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Subordinate submissive responses are predicted by dominant behaviour in a cooperatively breeding fish

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Abstract

In complex social groups, animals rely on communication to facilitate priority access to resources and minimise the costs of conflict. Animals typically have more aggression signals than submission signals. However, some social species do show multiple submission signals, and the context in which these different signals are used is often not well understood. In the current study, we assessed agonistic interactions within groups of the cooperatively breeding daffodil cichlid fish (*Neolamprologus pulcher*) to investigate the relationship between the aggressive behaviours of the dominant breeding pair, and the submissive responses of the highest ranked subordinate within the group. Daffodil cichlids may respond to aggression by fleeing or by the production of either a tail quiver display or a head up display. Among the two submission signals, the tail quiver display was used more frequently in response to a threat display while head up displays were produced approximately equally in response to both threat displays and overt aggression. An exaggerated version of the head up display was given more often in response to overt aggressions, suggesting a graded submissive response both within and between the two submission signals. Within fish, the frequency of head up displays, but not tail quiver displays, correlated positively with the frequency of threat displays received. The current study helps us to better understand the use of submission signals in a highly social vertebrate and sheds light on submission as an understudied aspect of communication.

Keywords: aggression; communication; daffodil cichlid; *Neolamprologus pulcher*; signalling; submission

1. Introduction

Conflict is costly, and costs may not differ substantially between the winner and loser of an interaction, with energetic expenditure, stress induced physiological responses, and the risk of injury or death, not differing between competitors (Huntingford et al., 1987; Morrell et al., 2005; Hardy & Briffa, 2013). Conflict may also lead to secondary costs, such as reduced vigilance, while increasing the risk of attracting predators (Jakobsson et al., 1995). As a result, animals have evolved strategies to mitigate the costs of conflict (Briffa & Sneddon, 2010). For example, threat displays, opponent assessment, avoidance, and submission may help to minimise costs (Archer, 1988; Hardy & Briffa, 2013; Briffa, 2014). Signals that reliably convey information about aggressive motivation and fighting ability have evolved to benefit both the sender and the receiver of the signal (Smith and Harper, 2003). Aggressive signals usually involve displays that emphasise traits relevant to fighting ability, such as physical strength, size, and weaponry (Huntingford et al., 1987). Conversely, signals that convey submission, typically deemphasise these traits (Bernstein, 1981; Reddon et al., 2022).

Submission signals are less well characterised in the literature than are aggression or dominance signals, despite being widespread (e.g., Fox & Cohen, 1977; O'Connor et al., 1999; Ligon, 2014; Ruberto et al., 2020). Most species have fewer submission signals than aggression signals (Bradbury & Vehrencamp, 1998). Although submission signals may be repeated or intensified (Eaton & Sloman, 2011) to ensure the signal is successfully transmitted, multiple distinct submission signals which indicate varying levels of submissive intent are uncommon, as a receiver may be less likely to accept a partial gesture of submission (Matsumura & Hayden, 2006).

Agonistic communication is important for animals that live in groups. Group living is beneficial to the members of the group (Hamilton, 1971; Roberts, 1996; Lehtonen & Jaatinen, 2016). However living in proximity can increase the likelihood of conflict, which may offset these advantages, and thus conflict within groups must be managed for group living to be a stable strategy (Lorenz, 1966; King, 1973; Krause & Ruxton, 2002). In social groups, the opportunity to flee from an attack may also be reduced by ecological or social constraints (Matsumura & Hayden, 2006; Wong, 2010; Reddon et al., 2022), and therefore social stability relies on effective communication (Frommen, 2020).

The daffodil cichlid (*Neolamprologus pulcher*) is a cooperatively breeding freshwater fish endemic to Lake Tanganyika, East Africa (Taborsky & Limberger, 1981; Balshine et al., 1998). In the wild, daffodil cichlids form stable groups, typically of 3-20 individuals, organised into linear dominance hierarchies based on body size, which correlates with age (Balshine et al., 2001; Dey et al., 2013). The largest male and largest female form a breeding pair who are socially dominant and engage in most of the reproduction in the group. Dominant fish may be replaced due to predation events or territory takeovers, and larger subordinates occasionally disperse between groups (Stiver et al. 2004). Subordinate group members assist the dominant pair in raising their offspring and in defending the territory from predators and competitors (Wong & Balshine, 2011). Within-group agonism is frequent and can result from disputes over status, workload, and resource access (Wong & Balshine, 2011; Fischer et al., 2014; Hick et al., 2014; Balshine et al., 2017). Dominant status is reinforced through threat displays and overt aggression (Dey et al., 2013; Balzarini et al., 2017). Subordinates often flee from dominant aggression by swimming away rapidly (Balshine et al., 2017), however, the ability to flee from aggression may be constrained by the danger of predation outside of the group's territory (Groenewoud et al., 2016) and by the availability of shelters to flee to inside the territory (Reddon et al., 2019). Subordinates may also signal submission through one of two displays: the head up display (HUD; Table 1), or the tail quiver display (TQD; Table 1). Tail quiver displays are given in a variety of social contexts including affiliation and courtship (Pisanski et al., 2015), as well as submission (Bayani et al., 2017; Naef & Taborsky, 2020; Antunes et al., 2022). Head up displays by contrast seem to be used primarily as a submission signal (Ruberto et al., 2020). The HUD may vary in intensity from a slight upwards tilt to the adoption of a near vertical posture in the water column (Sopinka et al., 2009). The cause of this variation in signal expression is unknown, it may be exaggerated to ensure signal transmission in more challenging signalling environments such as the low visibility that occurs seasonally in Lake Tanganyika, or may represent quantitative variation in submissive motivation, though the latter is not predicted by a model of submission signalling (Matsumura & Hayden, 2006). Head up and tail quiver displays may occur in isolation or together, either sequentially or simultaneously. Previous studies have often focused on one signal or the other (TQD: (Bayani et al., 2017; Naef & Taborsky, 2020; Antunes et al., 2022); HUD: (Reddon et al., 2012; Hick et al., 2014; Ruberto et al., 2020) or combined the two displays into a general submissive display category (Taves et al., 2009). These two different displays may be used in different contexts or may depend on the level of submissive motivation.

In this study, daffodil cichlids belonging to 19 different social groups were observed under standardised environmental conditions. The relationships between aggressive behaviours of the dominant breeding pair, and the submissive responses of the largest subordinate in the group were recorded to examine what predicts the response of the focal subordinate fish. Specifically, how submissive responses varied based on the escalation level of the instigating aggression (threat display or overt aggression). We predicted that the TQD would mostly be used in response to less escalated threat displays, whereas HUDs and fleeing responses would be more frequent in response to overt aggression.

2. Methods

2.1 Study subjects and housing conditions

The research subjects were laboratory reared daffodil cichlids, *Neolamprologus pulcher*, which were descendants (F5-F7) of fish captured on the southern shore of Lake Tanganyika, Africa. Prior to the experiment, all fish were kept in mixed-sex groups of approximately 50 fish per aquarium (105 × 43 x 40cm, 180-litre). The housing tanks were equipped with a heater, a thermometer, two powered filters, an air stone, and 3 cm of fine coral sand. Temperature was maintained at 27 ± 1 C on a 12:12 h light:dark cycle with 15 min of gradual transition in lighting simulating sunrise and sunset. Fish were fed daily with a variety of dried prepared cichlid foods.

The study subjects were later moved into social groups (n = 19) housed in 90L aquaria (53 × 43 x 38 cm), each equipped with two foam filters, a heater, and a thermometer, along with 3 cm of fine coral sand. Each aquarium was furnished with 4 terracotta caves, used by the fish as breeding substrate as well as shelter, and two floating translucent green PET bottles, providing additional refuge. All animals were kept under the same husbandry regime previously described. Fish were housed in either small groups (n = 9), comprised of two dominant individuals and two subordinates, or larger groups (n = 10), which included the two dominant fish and 6-7 subordinates. Sex was only determined in the dominant pair, as many of the subordinates were too small to be sexed by examination of the genital papillae. For each group, the sizes of the dominant breeding pair and of the largest subordinate were recorded, by measuring the standard length of each fish from the tip of the snout to the end of the caudal peduncle (standard length mean±SD: dominant male = 5.25±0.48cm; dominant female = 4.75±0.52cm; focal subordinate = 3.5±0.45cm). We aimed for approximately a 5-10% difference in body size between breeders, and between adjacent subordinate ranks, with size difference of approximately 25-35% difference between breeder female and largest subordinate, mimicking the size distribution of wild groups. Subordinate individuals were moved into the experimental tanks 24 h before the dominant pair. Groups were housed together for at least one month prior to observation to allow for groups to stabilise while reducing variation across the observation period due to uncertainties in the hierarchy. On the rare occasions that members were rejected during group formation, groups were dissolved and new ones were formed using new fish from the stock aquaria.

2.2 Video recordings

The 19 groups were recorded with a camera (CX240E Full HD Camcorder, Sony Corp., Japan), from a frontal perspective capturing the entire aquarium. Each group was recorded four times over a period of two weeks between 10:00-15:00, with only one recording captured per day. Each recording was 30 minutes long, leading to a total of 120-minutes of recording per group. The first 10 minutes of each recording were treated as a habituation period and were therefore not coded, resulting in 80 total minutes of coded observation per group, which were summed together for analysis purposes.

2.3 Behavioural coding

We recorded each instance of aggression from either member of the dominant pair towards the largest subordinate (focal fish) and the focal fish's response to that aggression (see Table 1 for a detailed description of the coded behaviours). For each agonistic interaction (n = 369) we recorded the sex of the aggressor and whether they performed an overt aggression or a threat display. The subordinate response was recorded for each interaction as either a submission display (HUD or TQD) or a flee. Moreover, if the submission display was a HUD, the angle of the tilt was visually estimated as being above or below 45° relative to the substrate by pausing the video at the apex of the display. HUD and TQD were recorded as separate responses when they were carried out sequentially or simultaneously in response to a single aggressive act.

Table 1. Ethogram of agonistic behaviour for the daffodil cichlid, *Neolamprologus pulcher*.

TYPE OF INTERACTION	**BEHAVIOUR**	**DESCRIPTION**
Overt aggression	Chase	The dominant fish swims rapidly towards the subordinate for a distance of at least 4 cm.
	Bite/ram	The dominant fish makes contact between their mouth and the body of the subordinate
Threat displays	Aggressive posture	The dominant fish faces the subordinate with their head lowered, tail raised upwards, and fins extended.
	Opercular threat	The dominant fish swims towards the subordinate with its jaws open and opercula extended outward. The pectoral fins are spread, while the body is tilted slightly downwards.
Submission (display)	Head up display (HUD)	The subordinate fish responds to an aggression by tilting their body upwards and exposing their ventral aspect to the receiver.
	Tail quiver display (TQD)	The subordinate fish responds to aggression by producing a bilateral oscillation of the tail. Motion originates at the tail and can extend to the entire body.
Submission (escape)	Flee	The subordinate fish responds to aggression by quickly swimming away, for a distance of at least 4 cm.

2.4 Data analysis

To examine the effect of the type of inciting aggression on the type of submissive behaviour elicited, we fit a generalised linear mixed model to a multinomial logistic distribution. Dominant aggression was coded categorically as an overt aggression or a threat display and included as a predictor variable. Although not the focus of this study, we also included the group size (small, large) and the sex of the aggressor (male, female) as predictor variables as the broader social context could affect the submissive responses observed. The behavioural response from the focal fish was coded categorically as a HUD, a TQD, or a flee and included as the response measure. Focal identity was included as a random factor to account for multiple interactions per focal individual contributing to the dataset.

In a follow-up analysis, we compared only the subordinate submission signals (HUD, TQD) depending on the dominant behaviour (overt aggression, threat display), the sex of the dominant and the size of the group, using a generalised linear mixed model fit to a binomial logistic distribution. Group size and dominant sex were included as fixed factors and focal identity was included as a random factor.

The frequencies of HUDs above or below an angle of 45° in response to overt aggression or threat displays were examined with a generalised linear mixed model fit to a binomial logistic distribution. Type of aggressive behaviour received, the size of the group, and the sex of the aggressor were included as predictor variables. The response variable was the angle of the HUD relative to the substrate, coded categorically as HUD >45° or HUD <45°. The identity of the focal fish was included as a random factor. For all models, fixed effects were tested with Wald F tests.

We examined the Pearson product-moment correlation between the number of threat displays or the number of overt aggressions that the focal fish received from the dominant pair with the number of HUDs or TQDs they showed in response (n = 19). Data analysis and visualisation were conducted using SPSS (v. 27) and R (v. 3.6.2).

2.5 Ethical statement

Animal housing and handling protocols were approved by the Liverpool John Moores Animal Welfare and Ethics Steering Group (approval number: AR_TR/2018-4) and adhered to the guidelines of the Animal Behaviour Society and the Association for the Study of Animal Behaviour.

3. Results

The type of aggression shown by the dominant fish (overt aggression or threat display) significantly predicted the submissive response of the focal fish ($F_{2,361} = 27.69$, $p < 0.001$, Fig. 1) with flees being much more common in response to overt aggression and TQD being more likely in response to threat displays. Neither group size nor the sex of the aggressor had a significant effect on the focal response (Group size: $F_{2,361}=1.89$, $p = 0.15$; Sex: $F_{2,361}=1.18$, $p = 0.31$).

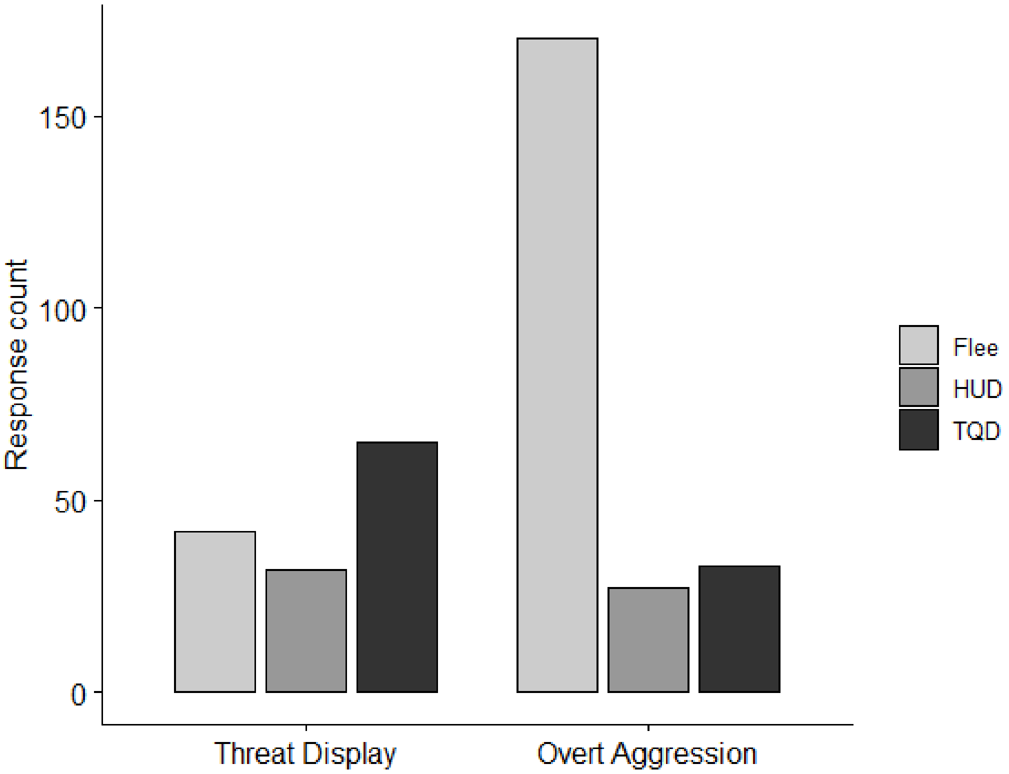

Figure 1. Counts of submissive behaviour in the largest subordinate in response to dominant aggression in 80 minutes of observation. The type of submissive response depended on the type of aggression received ($p < 0.001$).

Restricting the analysis to only submission display responses (i.e., excluding those interactions in which the focal fish fled from the dominant aggression), there was no significant effect of aggression type ($F_{1,153} = 1.49$, $p = 0.22$), dominant sex ($F_{1,153} = 0.03$, $p = 0.86$), nor group size ($F_{1,153} = 0.09$, $p = 0.76$), on the likelihood of the focal fish producing a TQD compared to a HUD in response to dominant aggression.

Aggression type received significantly predicted the likelihood of a HUD being greater than 45° ($F_{1,54}=5.68$, $p=0.021$; Fig. 2), while group size ($F_{1,54}=0.23$, $p=0.64$) and sex ($F_{1,54}=0.04$, $p=0.84$) did not.

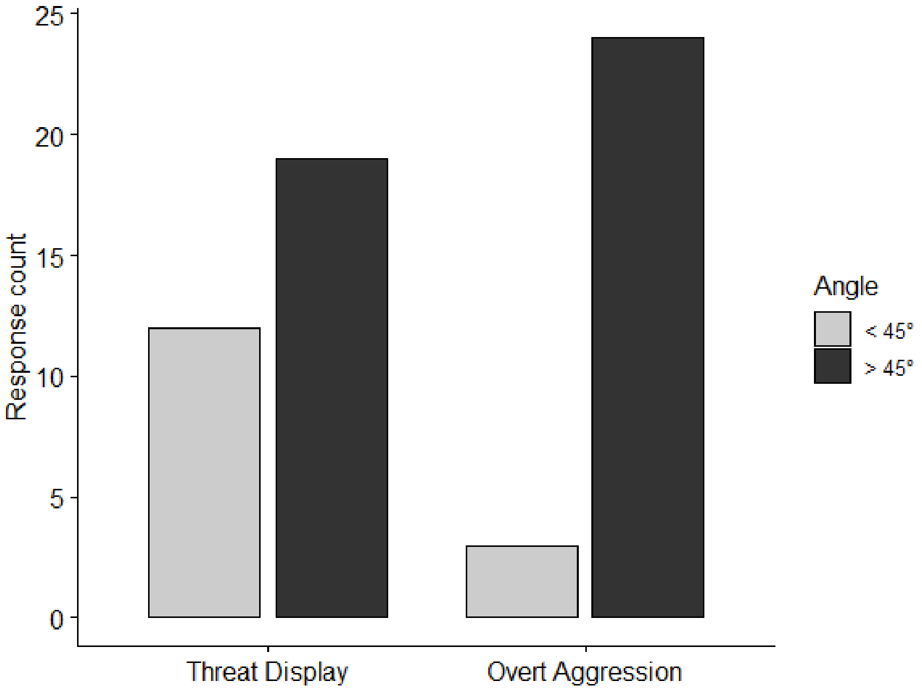

Figure 2. Counts of HUDs elicited above or below 45° relative to the substrate in response to dominant aggression in 80 minutes of observation. The tendency for the HUD to involve a greater tilt of the body was predicted by dominant behaviour ($p = 0.02$).

There was a significant positive relationship between the number of threat displays from the dominant fish and the number of the HUDs shown by the focal subordinate ($r = 0.65$, $N = 19$, $p = 0.002$, Fig. 3A). In contrast the number of TQDs shown by the focal fish was not significantly predicted by the number of threat displays received ($r = 0.27$, $p = 0.26$, $N = 19$, Fig. 3C). The number of overt aggressions from the dominant pair was not significantly related to the number of HUD shown ($r = 0.40$, $N = 19$, $p = 0.09$, Fig 3B) or the TQD shown by the focal fish ($r = -0.11$, $N = 19$, $p = 0.67$, Fig. 3D).

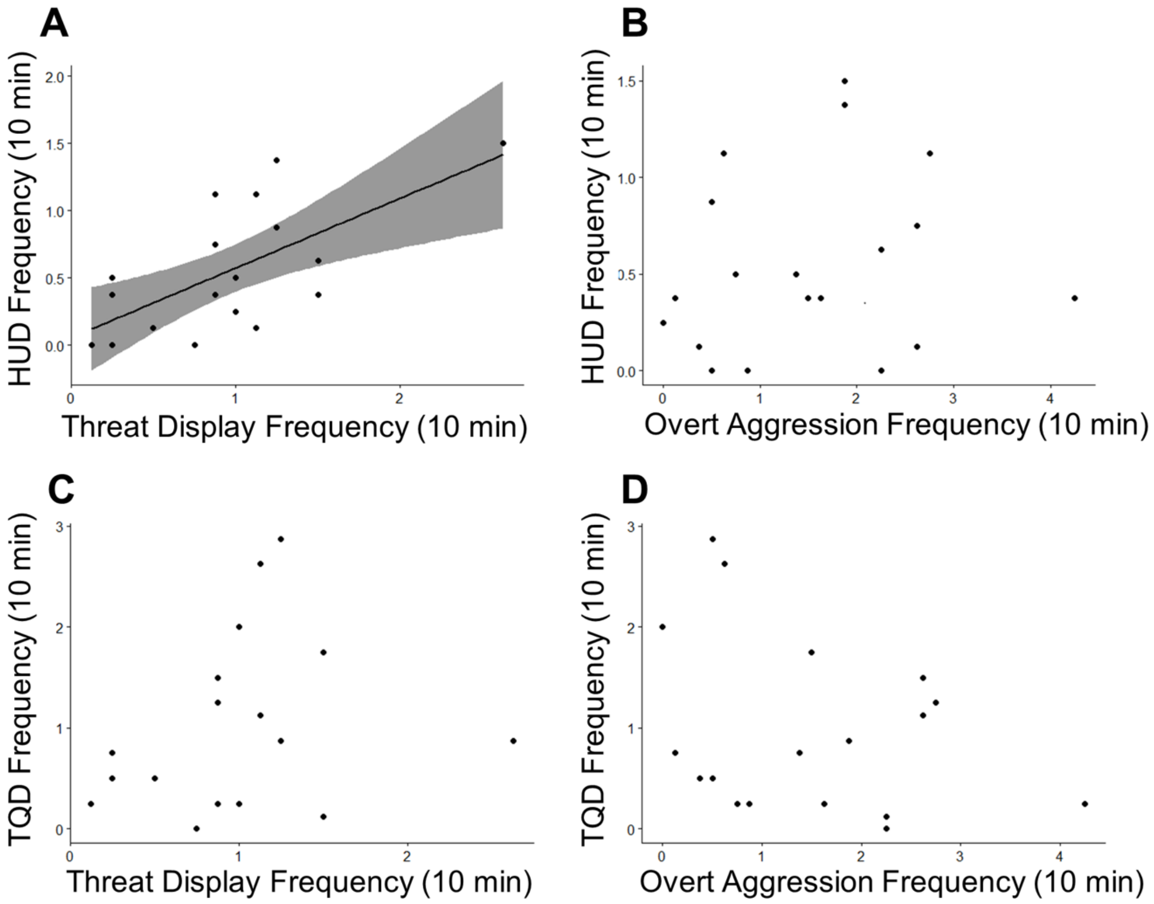

Figure 3. The linear relationship between the aggression received from the dominant fish and the number of submission displays shown by the focal subordinate per 10 minutes of observation. (A) There is a significant positive relationship between the number of threat displays received and the number of HUD shown ($p = 0.002$). (B) The number of overt aggressions was not significantly related to the number of HUD ($p = 0.09$). Neither (C) threat displays ($p = 0.26$) nor (D) overt aggressions ($p = 0.67$) received from the dominant pair significantly predicted the number of TQD by the focal fish. Linear best fit lines for significant relationships are shown with 95% CI.

4. Discussion

Using detailed observations of 19 laboratory housed groups of daffodil cichlids (*Neolamprologus pulcher*), we found that the escalation level of the aggression shown by the dominant breeding pair towards their largest subordinate helper strongly predicted the resultant submissive response. When one of the dominant pair attacked the focal subordinate by chasing or biting, the subordinate most often fled from the interaction.

When the dominant showed a threat display, the focal fish was more likely to show a submission display in response. Both head up displays (HUD) and tail quiver displays (TQD), were produced in response to both overt aggression and threat displays from the dominant pair. The number of head up displays was predicted by the number of threat displays received. By contrast, the TQD was not significantly associated with the number of overt aggressions or threat displays received, suggesting the HUD may be a more specialised submission display than the TQD. We also found that the HUD was more likely to be exaggerated through a more dramatic tilt of the body axis when the inciting aggressive act was an overt aggression rather than a threat display, suggesting possible gradation of submissive motivation within signal type.

Both the HUD and the TQD are used as submission signals in the daffodil cichlid, and the context in which they are used overlaps substantially. Submission signals need to be effectively received by dominant individuals to modulate their aggression (Reddon et al., 2022), and daffodil cichlids could use graded or differentiated displays to ensure that signals are efficiently transmitted and received across signalling contexts. For example, brown trout (*Salmo trutta*) show submission by darkening their body colouration, and do so more dramatically in turbid water, possibly to enhance signal transmission when visibility is reduced (Eaton & Sloman, 2011).

Although submissive repertoires are generally not as diverse as aggressive repertoires (Bradbury & Vehrencamp, 1998), examples of animals using more than one submission display are known. For example, Jacky dragons (*Amphibolurus muricatus*) deploy two different submission displays (slow arm waves and slow head bows) during opponent assessment (Carpenter et al., 1970; Van Dyk & Evans, 2008). In canids such as dogs (*Canis l. familiaris*), wolves (*Canis lupus*) and African wild dogs (*Lycaon pictus*), subordinate group members communicate submissive intentions through what is referred to as passive vs. active submission (Schenkel, 1967; Cordoni & Palagi, 2008; Baan et al., 2014; Van den Berghe et al., 2019). These passive and active displays can take place separately or in combination. Both displays involve crouched posture and lowered tail and ears, but these are performed more dramatically in passive displays (Schenkel, 1967). Both displays convey submission, but active displays may also signal affiliative motivation (e.g., during greeting ceremonies), while passive displays are given primarily in response to dominant inquisitive behaviours (e.g., sniffing urogenital areas) or aggression (Schenkel, 1967). Even greater complexity in signal repertoire is observed in social primates such as ring-tailed lemurs (*Lemur catta*). This species' visual and acoustic repertoire includes several submissive postures (Pereira & Kappeler, 1997) and vocalisations (Macedonia, 1993). Interestingly, vocal signals are elicited in context dependent agonistic interactions, with yips, cackles and twitters given by submissive individuals when losing agonistic interactions, and chutters elicited by both winners and losers (Bolt, 2021), supporting the existence of intricate signalling systems to mitigate conflict.

As with the canid and primate examples, the relatively rich submissive repertoire of daffodil cichlids could be explained by the social complexity hypothesis for communicative complexity. Socially complex environments can promote the evolution of social cognition, which in turn favours the greater nuance in communication necessary to support behavioural coordination (Freeberg et al., 2012; Sewall, 2015). Cooperative breeding systems are commonly affected by high levels of intra-group social conflict, and the evolution of multiple submission signals in daffodil cichlids could fulfil the need for social context-dependent communication. Alternatively, cooperation per se may not necessarily lead to richer submissive repertoires, but rather may increase the frequency of submission signals and/or reduce the threshold at which they are elicited (Reddon et al., 2022). Recent studies testing these predictions in birds have produced conflicting results (Rosa et al., 2016; Leighton, 2017), highlighting the current lack of agreement surrounding signal evolution in cooperative systems. According to previous analyses comparing cooperative and non-cooperative cichlids, communication repertoires appear to be similarly structured in close relatives across social systems (Hick et al., 2014; Balshine et al., 2017). Furthermore, social complexity should be enhanced when multiple individuals interact, due to eavesdropping and audience effects (Valone, 2007; Zuberbühler, 2008). In the current study however, group size did not affect submission responses, suggesting that eavesdroppers and rank conflict elsewhere in the hierarchy may not have a major effect. Breeder sex also did not affect the subordinate response, but it should be noted that the sex of the subordinate was unknown, as many individuals were too small to be visually sexed. This lack of information may have obscured sex specific patterns, as aggression from the dominant breeders could depend on whether they are interacting with same versus opposite sex group mates.

The observed overlap between the HUD and TQD may be the result of these signals fulfilling subtly distinct, context-dependent functions. Both signals were elicited in response to dominant aggression, but the

HUD was more strongly correlated with threat displays. We recently reported that individuals performing more HUDs receive less aggression from dominant fish (Ruberto et al., 2020). Our current findings seem to indicate that HUDs may be a more specific social signal in daffodil cichlids, serving to de-escalate the dominant aggressive interactions. Moreover, it is possible that differences in the angle of the body tilt in the HUD indicates gradation of submissive motivation in the subordinate fish, allowing the HUD to act as a nuanced submission signal.

The TQD occurred at higher rates in comparison to the HUD but in contrast, it wasn't significantly correlated with aggression received. This display may be co-opted from other communicative contexts to serve as a submissive-affiliative signal: the TQD is often observed during courtship, and it is conserved across a diverse array of cichlids in this context (Baerends & Baerends-van Roon, 1950; Barlow, 2008), while the HUD appears to be more species-specific. It is possible that subordinate cichlids use the TQD as a multipurpose affiliative-submissive signal, indicating both subordination and affiliation to higher ranked individuals, while the HUD is more strictly used to show immediate submission in response to acute aggression. In other animal species submission signals are reported to serve a multipurpose submissive-affiliative function, as in the case of wolves (Schenkel, 1967; Cafazzo et al., 2010). Displays can be differentiated when used to either de-escalate aggression or to pre-emptively appease the dominant individual, in what are sometimes referred to as submission signals (the former) or subordination (the latter; (Preuschoft & van Schaik, 2000). In wolves, submission signals, such as back rolling, are usually given as an immediate behavioural response following a dominant physical attack, particularly when avoidance behaviours or escape are not a viable option (van Hooff & Wensing, 1987). Subordination signals are primarily directed at dominants outside of the agonistic context, reinforcing subordinate status (van Hooff & Wensing, 1987). Dominant individuals are often approached and greeted by subordinates with stereotypical lowered posture, followed by lip licking and other appeasing behaviours to reemphasise status within an established social relationship. The use of signals for both submission and subordination purposes is also reported in other hierarchical species, such as Verreaux's sifakas (*Propithecus verreauxi;* (Flack & de Waal, 2007). Chatter vocalisations are used both as immediate response to aggression, but also when aggressive provocation is not apparent, to communicate peaceful intentions, and they are found to reduce within-group conflict while promoting hierarchical stability through reconciliation (Lewis, 2019).

It is possible that the space limitation imposed by the aquarium environment may have affected the conflict management strategies of daffodil cichlids, for example by making fleeing a less viable response, or by increasing the frequency or intensity of aggression from the dominants. Although the behaviour of daffodil cichlids is known to be broadly similar in the wild and in the laboratory (Taborsky & Grantner, 1998), we did find a higher rate of aggression in our observations than has previously been seen in the field (an average of 2 aggressions per 10 minutes in our sample vs. 0.4 per 10 minutes in the field; Hellmann et al., 2015). Some of this difference may be explained by the difficulty of recording behaviour in the field vs. laboratory setting, with more instances of aggression likely to be missed while observing live via SCUBA compared to coding from high-definition video. It should be noted that space limitations are also present in the wild as this species is highly territorial, defends a relatively small territory (~1m^3), and is exposed to extreme predation threat when venturing outside (Groenewoud et al., 2016). It is possible that subordinates in the wild may make greater use of shelters that are too small to be accessed by the dominants due to the difference in the body size, whereas all shelters were large enough to be used by all fish in our laboratory setup. Future work should examine the importance of shelter size and space limitation on conflict management in daffodil cichlids.

In conclusion, we found that dominant aggressive behaviour was a strong predictor of subordinate submissive responses in the cooperatively breeding daffodil cichlid. The most common response to an overt aggression was to flee, while submission signals were more common in response to a threat display. Both the HUD, and the TQD were used in response to both threat displays and overt aggression. However, the number of HUD shown was predicted by the number of threats received, while the angle of HUD varied with the type of aggressive behaviour received. These results suggest that the HUD is being used more specifically as a nuanced submission signal, while the TQD may have other social functions. We found evidence to suggest that daffodil cichlid subordinates communicate variation in submissive motivation with both multiple submission signals and variation in the expression of those signals. It is possible that socially complex groups may select for greater nuance in submission signalling than would be expected in one-off interactions.

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