The Colour of Cooperation: threadfin butterfly fish (*Chaetodon auriga*) preference for vivid colours

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Resumo

As cores vívidas são comuns na sinalização sexual e podem fornecer informação relevante sobre a qualidade dos indivíduos. Na sinalização interespecífica, estas cores têm sido principalmente interpretadas como tendo o objectivo de os animais indicarem a que espécie pertencem e também maximizar a detecção do sinal. No entanto, estudos recentes afirmam que, mesmo em relações interespecíficas, as cores vívidas também podem transmitir informações em relação à qualidade do indivíduo, podendo assim ser vantajosas para quem os avalia. Em mutualismos de limpeza, por exemplo, os clientes podem beneficiar ao escolher limpadores que forneçam um melhor serviço. O peixe bodião-limpador (*Labroides dimidiatus*) é uma espécie tropical, que vive em recifes de coral e que participa em interações sociais altamente complexas. Esta espécie apresenta uma coloração azul brilhante que permite aos clientes identificá-los como limpadores (ou seja, sinalização interespecífica). Para além disso, níveis mais elevados de saturação azul foram associados a um serviço de limpeza melhor, fornecendo aos seus clientes mais contacto físico (designada como estimulação tátil). Neste contexto, a cor dos limpadores pode fornecer pistas aos clientes sobre o seu comportamento de limpeza, que desta forma podem usar a informação para tomar decisões mais acertadas. Mas será que os clientes percecionam pequenas diferenças na saturação da cor azul ao avaliar limpadores individuais e usam essa informação na sua escolha? Se sim, a coloração azul do peixe bodião-limpador pode ter evoluído parcialmente como um sinal de qualidade do serviço de limpeza, de forma semelhante ao que ocorre na sinalização sexual em outros modelos animais.

Neste estudo, a espécie de cliente escolhida, o peixe-borboleta (*Chaetodon auriga*), conhecida por ter grandes territórios e assim poder escolher entre diferentes estações de limpeza, foi exposta a três sequências de vídeo de tratamento, com diferentes níveis de saturação de bodiões limpadores *L. dimidiatus* (saturação mínima, controlo e saturação máxima) com o objetivo de testar se os clientes conseguem distinguir entre as saturações apresentadas e se ativamente preferem alguma delas. Os resultados mostram que os clientes *C. auriga* evitam as sequências de vídeo com o limpador menos saturado quando comparadas com as saturações mais altas ou controlo, demonstrando pela primeira vez que os clientes distinguem diferentes saturações e que dessa forma podem usar a coloração azul dos *L. dimidiatus* como um sinal honesto de qualidade individual.

Palavras-Chave: Comunicação interspecífica, Cores vívidas, Mutualismo de limpeza, Saturação, bodião-limpador, Preferência do cliente

Abstract

Vivid colours are common in sexual signalling and can provide information on individual´s quality. In interspecific signalling, vivid colours have been interpreted mostly as a way to identify species and to maximize signal detection. Recent studies however, state that, even in interspecific relationships, vivid colours can also convey information on aspects of individual quality that could be advantageous for receivers to evaluate. In cleaning mutualisms for instance, clients could benefit from assessing and choosing individual cleaners that provide a better service. The Bluestreak cleaner wrasse (*Labroides dimidiatus*) lives in coral reefs and has highly complex social interactions. This species presents a bright blue colouration which enables clients to identify them (i.e., interspecific signalling) and higher saturations of blue have been linked to better cleaning service, with higher rates of physical contact (referred as tactile stimulation) towards clients. In this context, the colour of cleaners might provide cues to cleaner's behaviour, which clients could use to profitably make decisions. This poses the question if clients can perceive fine differences in colour saturation when evaluating individual cleaners, and if they use this information to select them. In this case, the vivid blue colouration of the Bluestreak cleaner wrasse may have partly evolved as a signal for the quality of the cleaning service, similarly to what happens in sexual signalling for other species.

In this study, the client species threadfin Butterflyfish (*Chaetodon auriga*) was subjected to three differently saturated treatment video-sequences of Bluestreak cleaner wrasses *L. dimidiatus* (minimum saturation, control, and maximum saturation) in order to understand if clients could distinguish between the saturations presented and if they would actively prefer any of these saturations. The results show that *C. auriga* actively preferred the video-sequences of the more saturated cleaner when played against lower saturations, providing for the first time, evidence that clients can use the blue colouration of *L. dimidiatus* as a true signal of individual quality when assessing individual cleaners. If *C. auriga* is able to distinguish these saturations it is likely that in the natural environment they use this condition-dependent signal to make an informed decision, this way selecting the cleaners within their territorial area that provide a better cleaning service.

Keywords: Interspecific communication, Vivid colours, Saturation, Cleaning mutualisms, Bluestreak cleaner wrasse, Client preference

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List of abbreviations

LED	Light emitting diode
UV	Ultra-Violet
RGB	Red, Blue, Green
HSV	Hue, Saturation, Value
LWS	Longwave Sensitive
MWS	Mediumwave Sensitive
SWS	Shortwave Sensitive
VIE	Visual Implant Elastomer
GLMM	Generalized Linear Mixed Model

1. Introduction

1.1. Vivid Colours in Animal Communication

Animal communication is the transfer of information by one or several individuals (i.e., sender/s) aiming to influence the decision-making of other(s) (i.e., receiver/s) (Wiley, 1983; Endler, 1993). Animal signals are traits that are specialized for communication and can range from pheromone release (chemical communication) and animal calls (auditory communication) to the bright colours commonly displayed by many birds and fish (visual communication) (Johnstone, 1996; Krebs and Davies, 2009). From the receiver's point of view, the signal is a potential source of information, while from the signaller's perspective a display can directly influence the receiver's behaviour (Krebs and Davies, 2009).

Animal signals can serve many different purposes (Krebs and Davies, 2009), and are present in both intraspecific and interspecific interactions. Intraspecific signals are defined as a stimulus produced and received by individuals of the same species (e.g., to attract a mate or alert offspring of the presence of a predator); interspecific signals, involve a stimulus produced by a sender of one species that has evolved to modify the behaviour of a receiver of another species, generally benefiting the sender (e.g., to deter predators, to attract prey, in competitor mediation and even to facilitate reproduction) (Bradbury and Vehrencamp, 2011; Stevens, 2013; Caro and Allen, 2017). Natural selection usually favours signallers who present eliciting displays (e.g., vivid colours, large ornaments, or distinctive sounds), and which provide beneficial responses, however, it also favours receivers who can better adjust their behaviour in response to the information provided by signals (which extends to ignoring displays that are uninformative) (Krebs and Davies, 2009). According to this theory, for signals to persist they must provide, over evolutionary time, some type of advantage for the signaller and consequently, the receiver will benefit from the information that the signal enables; these are called honest signals (Johnstone and Grafen, 1993; Stevens, 2013; Laidre and Johnstone, 2013; Caro and Allen, 2017); however, there are some signals which seem to be disadvantageous to the receiver (i.e., benefiting only the signaller), which are often referred to as dishonest signals (Caro and Allen, 2017); for example fangblennys (*Plagiotremus rhinorhynchos*) change their colour to mimic the appearance of Bluestreak cleaner wrasse (*Labroides dimidiatus*) in order to approach and exclusively feed on clients' tissue, benefiting only themselves (Cheney, 2013; Caro and Allen, 2017).

Vivid colours are a common example of honest signalling, since they can convey true information of the underlying quality of the sender (Andersson, 1994; Johnstone, 1996; Petak, 2019); these have been linked with enhanced immunity and overall individual health (Mougeot, 2008; Trigo *et al*., 2020) and depending on individual condition, colour traits may reflect different physiological properties. For instance, in sexual signalling (intraspecific communication), vivid colours are frequently displayed, where similarly to other sexual ornaments they are often used to attract mates, by showcasing the quality of the individuals of the selected sex (Anderson, 1994). For conspicuous sexual signals to be evolutionary stable, there must be a consistent correlation between ornament expression and the object of mate assessment (i.e., any type of quality in the selected sex that interests potential mates); therefore, in sexual signalling, vivid colours should only evolve as a conditional dependent signal for the fitness of individuals of the selected sex (Weaver *et al*., 2017). For example, in cowbirds (*Molothrus ater*), females select male partners based on their structural iridescent plumage, which is directly correlated with nutritional state, as shown by the significant reduction of coloration on food deprived males (McGraw *et al*., 2002). Nevertheless, vivid colours are not limited to sexual signalling, as these are also used in same species communication in social contexts (e.g., inform conspecifics of their location), as well as in interspecific communication where they have been interpreted to be selected for maximum effectiveness in terms of species identification (Young, 1971; Coates, 1980). Some flower species, for instance, use visual signals to attract pollinators (birds and insects) to improve the chances of reproduction by pollen transfer (Chittka and Thomson, 2001); consequently, pollinators memorize and discriminate flowers' traits and then associate them with rewards (i.e., flower nectar) (Chittka and Thomson, 2001; Grüter and Ratnieks, 2011). Vivid colours in interspecific signalling systems should therefore be selected as traits that improve memorability or distinctiveness in order to facilitate discrimination (Allen *et al*., 2014; Caro and Allen, 2017); this can be particularly important in different types of interspecific interactions, namely in cleaning mutualisms (Cheney *et al*., 2009).

1.2. Marine Cleaning Mutualisms

Cleaning mutualisms are widespread across both terrestrial and marine ecosystems (Dickman, 1992; Côté, 2000; Grutter 2002; Vaughan *et al*., 2017) and have been reported in many taxa (e.g., fishes, crustaceans, insects, reptiles, birds, and mammals) (Overall, 1980; Perry, 1990; Van Tassell *et al*., 1994). In marine cleaning

mutualisms, these interspecific associations occur between cleaner organisms (usually smaller fish or shrimps, which benefit from removing and eating parasites and infected tissue from the body surface, mouth, or gill chambers of their usually visiting organisms, referred to as “clients” (Losey, 1987; Côté, 2000). Clients also benefit from these interactions, since parasite infestations represent costs for the hosts, and can negatively affect their physiology (Shaw *et al*., 2009), morphology (Seppälä *et al*., 2004) and behaviour (Garnick and Margolis, 1990). When happening with dedicated/obligatory cleaners, these interactions usually occur at established sites, that represent the territories of cleaners (i.e., cleaning stations) (Youngbluth, 2010), which facilitate the repeated encounter between cleaners and clients (Stummer *et al*., 2004). The cleaning service provided by some species of cleaners can vary between different individuals (Wilson *et al*., 2014; Dunkley *et al*., 2019), with both clients and cleaners exerting a degree of selection in these interactions, since roaming clients can make decisions about which cleaners to interact with, by visiting their respective cleaning stations, and on the other hand, cleaners can also choose whether or not to clean visiting clients (Côté *et al*., 1998; Bshary and Noë, 2003). Several other factors can influence the frequency of cleaning interactions, for instance, the body size and abundance of clients are known to significantly impact these interactions (Grutter *et al*., 2005); larger hosts may provide better opportunities for cleaners, due to higher parasite loads (Grutter and Poulin, 1998a, b; Combes, 2001) and higher quantities of mucus (Grutter, 1995; Arnal and Morand, 2001), while on the other hand, more abundant clients increase the probability of encounter with cleaners (Hobson, 1971; Arnal *et al*., 2000; Sasal, 2003).

1.2.1. Cleaning Mutualism Between *Labroides dimidiatus* and their Client Fish

A classic model species to study cleaning interactions is the Indo-Pacific Bluestreak cleaner wrasse (*L. dimidiatus*) (Figure 1), which lives in coral reefs and has highly complex social interactions (Bshary and Côté, 2008) with several client species regularly visiting their cleaning stations (Grutter and Poulin, 1998b).

Fig 1 Bluestreak cleaner wrasse (Labroides dimidiatus), photograph taken by Diego Delso CC BY-SA delso.photo

This species is a protogynous hermaphrodite, which means that individuals first breed as females and eventually change sex to become male harem owners (Roberstson, 1972; Nakashima, 2000; Kuwamura *et al*., 2002). Males are found living and cleaning in pairs and the remaining females usually clean alone (Robertson, 1972). This species most commonly feeds on gnathiid isopods (Grutter, 1996, 1997) which negatively affect their hosts by consuming blood and propagating infections (Grutter *et al*., 2011). Even though cleaners can still act as potential spreaders of ectoparasites, as they are susceptible to infection by gnathiid isopods (Narvaez *et al*., 2022), the presence of cleaner organisms and in particular of *L. dimidiatus* is crucial, since as providers of cleaning services, they can consume up to 1200 parasites per day (Grutter, 1996), consequently reducing the gnathiid infestation rate, causing an overall positive impact on coral reef ecosystems (Grutter *et al*., 2017). However, it has been found that during cleaning interactions, instead of preying on ectoparasites, *L. dimidiatus* sometimes cheat, and opportunistically prefer to consume clients' mucus (Bshary and Grutter, 2005). This behaviour is harmful to clients, since mucus acts as a protective layer, (Soares *et al*., 2017); clients tend to usually respond to cheating by "jolting" (i.e., whole body shudders that appear to be painful) and aggressively chasing cleaners away, effectively ending the cleaning interaction (Bshary and Grutter, 2002; Soares *et al*., 2008; Morado *et al*., 2019). Because of these differences in service quality, visiting clients, must choose amongst potential cleaner fish partners that may or may not cooperate; as some cleaners will mostly forage on client's ectoparasites, while others will feed on mucus (Grutter and Bshary, 2004). As a way to manipulate client behaviour, *L. dimidiatus* provide tactile stimulation (i.e., hovering above the client and touching its dorsal fin area with its pectoral and pelvic fins) which attract clients to their cleaning stations and maintains them in their territories (Bshary and Wurth, 2001; Cheney *et al*., 2008). This not only is favourable to

L. dimidiatus but clients also benefit from this behaviour, since it has been demonstrated that by being exposed to tactile stimulation alone (provided by a mechanical fish model), clients can experience short term benefits, namely stress level reduction (Soares *et al.*, 2011).

1.2.1.1. Cleaners' Colour as a Condition Dependent Signal

Another relevant characteristic of *L. dimidiatus* individuals are their bright colours. Similarly, to other species of labrids and of the genus *Labroides,* which usually present a high array of colours, *L. dimidiatus* is known for their conspicuous bright blue colour, lateral black stripe, and some yellow details on the posterior part of their body (Arnal *et al*., 2006). These colour patterns are deemed the most important signal enabling clients to identify them as cleaners. Stummer *et al*. (2004) found that a larger black stripe increased the number of clients approaching by attracting them at distance, but not at a close range; which may indicate that when clients are near, their interest may be based on other cues such as behavioural (e.g., cheating), sensorial (e.g., tactile stimulation or chemical cues) or even finer colour trait differences (e.g., saturation and/or brightness – Trigo *et al*., 2020). Cheney *et al*. (2009) also hypothesized that the conspicuous blue colouration is the most important signal enabling clients to identify cleaners. *L. dimidiatus* lives in coral reefs of inner lagoons, subtidal reef flats and seaward reefs (Myers, 1991) which usually present shallow clear waters, making this environment highly suitable for signal detection. Because the combination of colours within cleaner fish patterns is highly contrasting against different environment backgrounds, a combination of blue, yellow, and black enables cleaner fish to be highly visible when viewed from different directions (Cheney *et al*., 2009) in a clear example of the importance of vivid colours in interspecific signalling.

It has been traditionally assumed that the blue colouration of *L. dimidiatus* did not evolve through sexual selection, as this species was previously described as sexually monochromatic, with males and females having similar coloration (Pandian, 2010). However, recent findings by Trigo *et al*. (2023) reported blue colour saturation to be positively correlated with size, as well as a trend for the sexes to differ in colour, with males showing a more saturated blue colour compared to that of females. It has also been found that this species coloration might also be a signal of individual quality, since oxidative stress and other challenges to the individual condition (i.e., lower food consumption) decrease their colour saturation, which is in turn correlated with the quality

of their service (Trigo *et al*., 2020). Fish colour is usually composed of pigment and structural components (Price *et al*., 2008), and for most fish, the blue colour is considered structural (Bagnara *et al*., 2007; Leclercq *et al*., 2010). Iridophores (i.e., chromatophores responsible for blue colouration) usually contain purines and pteridines; the spatial distribution of purines may be affected by the physiological state of individuals, which in turn affects their colouration (Grether *et al*., 2004); as for pteridines, depending on environmental conditions, these can either act as, antioxidants or pro-oxidants (Oettl and Reibnegger, 2002) and thus indirectly affect fish colouration (Trigo *et al*., 2020). Therefore, the blue colour saturation of *L. dimidiatus* might not only be an important signal in interspecific species recognition, but it may also be an honest signal for individuals' fitness (Trigo *et al*., 2020, 2023). Because the fitness level of cleaners might affect the quality of the cleaning interaction, as demonstrated by the negative effect of oxidative stress in *L. dimidiatus* service (Trigo *et al*., 2020), the blue colouration of this species might also be perceived by clients as a signal for the quality of their service.

1.2.2. The Model Client Fish: *Chaetodon auriga*

The threadfin butterfly fish (*Chaetodon auriga)* (Figure 2), is one of the most widespread reef fishes on the planet, occurring from Hawaii and French Polynesia to the Red Sea, being a regular client species of *L. dimidiatus* (Grutter and Poulin, 1998b).

Fig 2 Threadfin butterfly fish (Chaetodon auriga), photograph taken from monaco nature encyclopedia, authory by Giuseppe-Mazza

This species presents a black mask, a pattern of black and white stripes, a conspicuous bright yellow colour, and a conspicuous "eyespot" or *ocellum* on the soft dorsal fin (which is not present in the Red Sea population). They are considered an

omnivorous species, foraging mostly on corals and display a mixed social grouping behaviour, being found alone (however rarely), in pairs or in aggregations of three or more individuals (Yabuta, 2007). *C. auriga is* also classified as a territorial species, with territories ranging from $10m^2$ to $1000m^2$, as they need to maintain large territories in order to obtain sufficient resources (Rendall, 2013). As any gregarious animal, they are subjected to parasite infestations and need to have them removed; by maintaining large territories, they could visit different cleaning stations and therefore choose among cleaners who provide a better cleaning service.

1.2.2.1. Clients' Colour Visual Systems

In the past there have been some misconceptions regarding teleost fish visual perception, but recently, several studies have demonstrated that the visual ecology of fish species is more complex than previously assumed (e.g., Cheney and Marshall, 2009; Cheney *et al*., 2009; Siebeck *et al*., 2009; Siebeck *et al*., 2010; Cheney *et al*., 2013; Newport *et al*.,2013; Champ *et al*., 2014; Newport *et al*., 2014; Rosa Salva *et al*., 2014; Newport *et al*., 2017; Newport *et al*., 2018) as the visual systems of fish show similar aspects to other vertebrates (Hunt *et al*., 2014). For instance, fish can accurately discriminate between different objects and even recognize changes in size, which indicates some recognition flexibility (Schuster *et al*., 2004). It has even been observed that fish are able to recognize images displayed on monitors. For instance, Newport *et al*. (2016) tested archerfish (*Toxotes chatareus*) and exposed them to stimuli displayed on an computer monitor presenting them to a two-alternative choice test; the authors demonstrated that archerfish could be trained to discriminate a learned human face from a large number of other human faces displayed on monitor images, even when some trivial cues had been removed (i.e., brightness, colour and head-shape). Using a similar methodology, Newport *et al*. (2018) demonstrated that the archerfish could also recognize rotated objects displayed on monitors. It is also possible to use other tools such as LED (light emitting diode) monitors to study fish vision. LED displays can be very advantageous since they include a broad colour space, precise control over chromaticity and brightness, as well as the relative ease and speed of changing the displayed pattern (Powel *et al*., 2021) and have been used in several studies of vision across some taxa (e.g., insects and fish – Bok *et al*., 2018; Stoddard *et al*., 2020; Kócsi *et al*., 2020; Powel *et al*., 2021). For example, in a study conducted by Powel *et al*. (2021) they even demonstrated that LED displays can be used to study colour vision, including in the ultra-

violet (UV) spectrum, on the clownfish *Amphiprion ocellaris,* showing that similar methodologies can be used to study fish vision.

Reef ecosystems represent some of the most diverse marine habitats and are considered as biodiversity hotspots (Hubert *et al*., 2008). In these habitats, fish usually present bright conspicuous colours and bold patterns. Such colourful displays suggest that vision must be a key aspect in this ecosystem. In coral reef species, the basis of vision involves a set of light-sensitive receptors consisting of a visual opsin protein, bound to a vitamin A derived chromatophore; when excited, the chromophore induces a conformational change in the opsin gene, which initiates the phototransduction cascade resulting in an electric signal to the brain (Darnal, 1976; Cortesi *et al*., 2020). Reef fishes rely on a limited set of photoreceptor types and usually use two to four cone photoreceptors for colour vision (Cortesi *et al*., 2020).

Species from the genus *Chaetodon* in particular, usually present dichromatic visual systems, meaning they have only two photoreceptors involved in colour vision (Losey *et al*., 2003). Trichromats (e.g., humans), possess longwave (LWS), mediumwave (MWS), and shortwave (SWS) sensitive cone types, and these are used to create two opponent colour channels (red – green by comparing LWS to MWS, and blue – yellow by comparing the combination of LWS and MWS to SWS - Kelber *et al*., 2003). Dichromats lack either the LWS (protanopes) or MWS (deuteranopes) cone type, meaning they have no red – green opponent channel (Troscianko *et al*., 2017) not being able to distinguish between red and green, but being able to differentiate blue and yellow (Fig 3). This seems relevant since *Chaetodon* have been found to be visually attracted to their conspecifics and are also able to recognize other coral reef species (Ehrlich, 1977; Fricke, 1986; Reese, 1975) and to discriminate their mates by both visual and olfactory cues (Boyle and Tricas, 2014). The bright coloration and conspicuous colour patterns of many *Chaetodon* is historically thought to facilitate visual recognition (Lorenz, 1962) since territorial species from this genus live in an environment that is suited for visual species recognition; such a mechanism could, for instance, alert fish to the presence of a territorial competitor or the location of a mate after a period of separation (Boyle and Tricas, 2014). Since they are a regular client of *L. dimidiatus* it is also logic to assume that, according to their visual capabilities, they recognize cleaners by their bright blue colour and may exert some choice based on this colour signal.

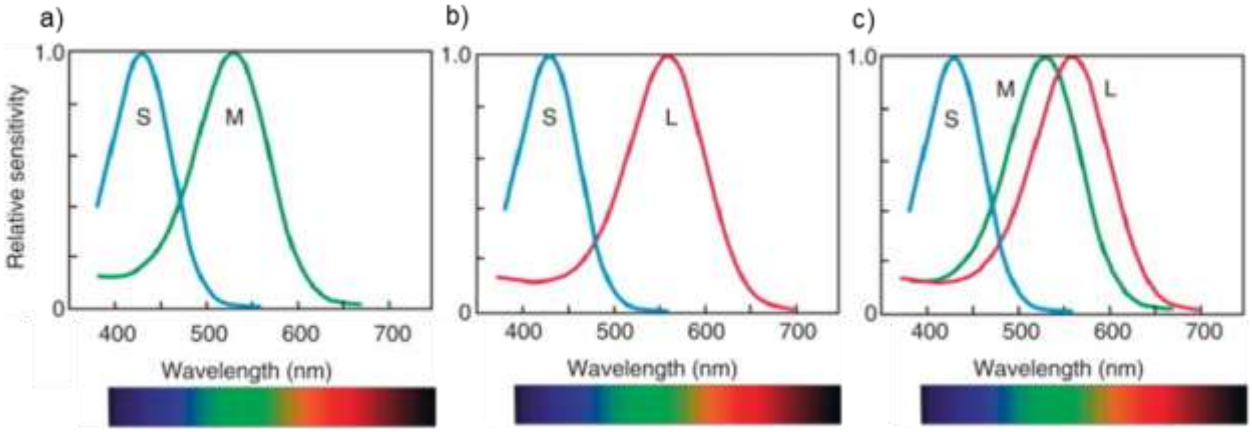

Fig 3 Spectra of SWS (S), MWS (M) and LWS (L) cones and their absence. (a) protanopes, (b) deuteranopes and (c) trichromats. Adapted from Hunt. (2013).

1.3. Objectives

Visual signals seem to be particularly relevant in the mutualistic interactions associated with *L. dimidiatus* cleaners, as these have been demonstrated to be socially complex, due to the frequent conflict of interests between cleaners and clients (Bshary, 2002; Bshary and Grutter, 2002; Bshary and Côté, 2008), underlined by *L. dimidiatus* preference to forage on clients' mucus (which have greater nutritional value) instead of simply removing ectoparasites (Bshary, 2002; Bshary and Grutter, 2002; Bshary and Côté, 2008).

Because cleaning service quality may vary tremendously between individual *L. dimidiatus*, the ability of clients with larger territories, as *C. auriga,* to choose between cleaning stations and individual cleaners is important (Bshary and Shaffer, 2002). In this context, can *L. dimidiatus* blue coloration (a condition dependent signal for individuals' fitness), be yet another tool used by clients to select the cleaners that provide a better cleaning service, given the correlation between fitness level and service quality? If clients can perceive fine differences in colour saturation when evaluating individual cleaners, and if they use this information to select between them, then the vivid blue colouration of *L. dimidiatus* may have partially evolved as a signal for the quality of the cleaning service, analogous to what happens in sexual signalling.

Here, individuals from the species *Chaetodon auriga* were exposed to video sequences of *L. dimidiatus* with different blue colour saturation levels, using a novel experimental design. I predicted that clients would spend more time and interact more with the video sequences of the more saturated cleaner, thus providing evidence that

they in fact can evaluate individual service quality based on visual signals provided by *L. dimidiatus.* To understand if *C. auriga* could also perceive cleaners in the displayed video-sequences (as there were no evidence if this species could perceive motion images on an LED monitor), a validation test was performed prior to the colour choice experience. *C. auriga* was exposed to a video-sequence of a blue ball against a video-sequence of *L. dimidiatus* and their preference was registered. This validation test is also extremely relevant as it provides more evidence of the visual capabilities of reef fish species.

2. Methods

This study was conducted during the months of January and February 2023, at LEOA research centre in CCMAR (Faro, Portugal). A total of 20 threadfin butterfly fish, *Chaetodon auriga* (Forsskål, 1775) and three Indo Pacific Bluestreak cleaner wrasses, *Labroides dimidiatus* (Valenciennes, 1839) were used for this experiment. All individuals were wild caught in the Maldives and directly imported to Portugal by an official supplier (Tropical Marine Centre, Lisbon, Portugal) where they were locally de-parasitized. Upon arrival all butterfly fish were transferred to a glass housing aquaria (82 x 46 x 50 cm) in groups of four individuals per tank, while the cleaner wrasses were kept alone in (41 x 23 x 25 cm) aquarium. All aquaria were connected through a flow-through system which were pumped from a large sump that was equipped with an UV light, a mechanical and biological filter, air supply, a saltwater skimmer and a water heater connected to a thermostat to keep water at a constant temperature rounding 25 ºC. A photoperiod of 12 h was kept throughout the experiment and the individuals were fed twice a day (morning and afternoon) with mysis shrimp. Vitamins and minerals were also given as a complement to diet in both species. Concentration of ammonia, nitrites and nitrates was regularly checked to ensure that the levels were kept to the lowest possible, never surpassing 0.3 mg. L^{-1}. The water in the housing tanks was also constantly monitored for other parameters including pH, O_2 concentration and salinity. Environmental enrichment such as PVC pipes (20 cm long; 10 cm diameter for the clients and 10 cm long; 3 cm diameter for the cleaners) and artificial corals were placed in the aquaria to serve as shelter for the fish.

Before the start of the experiments Visual Implant Elastomer (VIE) tags (Northwest Marine Technology, 2008) were implanted on all individuals which were previously anesthetized with 2-phenoxyethanol 150 ppm for 2 minutes. All tagging equipment was prepared and used following the respective user's manual. Individuals were acclimatized for 10 days in the housing tanks prior to the start of the experiment. Animal procedures used in this study were submitted to ORBEA of CIBIO and CCMAR (in review) and the facilities where the study was conducted are licenced by DGAV.

2.1. Video collection and colour manipulation

Prior to the start of the experiment, we collected several videos of the cleaners freely swimming in its housing tank for three days with a GoPro HERO7 positioned inside the tank. The videos were thoroughly analysed in order to select a sequence with the better quality, in which the cleaner was constantly in the video frame. After selecting the best sequence, Adobe Premiere Pro 23.4 (2023) was used to manipulate the blue colour saturation of the cleaner. To do this, we selected the point in the fish where the blue colour was most saturated and then using the Hue vs Saturation function, the saturation level was adjusted as desired. A total of three alternatives of the same video-sequence showing a cleaner were made: 1) the control (saturation = 0.75), an unedited video-sequence with the standard blue saturation of the cleaner fish that was recorded; 2) a maximum saturation video-sequence (saturation = 1), where the blue colour saturation was exaggerated to the maximum; and 3) a minimum saturation video-sequence (saturation = 0.08), where the blue colour saturation was minimized (Figure 4). To extract the saturation values from the images, the RGB (Red, Blue, Green) values were also collected and were then converted into HSB (hue, saturation, and brightness) with the R function rgb2hsv. This converts the red, green, and blue values of an RGB image into hue, saturation, and value (HSV) values of an HSV image. It is important to note that the video of the cleaner remained the same and only the blue saturation of the cleaner was manipulated. All three videos were edited to be in constant loop for 20 minutes. An animation of a blue ball (Figure 5) was also made using the Adobe Animate 23.0.0 (2023) software, where the ball moved across all sides of the monitor in a 20-minute motion loop. The colour blue was chosen to mimic the natural coloration of *L. dimidiatus*, as it represents the most important signal for clients to identify them (Cheney *et al*., 2009).

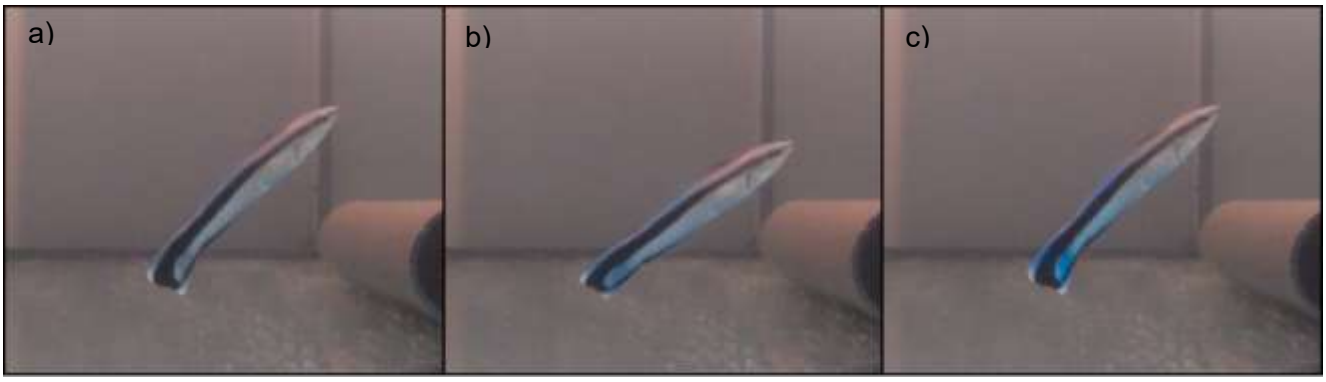

Fig 4 Representation of the colour manipulated video-sequences of *L. dimidiatus* presented to *C. auriga* (a) minimum saturation, (b) control and (c) maximum saturation.

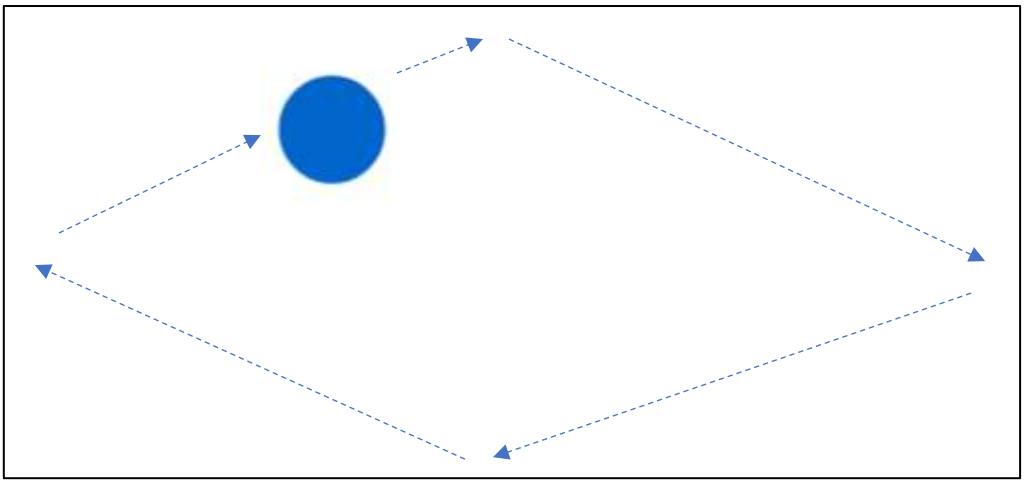

Fig 5 Visual representation of the video-sequence of the blue ball presented to *C. auriga.*

2.2. Experimental design

Because the client, *C. auriga* and the cleaner, *L. dimidiatus* both originate from Maldives, they were considered sympatric species and therefore clients were familiarized with the cleaner species. This experiment was conducted for five weeks, as each individual was used for four consecutive days. To perform this experiment, we had an experimental tank (200 x 40 x 46 cm) with two LED monitors (10.1 in., Huawei Matepad T10s) attached to opposite sides of the tank, each of which played different video-sequences that were remotely activated through a computer in order to avoid any human interference during the trials (Figure 6).

Fig 6 Visual representation of the experimental tank. Experimental tank was divided into two preference areas, (a) right and (b) left, and a neutral area which was not considered in the analysis. Each side of the tank had a monitor displaying the treatment videos.

Following 10 days of acclimatization, clients were individually placed in the experimental tank which was connected to the same sump as the housing aquaria. To

minimize any interference in the results due to the transportation to the experimental tank individuals were acclimatized for 5 minutes prior to the start of the observations, where they could freely explore the aquarium. After the acclimatization period the trials were divided into two phases: the first, to check if there were any a priori preferences for one of the sides of the tank, in which individuals were filmed for 10 minutes without any stimulus; and the second, where we filmed clients for 20 minutes being exposed to the treatment videos with the different colour saturations playing synchronously on each side of the tank. On the first day of each week, all tested individuals were subjected to a validation test, to see whether they could distinguish between the video-sequence of a cleaner and the video-sequence of novel object (a blue ball). To do this, one of the monitors was playing the control video-sequence of a cleaner wrasse, while the other was playing an animated video of a blue ball. After the validation test, on each test day, one of three video-sequences (i.e., treatments) was assigned to each focal individual in a balanced manner: minimum/control (minimum saturation against control); control/maximum (control against maximum saturation); and maximum/minimum (maximum saturation against minimum saturation) (Table 1). Each trial was recorded for a total of 30 minutes with a GoPro HERO7 positioned in front of the experimental tank. A total of 80 videos of 30 minutes were recorded, of which 20 corresponded to the validation test trials and 60 to the choice test trials. To minimize any possible chemical signals, a 30-minute interval was conducted before each trial in order to have water renovation. To minimize interfering in the results the experimenter left the room during the observation period.

2.3. Behavioural observations

To quantify the preference of the cleaner towards each treatment, the experimental tank was divided into two preference areas (left and right), one on each side with 20 cm from the edges (Fig 5). Four behaviors were recorded for each trial: 1) Time in preference area: total time spent in each preference area of the tank in seconds; 2) Frequency of entries in preference area: number of times a client entered the preference area; 3) Facing time: time spent facing each monitor (i.e., the client is directly looking at the monitor), in seconds; 4) Facing frequency: number of times a client faced each monitor. When performing the *a priori* preference analysis solely the first 10 minutes of every video (n= 80) were considered. Video recordings were blindly analysed to the

experimental treatment of each individual using the Behavioural Observation Research Interactive Software - BORIS (Friard and Gamba, 2016).

Table 1 Distribution of the treatments to each individual (n = 20). On the first day, a validation test was performed (fish vs ball), on following days treatments were played (control, maximum or minimum saturation) in a balanced design. Right/ left correspond to the side where the videos were displaying in each monitor.

		Treatments (right /left)			
ID	**week**	**Day 1**	**Day 2**	**Day 3**	**Day 4**
1	1	validation test	maximum / minimum	control / maximum	minimum /control
2	1	validation test	control / maximum	minimum / control	maximum / minimum
3	1	validation test	minimum / control	maximum / minimum	control / maximum
4	1	validation test	maximum / minimum	control / maximum	minimum / control
5	2	validation test	control / maximum	minimum / control	maximum / minimum
6	2	validation test	minimum / control	maximum / minimum	control / maximum
7	2	validation test	maximum / minimum	control / maximum	minimum / control
8	2	validation test	control / maximum	minimum / control	maximum / minimum
9	3	validation test	minimum / control	maximum / minimum	control / maximum
10	3	validation test	maximum / minimum	control / maximum	minimum / control
11	3	validation test	control / maximum	minimum / control	maximum / minimum
12	3	validation test	minimum / control	maximum / minimum	control / maximum
13	4	validation test	maximum / minimum	control / maximum	minimum / control
14	4	validation test	minimum / control	maximum / minimum	control / maximum
15	4	validation test	maximum / minimum	control / maximum	minimum / control
16	4	validation test	control / maximum	minimum / control	maximum / minimum
17	5	validation test	minimum / control	maximum / minimum	control / maximum
18	5	validation test	maximum / minimum	control / maximum	minimum / control
19	5	validation test	control / maximum	minimum / control	maximum / minimum
20	5	validation test	minimum / control	maximum / minimum	control / maximum

2.4. Statistical analysis

Data was checked for homogeneity, normality, collinearity, and possible outliers (Zuur *et al*., 2010). First, I analysed whether there were any *a priori* preferences for one of the areas of the tank (left or right), using a Wilcoxon test, with Time in preference area and Frequency of entries in preference area as response variables, and the Preference area as a two-level factor (left and right).

2.4.1. Validation Test

For the validation test, preference for either areas of the tank (right and left) was tested using a Generalized Linear Mixed Model (GLMM), where the behavioural variables (Time in preference area, Frequency of entries in preference area, Facing time and Facing frequency) were set as a dependent variable, treatment as a two-level factor (ball and cleaner), Preference area as another two-level factor (left and right), and focal individual as a random factor. When performing the validation test, the two-level factor Preference area (left and right) was removed from the analysis, since no a priori preferences for the areas of the tank were detected. Distributions and link functions for each model were chosen according to the distribution of the dependent variable and were evaluated through skewness-kurtosis plots and quantile-quantile plots. For Time in preference area and Facing time, a Gamma distribution with a log link function was chosen to accommodate the positively skewed nature of the response variables. For the dependent variables Frequency of entries in preference areas and Facing frequency, a gamma distribution with a zero-inflated model and an inverse link function was used.

2.4.2. Colour Choice Test

To test for the preference for different blue colour saturations a GLMM was also used. Here the same behaviours were set as a dependent variable, colour as a three-level factor (maximum saturation, control, and minimum saturation), Preference area as a two-level factor (left and right) and focal individual as random factor. Similarly, to what we did in the validation test, the Preference area was also removed from this part of the analysis, since no statistically significant differences were detected for the areas of the tank. Skewness-kurtosis plots and quantile-quantile plots were also used here to choose which distributions and link functions of the dependent variable were best for each model. A Gamma distribution with a log link function was used for the dependent variables: Time in preference area and Facing time. A gamma distribution with a zero-inflated model and an inverse link function was used for the dependent variables: Frequency of entries in preference area and Facing frequency. A zero-inflated model was incorporated in part of the analysis to address excessive zeros present in our dataset. By considering this, we aimed to accurately capture the complexities of our data and provide a more comprehensive analysis. Normality of model residuals was confirmed through residual plots (scatter plots), for each variable. When significant differences were found upon

conducting the GLMM analysis, a post hoc comparisons between treatments of each model were made using Tukey's Honestly Significant Difference test with the Bonferroni adjustment (Lenth, 2016). For all comparisons, the significance level was set at $p < 0.05$. Statistical analyses were carried out using the open-source software RStudio (version 2023.03.1-446). Residual plots (scatter plot) were made in R package RVAideMemoire (version 0.9-82-2; Herve, 2023), quantile-quantile plots were made in the R package car (version 3.1-2; Fox and Weisberg, 2011), the remaining GLMM analyses were made in R package lme4 (version 1.1-33; Bates *et al*., 2015) and glmmTMB (version 1.1.7, Brooks *et al*., 2017) and the post hoc test were performed in R package lsmean (version 2.30-0, Lenth 2016).

3. Results

Regarding the *a priori* preference test for the side areas of the tank no statistically significant side differences were detected for the variable Time in preference area found (Z = 1396, p = 0.473) and Facing time found (Z = 810, p = 0.725) (Figure 7) when performing the Wilcoxon test. Similarly, when testing for Frequency of enters in preference area (Z = 1686, p = 0.248) and Facing frequency (Z = 614, p = 0.592), no significant differences were also found (Figure 7).

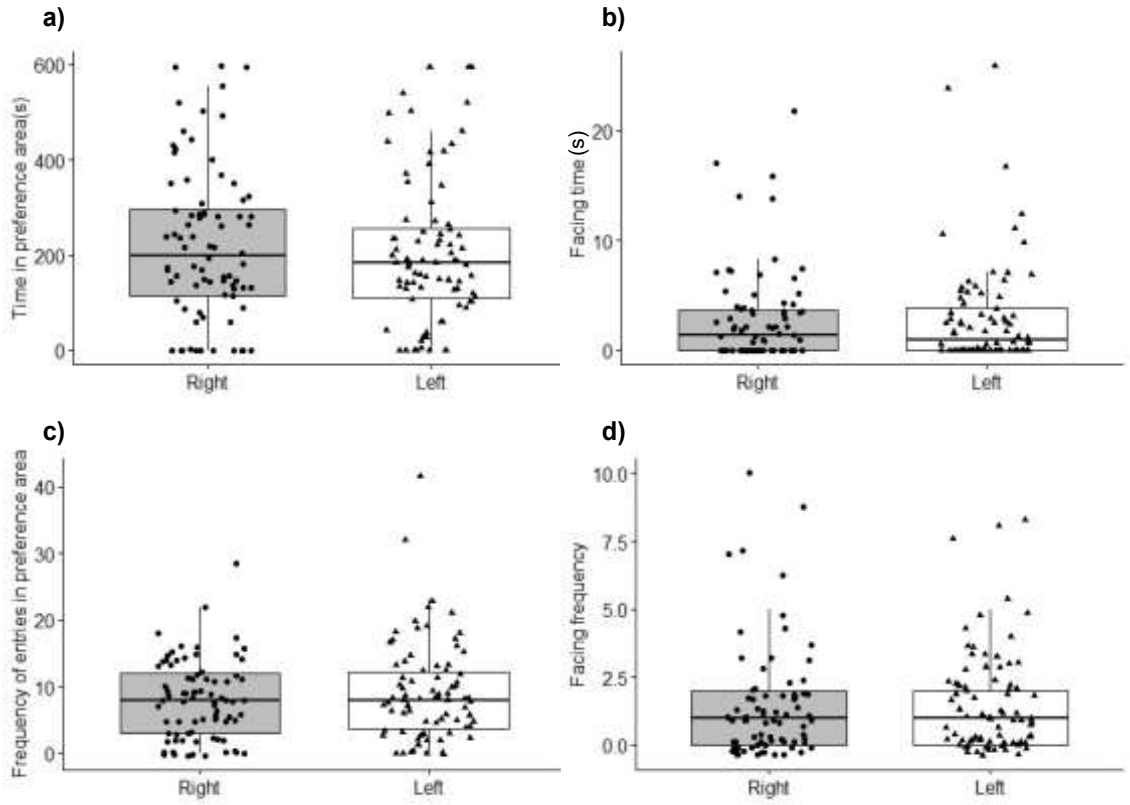

Fig 7 A priori preferences for the areas of the experimental tank. Individuals were placed in the experimental tank without any stimulus and (a)Time in preference area, (b) Facing Time, (c) Frequency of entries in preference area) and (d) Facing frequency were recorded. Wilcoxon test was performed for comparisons between groups.

3.1. Validation Test

Statistically significant differences were detected when comparing the Facing time (GLMM, $\chi 2$ = 6.21, df = 1, p = 0.013) as individuals significantly faced more time the monitor displaying the video-sequence of the cleaner (10.30 ± 9.80 s) compared to the video-sequence of the ball (4.05 ± 4.67 s) (Figure 8, Table 2). This was also observed

for the Facing frequency (ziGLMM, $\chi2 = 17.99$, df = 1, $p < 0.001$) where the video-sequence of the cleaner was also more frequently faced (4.08 ± 3.39) than the video-sequence of the ball (1.78 ± 1.61) (Figure 8, Table 2). No statistically significant differences were found for the variable Time in preference area (GLMM, $\chi2 = 1.63$, df = 1, $p = 0.202$) (Figure 8, Table 2) and Frequency of enters in preference area (ziGLMM, $\chi2 = 0.86$, df = 1, $p = 0.353$) towards the video sequence of the ball or the cleaner (Figure 8, Table 2). For more information refer to appendix.

Table 2 Generalized linear mixed models (GLMM) for the dependent variables Time in preference area, Frequency of entries in preference area, Facing time and Facing frequency. *C. auriga* were exposed to two treatments: video sequences of an animated blue ball (Ball) and a video-sequence of a cleaner (Cleaner) (BlueStreak cleaner wrasse). Presented are Mean ± standard deviation, Chi-square test (χ2), degrees of freedom (Df) and p_values.

	Ball	Fish	χ2	Df	p_value
Time in preference area (s)	155±123	232±164	1.63	1	0.202
Frequency of entries in preference area	7.08±4.66	8.45±6.35	0.86	1	0.353
Facing Time	4.05±4.67	10.3±9.80	6.21	1	**0.013**
Facing Frequency	1.78±1.61	4.08±3.39	17.99	1	**<0.001**

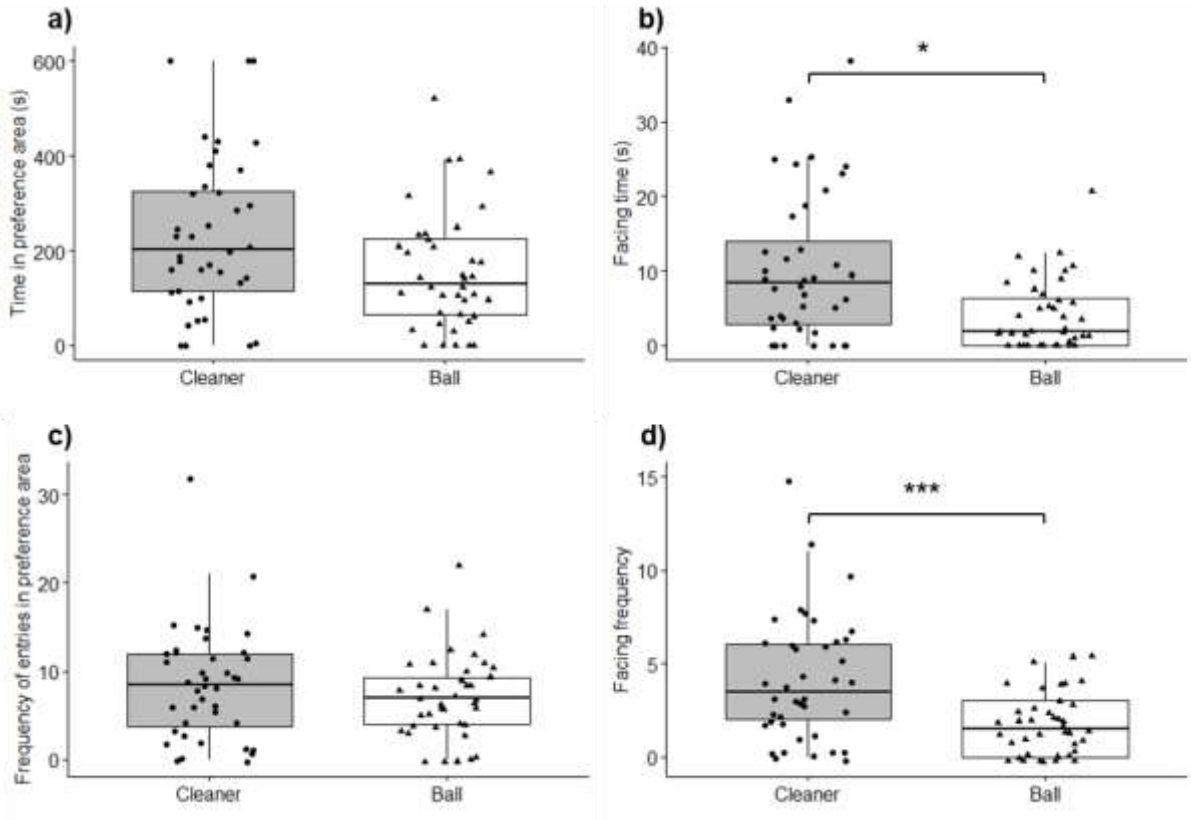

Fig 8 *C. auriga* preference for monitors playing video-sequences of a fish (BlueStreak cleaner wrasse) vs an animated blue ball. Individuals were placed in the experimental tank and (a)Time in preference area, (b) Facing Time, (c) Frequency of entries in preference area) and (d) Facing frequency were recorded.

3.2. Colour Choice Test

For the colour choice test analysis, the mixed model showed statistically significant differences for the Frequency of enters in preference area (GLMM, $\chi2 = 12.64$, df = 2, $p = 0.002$). This was also observed for the Facing frequency (GLMM, $\chi2 = 13.21$, df = 2, $p = 0.001$) (Table 3). In terms of Time in preference area, no statistically significant differences were detected (GLMM, $\chi2 = 4.12$, df = 2, $p = 0.128$) (Table 3). The same pattern was also observed for the Facing time, where no significant differences were also detected (GLMM, $\chi2 = 0.59$, df = 2, $p = 0.746$) (Figure 9, Table 3). The Tukey's post hoc comparison test showed that for the Frequency of enters in preference area, minimum saturation, and control ($p = 0.001$) and minimum saturation and maximum saturation ($p = 0.001$) differed significantly, where on average, the individuals entered more frequently the areas where the monitor was displaying the video-sequence of the maximum saturated cleaner ($\bar{x} = 2.71 \pm 2.90$), rather than the control ($\bar{x} = 1.56 \pm 1.82$) and the minimum saturation ($\bar{x} = 1.81 \pm 2.19$) (Figure 9 Table 3). Tukey´s test also demonstrated that for the Facing frequency, statistically significant differences were found between the maximum saturation and minimum saturation video-sequences ($p < 0.001$) where individuals faced more often the maximum saturated cleaner ($\bar{x} = 2.71 \pm 2.9$) rather than the minimum saturation cleaner ($\bar{x} = 1.56 \pm 1.82$) (Figure 9, Table 3).

Table 3 Generalized linear mixed models (GLMM) for the dependent variables Time in preference area and Facing time. Zero inflated Generalized linear mixed models (ziGLMM) for the dependent variables Frequency of enters in preference area and Facing frequency. *C. auriga* were exposed to three treatments of video-sequences of a *L. dimidiatus* across three different saturation levels (maximum saturation, minimum saturation, and control). Mean ± satandard deviation, Chisquare test (χ2), degrees of freedom (Df) and p_values.

	Maximum Saturation	**Minimum Saturation**	**Control**	**χ2**	**Df**	**p_value**
Time in preference area (s)	250±172	148±144	261±168	4.12	2	0.128
Frequency of entries in preference area	9.51±9.07	5.99±4.72	8.18±6.36	12.64	2	**0.002**
Facing Time	5.28±5.95	4.02±6.62	4.49±8.67	0.59	2	0.746
Facing Frequency	2.71±2.9	1.56±1.82	1.81±2.19	13.21	2	**0.001**

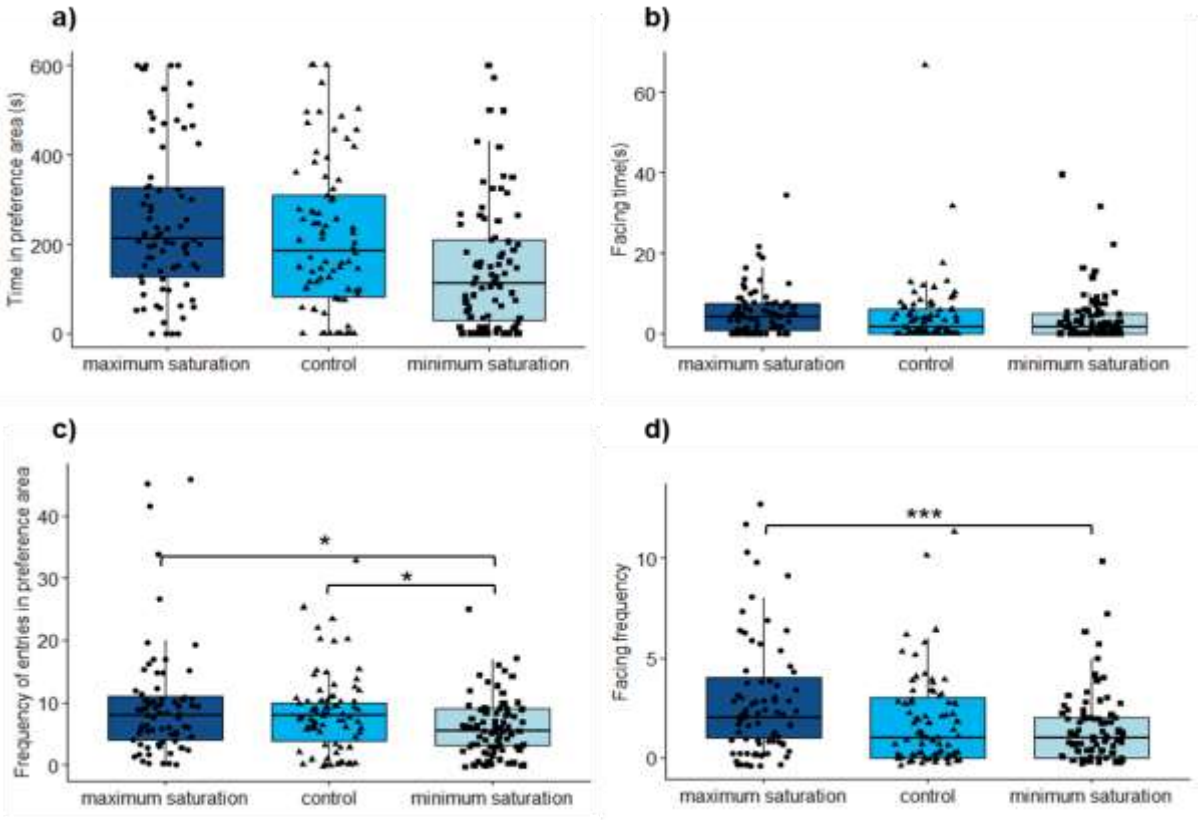

Fig 9 *C. auriga* preference for monitors playing video-sequences of a cleaner (*L. dimidiatus*) across three different saturation levels (maximum saturation, minimum saturation, and control). Individuals were placed in the experimental tank and (a)Time in preference area, (b) Facing Time, (c) Frequency of entries in preference area) and (d) Facing frequency were recorded.

4. Discussion

In the present study I aimed to understand if the client *C. auriga* demonstrates an active preference towards different saturation levels (maximum saturation, control, and minimum saturation) in the blue coloration of *L. dimidiatus*, by presenting the chosen model to differently saturated video-sequences. To demonstrate this, I also needed to validate the method by testing if the client model could perceive the presented video-sequences displayed as a cleaner. In this way, I repeatedly exposed the client species to video-sequences of a blue ball against a cleaner and recorded its preference. For the validation test, I found that the client could perceive both images by facing more time and more frequently the video-sequence of *L. dimidatus*. As for the colour choice test, I found that the chosen model was able to distinguish between different blue colour saturations of *L. dimidiatus* by having different responses to the treatments, by swimming in the proximity and by facing the more saturated video-sequences more frequently. The results demonstrate, for the first time, that clients respond to the blue colour saturation of this species as a signal of individual quality (Trigo *et al*., 2020; Trigo *et al*., 2023) using it as a relevant criterion during cleaning mutualistic relationships.

4.1. Can Clients Identify Cleaners in Video Images?

It is already known that some fish species can accurately distinguish different objects and can even discriminate between faces of other individuals (Schluessel *et al*., 2014; Newport *et al*., 2018; Khoda *et al*., 2023) which can be extremely relevant for predator deterrence, food selection and even in the context of social interactions. Clients can recognize *L. dimidiatus* in the natural environment, either by their bright blue coloration (Cheeney *et al*., 2009) or because of their size and lateral black stripe (Stummer *et al*., 2004). However, it was not clear if the client fish *C. auriga* were able to make sense of video images of *L. dimidiatus* and distinguish it from an unknown moving object. The results from the first experiment, the validation test, demonstrated that client fish are able to distinguish the video images of *L. dimidiatus*, when these are displayed on a monitor. Clients responded to the video-sequence of *L. dimidiatus* by significantly remaining nearby during longer bouts of time and by facing the *L. dimidiatus* stimulus more often, compared to the video-sequence of a blue ball. The fact that clients faced more the video-sequences of *L. dimidiatus* may be explained because they recognized

the video-sequence as familiar image, as *C. auriga* are a regular client of *L. dimidiatus* in the natural environment. No significant differences were however detected for the time and frequency of moving into the preference area, which might be related to the fact that while recognizing the video-sequence as a cleaner, because they were not able to interact with them, they would probably lose interest and leave. In the wild, the so-called clients (as *C. auriga*) are not willing to wait for a cleaning interaction (Bshary, 2002); in this way, either they are immediately inspected once they arrive to a cleaning station, or they leave in search for another cleaner. Thus, in captivity it also made sense to observe that *C. auriga* individuals maintained the same behavioural response: they were interested in approaching the video sequence of cleaners, but they did not stay for longer. This experiment acted as a validation of the methodology but has also shown that the chosen model species is able to differentiate between stimuli, similarly to other fish species that have been shown to distinguish objects in static images (e.g., Cheney *et al*., 2013; Newport *et al*., 2018). It is likely that other reef associated client species should also be able to distinguish between cleaners and other unknown objects displayed on monitor images. Thus, it would be interesting to extend this methodology to other fish species, in accordance with their territorial range size (species with larger territories vs others with smaller territories that may just visit the closest cleaner around).

4.2. Clients' preference for vivid colours

In the second experiment, the aim was to understand whether the model client fish could distinguish between the different blue colour saturations of *L. dimidiatus*. Indeed, comparing to lower saturations, clients faced more frequently and entered the preference area more often if more saturated *L. dimidiatus* were being displayed (Fig. 8); however, again, as in the validation experiment, while responding more frequently to more saturated stimuli, the client fish model did not necessarily spend more time nearby. This could be explained because, while more saturated stimuli could still evoke a response by clients, they at some point learn that no physical interaction is possible and therefore, there is no reason to stay for longer. Clients usually go to *L. dimidiatus* with the intention of being cleaned and expect some physical contact and parasite removal. Moreover, as explained above, clients with larger territories choose between cleaners and if ignored, clients just leave, this way punishing the cleaner as these lose the opportunity to feed on their surface and to provide a cleaning service (Bshary and Shaffer, 2002). Since clients were only subjected to video-sequences and could not

engage in any physical interaction, they most likely lost interest after some time and consequently, stopped facing the video-sequence of the cleaner and left the preference area, in search of another cleaner that would interact. Significant behavioural differences were only observed in frequencies (entering the preference area and facing the more saturated stimuli), which indicates that *C. auriga* individuals were more interested in more saturated video-sequences and therefore are more responsive towards more saturated cleaners.

Clients responded differently across the three levels of saturation tested. Indeed, *C. auriga* entered more often the areas in which both the maximum saturated and the control video-sequences were being shown, compared to the minimum saturated videos; moreover, clients did not demonstrate a significant preference when the maximum saturated video-sequence was tested against the control (Fig 8). As for the observed differences in facing frequency, these were only significant when the maximum saturation was compared against the minimum saturation. This means that clients may be able to detect cleaners in very low condition (with very low blue colour saturation), however above a certain threshold there may not be much difference in the quality of service provided by cleaners with higher saturation levels, when compared to medium saturations. In the wild, cleaners are known to show tremendous variation of cleaning service, especially those that are found alone (most likely females). For instance, Bshary, (2002) suggested that cleaner fish could be separated in two distinct classes; one that rarely bites larger and/or choosier clients (including *C. auriga* for instance), and one that frequently bites larger clients. Later, Soares *et al*. (2014) discovered that cortisol was mediating cleaners behavioural switch, from those that bite (cheat), to those that cooperate with these more important and choosier clients. Considering that cortisol is associated with body energetic demands, associated with life-changes and health, it could be that these cheating individuals, that have higher levels of cortisol may also show lower saturation levels of blue. For instance, Lewis *et al*. (2017) have demonstrated that high glucocorticoid levels decrease structural ornamental colour expression in tawny dragon lizards (*Ctenophorus decresii*), which use their colour as an honest signal of their individual quality. Furthermore, higher glucocorticoid concentrations in birds result in poorer quality feathers in terms of colouration (Roulin *et al*., 2008), which have also been linked to their reproductive investment (Love *et al*., 2014). Hence, if this blue colouration of *L. dimidiatus* is related to their cortisol levels, which in turn reflects in their service quality, clients could benefit from this colour-dependent signal as another tool to be able to distinguish these less cooperative and low saturated cleaners.

Another possible explanation might be related to the visual acuity of the clients. Several studies have reported that marine fish can discriminate colours (e.g., Marshall *et al*., 2003; Siebeck *et al*., 2008; Champ and Marshal, 2016; Escobar-Camacho *et al*., 2019) however when we focus on saturation only it is not clear whether clients can perceive these fine differences (particularly for higher saturation levels) and distinguish between very similar saturations of blue. They may be able to differentiate the highest and lowest saturation levels of the spectrum, but when clients are comparing them with medium saturations (i.e., the control video-sequence) they may not be able to detect these differences. The main signal that enables clients to detect *L. dimidiatus* is through the conspicuous blue colouration (Cheney *et al*., 2009) and therefore it is logic to assume that more saturated levels of blue might be preferred and potentially those that provide better cleaning service.

Our findings suggest that clients are able to use the blue colour saturation as a selection criterion when choosing a cleaner. Given that this client species can differentiate between the saturations of blue of *L. dimidiatus*, it is also possible that this system works in a similar way to those of pollinated plants, which also use colour (and other traits) to attract flower visitors to achieve pollination (Giurfa *et al*., 1995; Lunau and Maier, 1995; Goyret *et al*., 2008). The flower visitors identify specific plants by associating reward (pollen) quantity and quality after visiting a particular-coloured flower. After several visits, pollinators can predict the reward probability of a particular flower type and thus may develop floral (colour) preferences based on previous experience (Streinzer *et al*., 2019, 2021). This could also be the case for *L. dimidiatus* clients. The quality of the cleaning service can vary significantly among individual cleaners, which makes clients very selective when visiting cleaning stations (Bshary and Shaffer, 2002) and it has also been shown that clients usually eavesdrop on cleaners and are able to select the ones who provide better cleaning service (Pinto *et al.,* 2011) and perhaps they can also associate the colour of *L. dimidiatus* with reward (e.g., less cheating and more tactile stimulation). By being able to differentiate the blue saturation of cleaners, clients likely use this condition-dependent signal to select the best cleaning service. This can be explained since the blue colour saturation of the Bluestreak cleaner wrasse represents an honest signal of the individual's health, being also related to the quality of their cleaning service (Trigo *et al*., 2020).

4.3. Environmental Implications

It is widely known that the natural environment has been shaped by human intervention. In the last decades, it has been observed a rapid acceleration of habitat loss, and the rate at which these alterations have occurred, do not allow for individuals to naturally adapt (Heinrichs *et al*., 2016; Greenspoon and Spencer, 2021). This can have consequences in several aspects of the visual ecology of many taxa, and the cleaning mutualism between *L. dimidiatus* and their clients, may also be subjected to these changes. For instance, only individuals with good access to their necessary resources can express costly phenotypes. As mentioned before, the colouration of *L. dimidiatus* is composed mainly by structural components (purines and pteridines) which give them their natural blue colour, and which have been linked to individual quality. Even though structural coloration is primarily dependent on the incidence of light in the environment, some aspects of structural colours are constrained by protein availability in the diet (Prum, 2003, 2006). Anthropogenic pressures are causing changes in habitats, particularly in coral reefs, with serious losses of coral species and total cover, with increase algae cover and rubble. This may change colour background and hence the potential relative value of species colour nuances, leading to a putative change of food resources and influencing individual's physiology and pigment production (Koneru and Caro, 2022). Furthermore, individual pigmentation could also be affected by pollution as pharmaceuticals, pesticides, and heavy metals, which have been linked to impact animal colouration (Pacyna *et al*., 2018).

In this context, if habitat change can lead to a decrease in pigment production, colour value and salience, and if clients really do use the coloration of *L. dimidiatus* as a signal for individual quality, this can impact their cleaning interactions and possibly lead to a decrease in the number of clients visiting the cleaning stations. Therefore, further investigating how habitat chance can influence colour signalling in cleaning mutualisms is extremely relevant.

5. Conclusions

In summary, it appears that the selected model client fish *C. auriga* is able to respond to different saturations of the blue colouration of *L. dimidiatus* being less responsive towards less saturated individuals. Since clients need to choose between individual *L. dimidiatus*, due to differences in service quality (e.g., receiving a poor service is costly since it can manifest in higher parasite loads, losing live tissue and mucus and/or by wasting time) perhaps by associating this blue colour saturation to the quality of service, clients may use this colour signal to make an informed decision before starting the interaction. It is also noteworthy that because the saturations in the video-sequences were manipulated, these might not correspond to real variations on the blue colour of the *L. dimidiatus* in the natural environment, as no studies have yet tested for this preference in real individuals. In this context it is worth exploring in future research how client fish make use of their visual mechanisms to distinguish between different saturations of blue, and also if those with higher saturations are indeed visited more frequently.

6. References

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Appendix

Table A 1 GLMM results from the Validation test of the relation between Time in the preference area and Facing time with the monitor. Coefficients (β), standard errors (SE), t and p-values

	β	SE	t value	p_value
Time in prefrence area Monitor: Cleaner	0,4046	0,32	1,277	0,202
Facing time Monitor: Cleaner	0,9372	0,38	2,493	0,0127

Table A 2 Conditional models of zero-inflated GLMMs for the Validation test, relating Time in preference area and Facing time, with the monitor. Coefficients (β), standard errors (SE), Z and p-values

conditional	β	SE	z value	p_value
Frequency of entries in preference area Monitor: Cleaner	-0,01445	0,02	-0,929	0,353
Facing frequency Monitor: Cleaner	-0,206	0,05	-4,242	0,0000222

Table A 3 Zero-inflated models of zero-inflated GLMMs for the Validation test. Coefficients (β), standard errors (SE), Z and p-values

zero-inflated	β	SE	z value	p_value
Frequency of entries in preference area Monitor: Cleaner	-0,8398	0,97	-0,867	0,386
Facing frequency Monitor: Cleaner	-0,5812	0,55	-1,064	0,28747

Table A 4 GLMM results from the Choice test of the relation between Time in the preference area and Facing time with the monitor. Coefficients (β), standard errors (SE), t and p-value

	β	SE	t value	p_value
Time in prefrence area Monitor: maximum saturation	0,1461	0,2684	0,544	0,586
Monitor: minimum saturation	-0,3814	0,2684	-1,421	0,155
Facing time Monitor: maximum saturation	0,1637	0,3587	0,456	0,648
Monitor: minimum saturation	-0,1088	0,3587	-0,303	0,762

Table A 5 Conditional models of zero-inflated GLMMs for the Choice test, relating Time in preference area and Facing time, with the monitor. Coefficients (β), standard errors (SE), Z and p-values

conditional	β	SE	z value	p_value
Frequency of entries in preference area				
Monitor: maximum saturation	-0,005595	0,009801	-0,571	0,56811
Monitor: minimum saturation	0,036819	0,012628	2,916	0,00355
Facing frequency				
Monitor: maximum saturation	-0,05622	0,03865	-1,454	0,1458
Monitor: minimum saturation	0,10219	0,04883	2,093	0,0364

Table A 6 Zero-inflated models of zero-inflated GLMMs for the Choice test. Coefficients (β), standard errors (SE), Z and p-values

zero-inflated	β	SE	z value	p_value
Frequency of entries in preference area				
Monitor: maximum saturation	0,0874	0,009801	-0,571	0,56811
Monitor: minimum saturation	-8,759E-07	0,01268	2,916	0,00355
Facing frequency				
Monitor: maximum saturation	-0,7424	0,3739	-1,986	0,0471
Monitor: minimum saturation	-0,3166	0,357	-0,887	0,3751