the four types, and across the conspecific introduction phase and the three discrimination assays (Table 1).

	CONSPECIFIC INTRODUCTION			OBJECT ASSAY			CONSPECIFIC ASSAY			1 Vs. 3 FEMALES ASSAY		
	Total N	Participating N	Proportion	Total N	Participating N	Proportion	Total N	Participating N	Proportion	Total N	Participating N	Proportion
Focal females	41	18	44%	41	23	56%	41	31	76%	31	25	81%
Nesting males	39	12	31%	38	8	20%	39	30	78%	32	25	78%
Satellite males	41	18	44%	42	16	38%	42	36	86%	33	31	94%
Sneaker males	42	20	48%	42	20	49%	41	37	90%	33	30	91%
TOTAL SAMPLE SIZE	163	68		163	67		163	134		129	111	

Table 1. Proportion of focal fish by type that participated (i.e. sampled both sides) in the conspecific introduction phase and each of the three discrimination assays (familiar versus unfamiliar object assay, familiar versus unfamiliar conspecific female assay, one female versus group of three females).

Object Discrimination Assay

Preference/discrimination in the object assay:

In order to ask whether the four types differ in preference for a novel object, I analyzed the preference score (time spent next to the familiar object – time spent next to the unfamiliar object). I found that none of the four types showed a significant preference to spend time with either the familiar or unfamiliar object (Wilcoxon-test: females, $Z = -0.03$, $p = 0.98$, $n = 23$; nesting males, $Z = -1.68$, $p = 0.093$, $n = 8$; satellite males, $Z = -0.103$, $p = 0.92$, $n = 16$; sneaker males, $Z = -0.34$, $p = 0.74$, $n = 20$). I also found no differences when comparing the preference score across the four types (Kruskal-Wallis ANOVA: $H(3,67) = 3.68$, $p = 0.3$).

Side switches in the object assay:

In order to ask whether the four types differ in exploratory behavior, I compared the number of side switches in all good trials. I found a significant difference in the number of side switches between the four phenotypes during the object discrimination assay (Kruskal-Wallis ANOVA: $H(3,163) = 10.11$, $p = 0.01763$) (Figure 5). However post-hoc comparisons found no significant differences between types.

When excluding fish that did not sample both sides, however, I found no significant difference across the four types in the number of side switches between familiar and unfamiliar objects (Kruskal-Wallis ANOVA: $H(3,67) = 6.89$, $p = 0.075$).

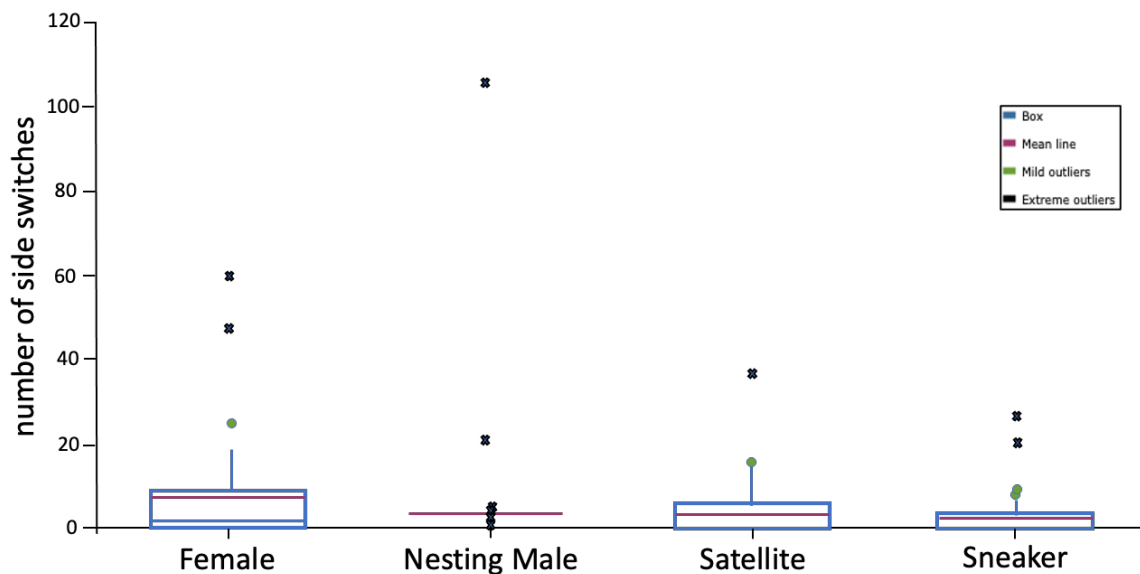


Figure 5. In the object discrimination assay, I found there was a significant difference in side switches across the four types, but only when we did not exclude focal fish that did not sample both sides.

Latency to explore in the object assay:

In order to ask whether the four types differ in the time they take to start exploring objects I compared the latency to explore (i.e. time to first movement) between the four types. First I analyzed all good trials and found a significant difference in the latency to explore during the object discrimination assay (Kruskal-Wallis ANOVA: $H(3,163) = 8.09$ $p = 0.044$). Post-hoc pairwise comparison found no significant differences between types, but there was a non-significant trend of focal females making their first movement faster than nesting males (Bonferroni post-hoc, female versus nesting male $p = 0.056$) (Figure 6a). I again analyzed latency to explore, excluding fish that did not sample both sides, and still found a significant difference among the four types (Kruskal-Wallis ANOVA: $H(3,67) = 9.24$ $p = 0.026$). However, in this second analysis, post-hoc pairwise

comparisons found that focal females made their first movement significantly faster than sneaker males (Bonferroni post-hoc, female versus sneaker male $p = 0.013$) (Figure 6b).

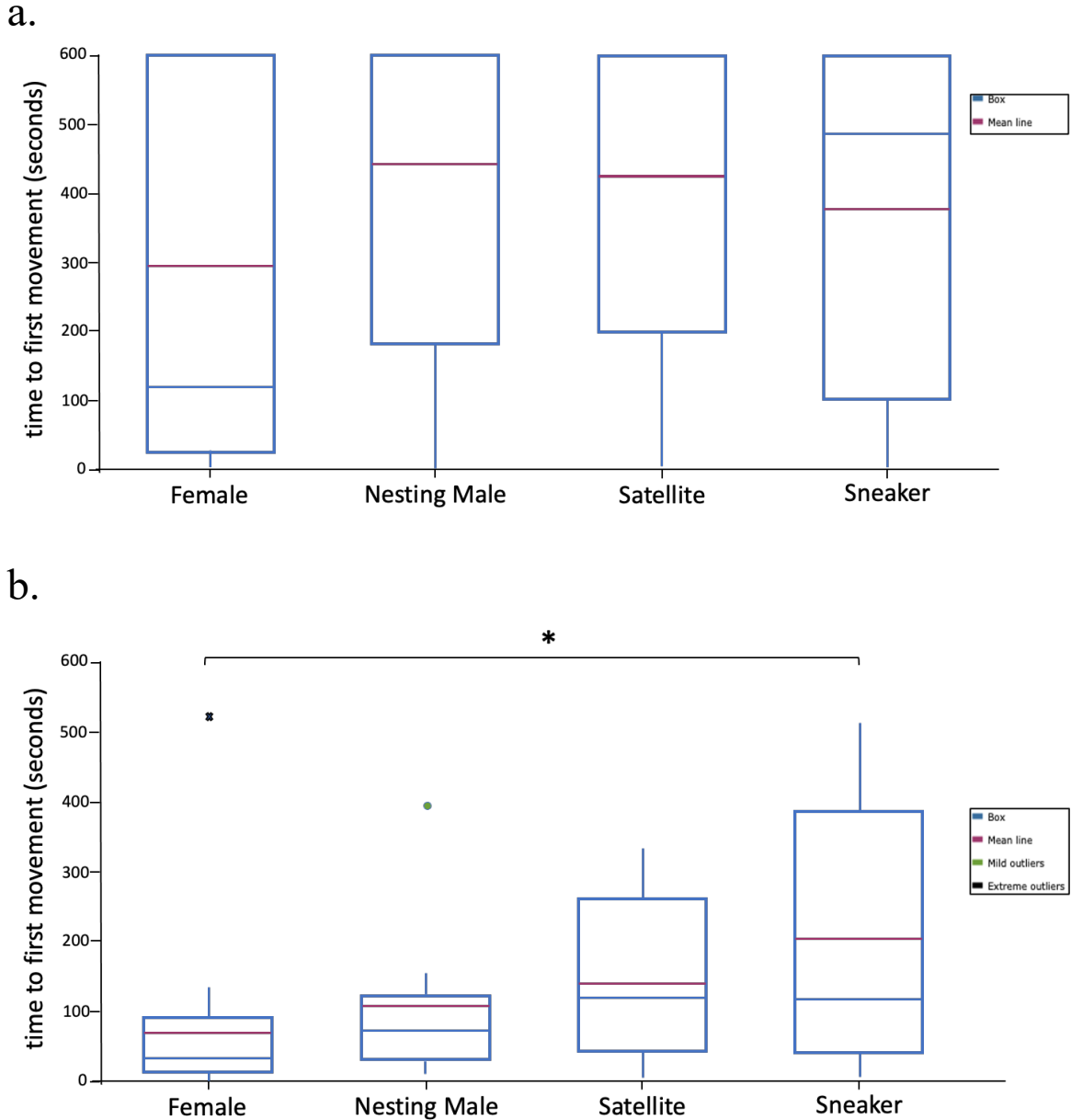


Figure 6. In the object discrimination assay, **a.** when I included all trials, a non-significant trend indicated that focal females made their first movement more quickly than nesting males but **b.** when I excluded fish that did not sample both sides, focal females made their first movement significantly more quickly than sneaker males.

Conspecific Introduction Phase

Preference/discrimination in the conspecific introduction:

In order to ask whether the wrasses generally prefer to associate with a conspecific overall, I analyzed the time spent next to a conspecific versus time spent next to an empty chamber. First I analysed the preference score (time spent next to the conspecific female – time spent next to the empty stimulus zone) for each of the four types separately and found that all four types spent significantly more time next to a conspecific female relative to an empty chamber (Wilcoxon-test: females, $Z = -4.58$, $p < 0.0001$, $n = 41$; nesting males, $Z = -2.82$, $p = 0.005$, $n = 39$; satellite males, $Z = -4.92$, $p < 0.0001$, $n = 41$; sneaker males, $Z = -4.49$, $p < 0.00001$, $n = 42$). I found no significant difference in the strength of this preference among the four types (Kruskal-Wallis ANOVA: $H(3,163) = 4.78$, $p = 0.19$) (Figure 7). I excluded 96 trials in the analyses above because the focal fish did not sample both sides. While this is a standard for forced-choice trials, I want to provide further information about these excluded trials. In 59 of those trials the focal fish were next to the choice zone with the female in it at start of the observation and never left that zone. In 25 trials, the focal fish started in the centre, then moved to the choice zone with the female in it and then never left (i.e. did not switch sides). In the remaining 12 trials I excluded, focal fish started on the empty chamber side and remained there throughout the observation.

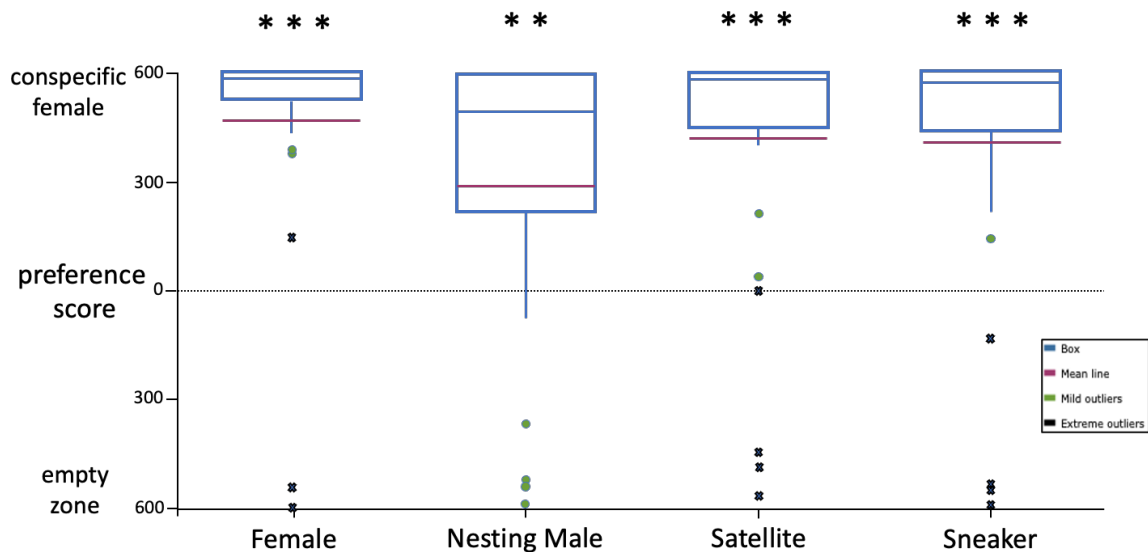


Figure 7. When analyzing preference scores during the conspecific introduction trial all four types preferred to spend time next to a conspecific female over an empty stimuli zone. There were no significant differences in preference between the four phenotypes.

Social Discrimination Assay

Preference/discrimination in the social assay:

In order to ask whether the four types have the capacity for individual recognition of conspecific females I analyzed the preference score (time spent next to the familiar female – time spent next to the unfamiliar female) for each of the four types. I found that only nesting males spent significantly more time with familiar females (Wilcoxon-test: females, $Z = -0.37$, $p = 0.71$, $n = 31$; nesting males, $Z = -2.21$, $p = 0.027$, $n = 30$; satellite males, $Z = -0.35$, $p = 0.73$, $n = 36$; sneaker males, $Z = -0.87$, $p = 0.39$, $n = 37$). I found no difference when comparing preference scores for conspecific females among the four types (Kruskal-Wallis ANOVA: $H(3,134) = 5.85$, $p = 0.12$) (Figure 8).

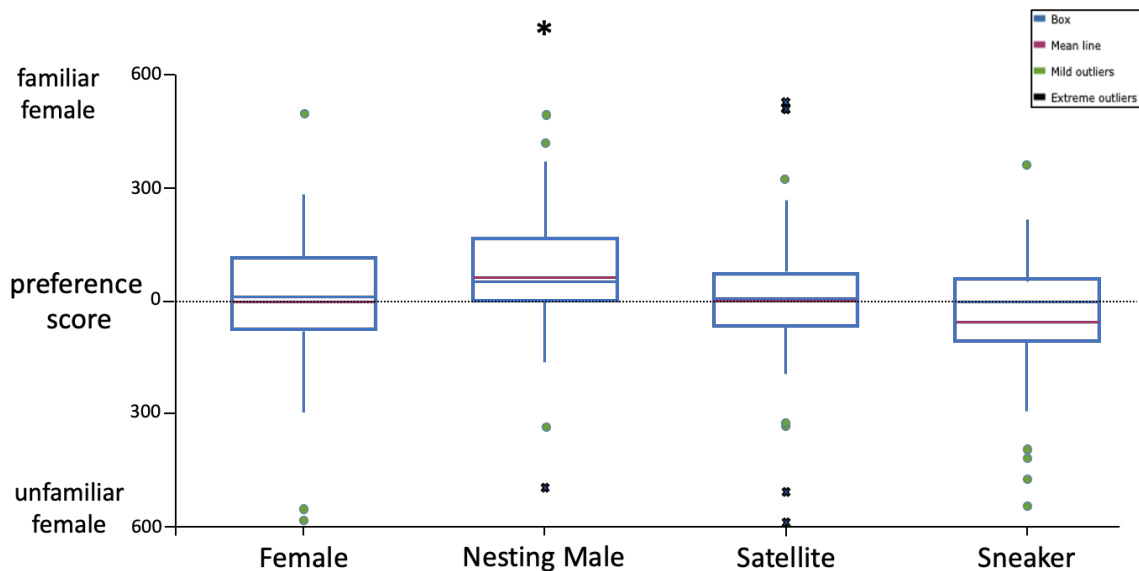


Figure 8. In the social discrimination assay nesting males spent more time with familiar females. Preference scores did not differ across the 4 types.

Side switches in the social assay:

In order to ask whether the four types differ in exploratory behavior of conspecific females, I compared the number of side switches in all good trials. First I analyzed all trials and found a significant difference in the number of side switches during the conspecific discrimination assay (Kruskal-Wallis ANOVA: $H(3,163) = 9.77$, $p = 0.02067$). Post-hoc pairwise comparisons found satellite males made significantly more side switches between female conspecifics than nesting males (Bonferroni post-hoc, satellite male versus nesting male $p = 0.0015$ (Figure 9a).

When excluding fish that did not sample both sides I still found a significant difference in the number of side switches during the conspecific discrimination assay (Kruskal-Wallis ANOVA: $H(3,134) = 11.79$, $p = 0.00815$). However, post-hoc pairwise comparisons now found that satellite males still made significantly more side switches

than nesting males but also more side switches than sneaker males (Bonferroni post-hoc: satellite male versus nesting male $p = 0.002$, satellite male versus sneaker male $p = 0.049$) (Figure 9b).

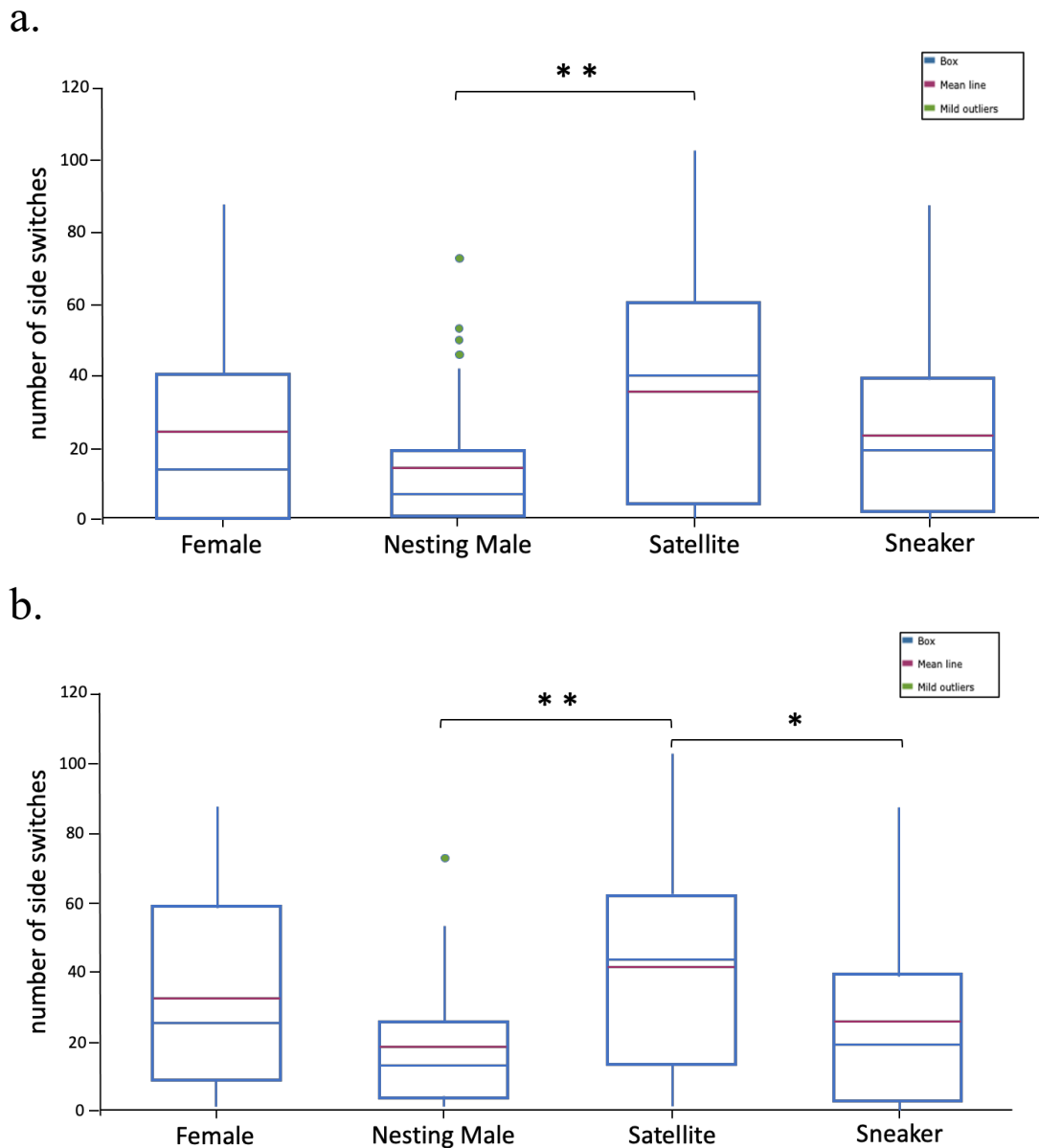


Figure 9. In the social discrimination assay, **a)** satellite males made more side switches between conspecific females than nesting males but **b)** when excluding fish that did not sample both sides, satellite males made more side switches than nesting males as well as sneaker males.

Latency to explore in the social assay:

In order to ask whether the four types differ in the time they take to start exploring conspecifics I compared the latency to explore (i.e. time to first movement) between the four types. First I analyzed all good trials and found a significant difference in the latency to explore during the conspecific discrimination assay (Kruskal-Wallis ANOVA: $H(3,163) = 8.2$ $p = 0.042$, Figure 10). However, post-hoc pairwise comparisons found no differences in the latency to explore between the four types.

I again analyzed latency to explore, excluding fish that did not sample both sides, and found no difference in the time to first movement across all four types (Kruskal-Wallis ANOVA: $H(3,134) = 5.28$ $p = 0.15$).

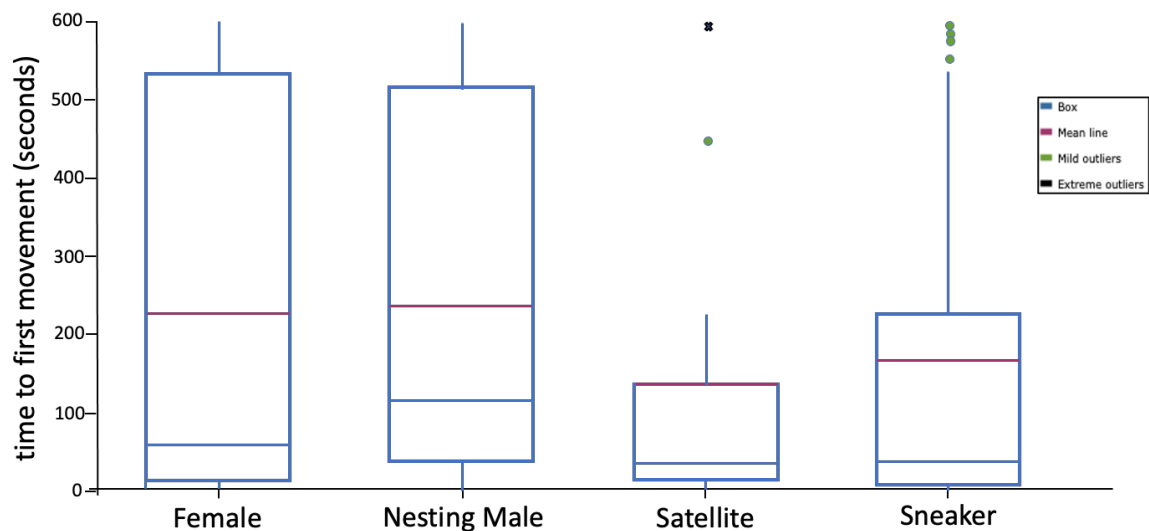


Figure 10. In the social discrimination assay I found there was a significant difference in time to explore across the four types, but only when we did not exclude focal fish that did not sample both sides.

Group Size Discrimination Assay

Preference/discrimination in the group size assay:

In order to ask whether the four types have the capacity for numerical abilities, I analyzed the preference score for group size (time spent next to one female – time spent next to three females) within all four types. I found that all four types spent more time with the group of three females (Wilcoxon-test: females, $Z = -3.11, p = 0.00189, n = 25$; nesting males, $Z = -2.92, p = 0.00351, n = 25$; satellite males, $Z = -2.84, p = 0.03114, n = 31$; sneaker males, $Z = -3.38, p = 0.00072, n = 30$). There were no differences in the preference scores for group size when comparing across the four types (Kruskal-Wallis ANOVA: $H(3,111) = 1.69, p = 0.64$) (Figure 11).

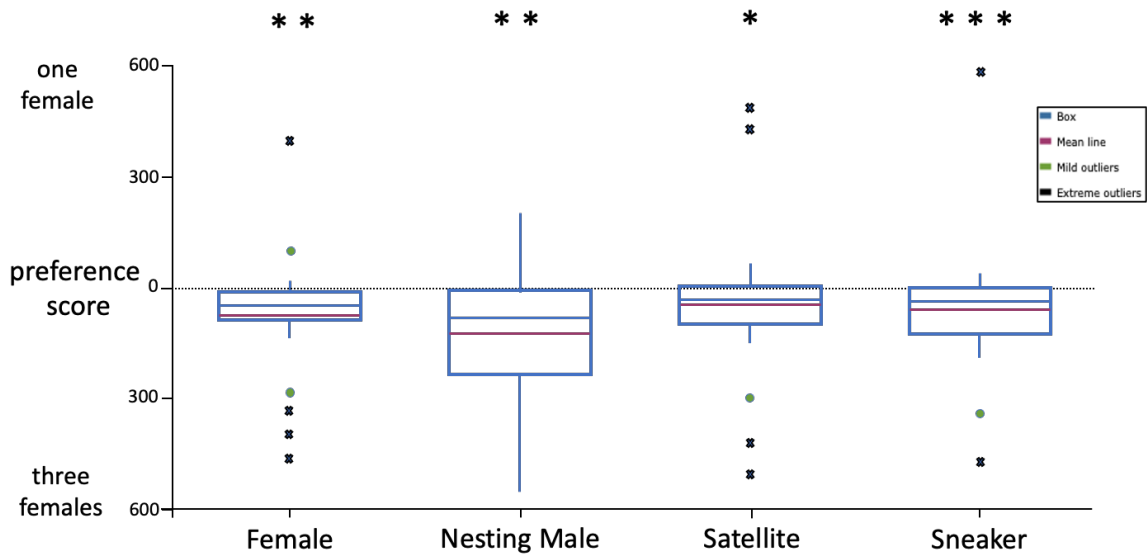


Figure 11. In the group size discrimination assay all four types spent more time with the group of three female over the single female.

Side switches in the group size assay:

In order to ask whether the four types differ in exploratory behavior of different sized groups of conspecifics, I compared the number of side switches in all good trials. First I analyzed all good trials and found that the number of side switches between the one conspecific female and the three conspecific females did not differ across the four types (Kruskal-Wallis ANOVA: $H(3,129) = 5.54$ $p = 0.14$). When excluding fish that did not sample both sides, number of side switches between the one conspecific female and the three conspecific females did not differ across the four types (Kruskal-Wallis ANOVA: $H(3,111) = 2.75$ $p = 0.43$). I therefore find that all four types do not differ in the number of side switches they made during the group size discrimination assay.

Latency to explore in the group size assay:

In order to ask whether the four types differ in the time they take to start exploring groups of conspecifics I compared the latency to explore (i.e. time to first movement) between the four types. First I analyzed all good trials and found that latency to explore the groups of conspecifics did not differ across the four types (Kruskal-Wallis ANOVA: $H(3,129) = 5.99$ $p = 0.11$).

I again analyzed latency to explore the different groups of conspecifics, excluding fish that did not sample both sides, and still found that latency to explore did not differ across the four types (Kruskal-Wallis ANOVA: $H(3,111) = 4.72$ $p = 0.19$). I therefore find that all four types do not differ in their latency to explore during the group size discrimination assay.

Discussion

As reviewed above, extensive research has looked at cognitive differences between species and between the sexes, but very little research has looked at differences among alternative male types. In addition, most research has been conducted on lab-reared or acclimated individuals rather than recently wild-caught individuals. Here I asked whether alternative reproductive phenotypes that change over the lifetime of the individual differ in memory, individual recognition, activity, boldness/anxiety and numerical ability in three forced-choice discrimination assays. I found that only nesting males showed evidence for memory and preferred a familiar female in the conspecific assay. This does, however provide strong evidence that individual recognition is found in this species. When examining activity levels and boldness, I found that females were the most exploratory and bolder in the object assay while satellite males were more exploratory and bolder in the conspecific female assay. Similar to many other fish species (Agrillo et al., 2017), all four types preferred the larger group of conspecific females, showing both the ability to discriminate group size (at least one versus many) and a preference for associating with larger groups. Thus my study confirms that the ocellated wrasse possesses at least basic numerical abilities. Further research is needed to explore motivation within each of the four phenotypes for their preference for a larger group of conspecifics.

Object Discrimination Assay

As reviewed above, my object discrimination assay was modelled after the one-trial or spontaneous object cognition test, widely used as a measure of memory and individual recognition in research on rodents (Blaser & Heyser, 2015) and lab-reared fish (Braida et al., 2014; Lucon-Xiccato and Dadda, 2014; Miletto Petrazzini et al. 2012). I used this assay to not only assess object recognition (and more generally memory), but also exploration behavior in the four wrasse types. While I observed no preference for either the familiar or unfamiliar object, I did find that females made their first move significantly faster than nesting males (all trials) or sneaker males (trials where fish sampled both sides). Previous research on the object recognition task has shown species specific preferences for either a familiar or novel object but did not report differences in measures of activity (Gaspary et al., 2018; May et al., 2016; Wallace and Hofmann, 2021b). My results could reflect spatial exploration and/or that females are less neophobic than at least two of the three male types. Paternity studies have shown that female ocellated wrasses revisit and move among multiple nests (Stiver et al., 2018). Males also have clear social consequences moving between nests, regardless of who they are, since at minimum there is a nesting male that may attack them. This likely holds true for moving around their environment generally. Females on average meet more neutral to positive social interactions when they move and so may be less inhibited to move more freely. My study showed that female wrasses were quicker to explore in the object assay that used a Rubiks cube. Future studies using more ecologically relevant non-social stimuli (e.g. natural objects found in the wrasse underwater environment) could be used to further explore potential differences in memory across the four wrasse types.

Conspecific Introduction

Many species of fish exhibit a preference to be near a conspecific rather than be alone (Krause et al., 2000) particularly in the presence of predation cues (Magurran and Pitcher, 1987), with some species even preferring to associate with heterospecifics if conspecifics were not available (Camacho-Cervantes et al., 2014). I found that all four types did prefer to spend time next to a conspecific instead of an empty chamber regardless of whether I analyzed all trials or only those where focal fish sampled both sides. This result is consistent with findings from a shoaling study in the ocellated wrasse (Cummings et al., 2022). In that study, all four types spent similar amounts of time next to conspecifics (social interaction) or time in a social zone (social boldness) and all were positively socially motivated. All four types had a positive relationship between social motivation and social boldness which was strongest in females and nesting males (Cummings et al., 2022).

Social Discrimination Assay

I found that the ocellated wrasse does have the capacity to recognize individuals but it was only the nesting male who exhibited this in the context of our study. Female wrasses visit the same nest multiple times (Stiver et al., 2018), and nesting males may prefer familiar females as a mechanism for preferentially spawning with females that are known performers. In my study, we had a clear barrier in front of the conspecific but we did not exclude olfactory cues. Due to this, it is not clear whether nesting males used strictly visual or olfactory cues (or both) to recognize familiar female conspecifics. The ability for individual recognition has been shown in a number of other fish species, though preferences for familiar or unfamiliar conspecifics varies between species

(Griffiths and Magurran, 1997; Salena and Balshine, 2020). Individual recognition can confer benefits such as the ability to recognize a previously cooperative partner (stickleback, Milinski et al., 1990) or for selecting an unfamiliar female to mate with (guppies, Kelley et al., 1999). When assessing exploration between the conspecific females, I found that satellite males had higher activity than nesting males. At an active nest, satellite males who are partnered with a nesting male search for and guide females to the nest. More repeated sampling or “assessment” between familiar and unfamiliar females may reflect a satellite’s activity at a nest. Though I did not find similar patterns between satellite males and females within assays as hypothesized, it is interesting that both showed the highest levels of activity in two of our assays.

Group Size Discrimination Assay

A diverse set of fish species possess numerical abilities (Agrillo et al., 2017). In my study, I demonstrated that all four types of the ocellated wrasse have this basic ability (i.e. able to discriminate between one versus many). All four types spent more time with the group of three conspecifics demonstrating their ability to discriminate group size or changes in density. Preferences for a larger group of conspecifics can be driven by risk of predation, access to social information or an opportunity for reproduction (Agrillo et al., 2017; Hager et al., 1991; Lindström and Ranta, 1993; Pritchard et al., 2001). However, I did not include any cues of predation risk in my assay unlike previous work assessing numerical ability in minnows and zebrafish that found, when tested with cues of predation present, they preferred larger groups (Hager et al., 1991; Pritchard et al., 2001). So what does a preference for a larger group of female conspecifics mean in the ocellated wrasse?

This preference was possibly driven by a number of factors that differed by type. Females are attracted to nests where other females are or have spawned recently (Alonzo, 2008) and sneakers are also attracted to nests with higher numbers of females present (Alonzo and Warner, 2000). Both nesting males and satellite males actively search out females and guide them to the nest (Stiver and Alonzo, 2013). Thus the ability to discern and prefer larger groups of conspecifics has reproductive benefits for all four types, at least during the reproductive season. Given that nesting males shut down reproduction in the presence of a large number of sneakers (Alonzo and Warner, 1999) it is likely that they may possess more complex numerical abilities than was demonstrated in our study.

Limitations of this study:

Based on the life history and what we know of the ecology and social system of the ocellated wrasse I expected there to be differences in discrimination, memory and exploratory behaviour in the discrimination assays. The assays I employed were relatively novel for wild-living or wild-caught fish and used abstract scenarios as opposed to mating scenarios. The novelty of the setup and brief acclimation to the lab environment may therefore have underestimated the discrimination and memory in general as well as the differences among types. Further research is warranted, including in situ assays in the wild as well as studies on wild-reared but fully acclimated individuals in this species for comparison. It is important to note that I am not stating the absence of other cognitive abilities not assayed here in the four wrasse types. The choice of these particular tests, the experimental design or choice of stimuli may not have been ecologically relevant or a

salient stimuli to elicit responses. However, given that these assays have been used successfully in many other fish species for cognitive assessments (Agrillo, 2017; Braida et al., 2014; Bshary et al., 2014) I considered them an excellent starting point for examining cognition in the ocellated wrasse. While fish were acclimated only briefly, I did observe that most fish moved freely in the tanks and a previous study successfully measured behaviour in the ocellated wrasse using a similar acclimation time (Cummings et al., 2022). One challenge of working with fish from the wild is that they are not as acclimated to the lab/test environment. But a strength of the study is my ability to assay wild-caught animals that are not lab acclimated- and we can't have one strength without the other "limitation". In future research it might be interesting to look at longer acclimation periods or compare these results to lab-reared fish.

Though the assays may not be in the context of mating behaviour, we can use behavioural measures such as memory, recognition, and latency to explore to give insight into differences in cognitive capacities or flexibility across the four wrasse types. Differential responses in the three male types when assessing cognitive performance may not be an indicator of cognitive ability but rather be a result of their lived experiences or age (Bshary & Triki 2022). The resulting male wrasse phenotypes share a genome and evolutionary history and thus we can study the whole organism outcome of that selection within each of the three male types.

Implications and Future Directions

A strength of comparing the cognition of the alternate phenotypes of this species is that we already know a lot about the selective pressures that have shaped these phenotypes and the many ways in which the growth, physiology, reproductive behavior, and social roles of these types differ. The differences in phenotype are likely shaped by sex-specific selection (when comparing males and females) and sexual selection (when comparing the male types). Therefore when we see differences between the sexes, we can assume that the sexes experience different selection pressures. And same for the three male types (likely differences in sexual selection). Based on this we can infer that sexual selection (in combination with natural selection- since survival and fecundity/fertility tradeoffs with sexual selection are likely always present) has shaped the differences we see. We can explore the role that cognition may have played in the evolution and maintenance of the different male phenotypes and particularly for the satellite males which seem to display the widest range of behavioural flexibility based on observations at active nests (Stiver and Alonzo, 2013).

Showing that we are able to get the wild-living wrasses to participate in lab-based studies opens up a suite of possibilities for future studies. Identifying cognitive differences/similarities could give us some insight into underlying proximate mechanisms and allow us to test for them, such as examining the effects of isotocin or 11-Ketotestosterone antagonists on social relationships between the nesting and satellite male. We can use assay measures as a means to identify different behavioural syndromes or coping styles as males transition phenotypes or ARTs. Outcomes of cognitive assays

can lead to targeting and comparing brain regions across male phenotypes and especially between one- and two-year-old satellite males (O’Connell and Hofmann, 2012).

Taking an ecological approach to view the differences between the male phenotypes is important for future work (Bshary and Triki, 2022). A recent metanalysis assessed the most commonly studied areas of fish cognition (simple learning, numeracy, spatial cognition and social cognition) and suggested possible ways to improve futures studies (Salena et al., 2021). This assessment of just over 600 studies found the majority of studies used lab-reared fish and were performed in a laboratory setting. Salena et al. (2021) proposed that studies of fish cognition would be furthered by using a larger variety of wild-caught fish species and performing that work in the field when possible. Other recommendations included increasing the size of the testing arena, considering testing in dyads rather than individuals only, use of ecologically valid or more salient stimuli and increasing acclimation time to reduce stress (Salena et al., 2021).

Conclusions

Though studying wild-living or wild-caught animals is challenging, taking an ecological approach to view the differences between the male phenotypes is important for our understanding of cognitive and behavioral evolution in the wild (Bshary and Triki, 2022) and for understanding the role cognition may play in the evolution of reproductive and social systems within and between species (Pritchard et al. 2016; Rosati et al., 2022). This approach could also inform conservation efforts such as aquaculture where fish are reared in captivity but then released in stock ponds and expected to have all the required

behaviours to survive (Näslund 2021). Ocean acidification is known to affect fish behavior (Munday et al., 2009) and possibly fish cognition (Ferrari et al., 2021), so studies of this kind may help us predict how climate change will affect social interactions and fish populations.

Studies of fish cognition often compare differences between the sexes or populations (Lucon-Xiccato and Bisazza, 2017a). We can build on studies that already have shown differences in cognition between sexes (Carbia and Brown, 2020; Cummings, 2018; Triki and Bshary, 2021) to continue examining differences within a sex that has multiple phenotypes or morphs such as the ocellated wrasse (Cummings et al., 2022). In this study, I explored how cognition may change within a species as males transition through different reproductive types over their lifetime. Performance in a cognitive task can give insight into the cognitive process or decision rules that an animal uses. This study also explored using a novel technique, the one-shot recognition test (Blaser and Heyser, 2015), for assessing cognition in wild-living animals. These simple yet potentially revealing behavioural assays were used to probe the cognitive differences across the ocellated wrasse three male types and females in a controlled way. My study showed that the ocellated wrasse is capable of individual recognition but I could only detect this in nesting males. It is possible that the other male types and females are also capable of individual recognition perhaps with other types of conspecifics. Sneakers and satellite males may be primed to pay more attention to the identify of a specific nesting male rather than a specific female. Though I didn't find similarities between satellite males and focal females as originally expected, I did find that they were the most active

and exploratory, though in different contexts. Satellite males spend much of their time finding and guiding females to the nest and were the most active in the conspecific assay. Females spawn in multiple nests that they revisit several days later and were most active in the object assay. Females may pay more attention to landmarks in their environment and use them as they navigate their way back to familiar nests. I did consistently find that all four types preferred the larger group. Though this reveals that they all have at least basic numerical skills I can not infer motivation across the four types. It is also possible that all types may not have the same level of more complicated numerical skills for discerning differences in between two larger groups when they only differ by one. For example, a nesting male might shut down reproduction at a nest when there are too many sneakers around. Might nesting males also show greater numerical abilities because of this? Though I have shown that the ocellated wrasse possesses this cognitive ability, future research may be able to further explore how the evolution of cognition and its role in social decision-making has shaped cognitive traits in this species. I agree with other researchers in the field of animal cognition advocating for the need to focus on and conduct ecologically valid studies in the wild and on wild-living species. I would like to suggest that, along with ecologically valid studies, incorporating a number of strategies, such as employing well established and simple behaviour assays when possible, can be useful to identify, understand and interpret cognition in a wide variety of animal species.

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