# Original Article

# The role of serotonin in the modulation of cooperative behavior

José R. Paula,[a] João P. Messias,[a] Alexandra S. Grutter,[b] Redouan Bshary,[c] and Marta C. Soares[a]

[a]CIBIO, Centro de Investigação em Biodiversidade e Recursos Genéticos, Universidade do Porto, Campus Agrário de Vairão, 4485-661 Vairão, Portugal, [b]School of Biological Sciences, Goddard Building (8) Room 112, Laboratory 111, The University of Queensland, Brisbane, Queensland 4072, Australia, and [c]Université de Neuchâtel, Institut de Biologie, Eco-Ethologie, Rue Emilie-Argand 11, 2000 Neuchâtel, Switzerland

Cleaning behavior is known as a classic example of cooperation between unrelated individuals. Although much is known of the behavioral processes underlying cooperative behavior, the physiological pathways mediating cooperation remain relatively obscure. Here, we show that altering the activity of serotonin on wild cleaner wrasses *Labroides dimidiatus* has causal effects on both social and cooperative activities. These cleaners cooperate by removing ectoparasites from visiting "client" reef fishes but prefer to eat client mucus, which constitutes "cheating." We found that enhancing serotonin made cleaner wrasses more motivated to engage in cleaning behavior and more likely to provide physical contact to clients (tactile stimulation) without spending more time cleaning or cheating more often. Blocking serotonin-mediated response resulted in an apparent decrease in cleaners' cheating levels and in an increase in cleaners' aggressiveness toward smaller conspecifics. Our results provide first evidence that serotonin is a neuromodulatory driver of cooperative behavioral activities and contribute to the understanding of neural pathways of cooperation.

*Key words*: cooperation, cleaning behavior, *Labroides dimidiatus*, neuromodulator, serotonin, serotonin 1A receptor, 8-hydroxy-2-(dipropylamino)tetralin hydrobromide, WAY 100.635.

## INTRODUCTION

A great variety of concepts exists to explain cooperation, defined here as stable mutual helping between unrelated individuals (Axelrod and Hamilton 1981; Côté 2000; Trivers 1971). These theories have been particularly successful at identifying various partner control mechanisms, such as partner choice, sanctions, or punishment that select against a reduction in investment (Bshary and Grutter 2002; Johnstone and Bshary 2002). In contrast, we do not understand well what strategies and underlying decision rules individuals use in potentially cooperative interactions. For example, it is quite clear that animals, including humans, do not use precise counting strategies like tit-for-tat and its deviations (Raihani and Bshary 2011). However, mechanistic studies that identify the link between cooperation and its physiological basis are still relatively rare.

The role of the oxytocin as neuromodulator of social bonds has become a substance of major interest. Evolved apparently to promote bonds between mothers and their offspring, various species have co-opted the mechanism to promote bonds, both between pair partners and between friends (Young and Wang 2004). In humans,

oxytocin even promotes in-group cooperation along with separation from out-group members (De Dreu et al. 2012). Although oxytocin is one of the mechanisms involved in the cooperation between bonded individuals, many cases of cooperation in nature do not involve bonded partners. Moreover, the mechanisms underlying the effects of 1 neuroendocrine factor usually work in an integrative way, so we can expect that other compounds might also mediate aspects of cooperative behavior. For instance, central effects (i.e., involving the brain) of oxytocin have been described as important mediators of the stress response (Neumann 2008). In humans, some selective serotonin reuptake inhibitors (SSRIs) like fluvoxamine and citalopram are known to increase plasma levels of oxytocin. Because serotonin fibers overlap with oxytocin-containing cells in the paraventricular and supraoptic nuclei of the hypothalamus (Marazziti et al. 2012), there may be a relationship between serotonin and oxytocin. Also, serotonin fibers and arginine vasopressin (AVP)–producing neurons are located in the same areas in the brain (Ferris et al. 1997). Indeed, studies on hamsters revealed that treatment with fluoxetine decreases AVP and blocks AVP-induced aggression (Ferris et al. 1997). Thus, there are candidate substances that may play a key role in the modulation of cooperation between nonbonded individuals.

A major aim in recent years has been to identify neurohormonal candidates that may modulate levels of cooperation in marine

Address correspondence to M. C. Soares. E-mail: marta.soares@cibio.up.pt.

cleaning mutualisms (Cardoso et al. 2015a,b; Ros et al. 2012; Soares et al. 2011, 2012, 2014). One of the best-known examples of cooperation between unrelated individuals occurs between the Indo-Pacific cleaner wrasses *Labroides dimidiatus* and their visiting reef fishes, usually referred to as "clients," which inspect the body surface, gills, and sometimes mouth of clients (Côté 2000). During an interaction with clients, cleaners can choose whether to cooperate, by removing ectoparasites, or to cheat, by eating mucus from clients which they prefer (Grutter and Bshary 2003). Conflict usually happens when cleaners choose to cheat, which is normally measured by the reaction of clients to cleaners' bites, referred to as "body jolts" (Bshary and Grutter 2002; Soares et al. 2008). In order to reduce conflict and to further invest in the interaction, cleaners frequently provide tactile stimulation to their clients, which is characterized by a physical contact in the dorsal area of the clients with their pelvic fins (Bshary 2011; Bshary and Würth 2001; Grutter 2004). Cleaners have been demonstrated to have a diverse set of behaviors to increase the duration of inspection and to promote future interactions (Bshary 2011). On the physiological level, arginine vasotocin (AVT) plays an important role as a modulator of cleaning behavior, as high levels of this neuropeptide decrease cleaners' likelihood to interact with their clients (Soares et al. 2012). In contrast, evidence is yet to be found regarding the role of IT (isotocin, fish homolog for oxytocin) in this system. Recently, cortisol levels were also found to associate with changes in cleaner wrasse behavioral patterns (Soares et al. 2014). Considering the relationship between both AVP and cortisol (Höglund et al. 2002; Winberg et al. 1997) with serotonin, it is parsimonious to suggest a possible role of serotonin in this mutualism.

Serotonin is a major neurotransmitter that is responsible for the regulation of social behavior in vertebrates, including humans (Fox et al. 2009; Raleigh et al. 1991; Winberg et al. 1993). For example, in humans, serotonin is associated with vulnerability to mood disorders (Fox et al. 2009), as it is known to play an important role in depression and anxiety (Lesch and Mössner 1998). Recent studies have shown that lowering serotonin levels in humans increases reactions to unfairness (Crockett et al. 2008) and reduces cooperative play during an Iterated Prisoners Dilemma game (Wood et al. 2006). On the contrary, the enhancement of serotonin levels in adult humans resulted in less quarrelsome behaviors, increased dominant behavior, and cooperative communication and play during mixed-motive games (games in which the players preferences among the outcomes are neither identical, as they are in pure coordination games, nor diametrically opposed, as they are in zero-sum or constant-sum games, Knutson et al. 1998; Tse and Bond 2002). Serotonin is also one of the main neuromodulators influencing the quality of close intimate partnerships in humans and other animal models (Bilderbeck et al. 2011) and is associated with antisocial (impulsive) behaviors and aggressive responses (Booij et al. 2010; Coccaro et al. 2010). In fish, experimental enhancement or reduction of serotonin levels may produce behavioral changes. However, most studies have focused on the role of serotonin in social status and aggression (Clotfelter and O'Hare 2007; McDonald et al. 2011), with few exceptions (Beulig and Fowler 2008).

To determine whether the behavior of the cleaner wrasse is modulated by serotonin activity, manipulations and observations were conducted in the field. Here, we probed the effect of serotonin by testing if exogenous administration of 2 serotonin activity facilitators (fluoxetine and 8-hydroxy-2-(dipropylamino)tetralin hydrobromide [8-OH-DPAT]) and 2 serotonin activity blockers (*p*-chlorophenylalanine [PCPA] and WAY 100.635) (Table 1) through peripheral injections affected the likelihood to engage in cleaning behavior (by the proportion of clients that are inspected and the proportion of cleaners that change from one client to the next), whether it would directly affect the service quality (by calculating the time cleaners spent inspecting clients and providing tactile stimulation and jolt rate), and if it affected conspecific-directed behavior (between cleaners, antagonistic chases where one individual rapidly advanced toward the other and parallel swimming). Both fluoxetine and PCPA act through influencing serotonin content in the synaptic cleft, whereas 8-OH-DPAT and WAY 100.635 modulate serotonin 1A receptor as agonist and antagonist, respectively (Table 1). The serotonin 1A receptor is one of the most abundant serotoninergic receptor subtypes in the mammalian brain. It was the first to be cloned and it is presently the most thoroughly characterized (Albert et al. 1990; Laaris et al. 1995). This receptor has also been cloned for several species of fish, providing good evidence for similarity to the mammalian version (Medeiros and McDonald 2013).

## MATERIALS AND METHODS
### Field methods

This study was conducted on 10 different reefs around Lizard Island (Lizard Island Research Station, Australia, 14°40′S, 145°28′E) between August and October 2012. We used 45 females (as these are more common than males and easier to catch) of bluestreak cleaner wrasse *L. dimidiatus* to test the effect of serotonin on cleaning behavior. All observations and manipulations were made by 2 SCUBA diver, between 9:00 and 15:00h. Cleaner fish were selected haphazardly across the reefs and cleaning stations varied in depth between 1 and 10 m. All individuals were captured using hand and barrier nets and

**Table 1**
**Function, effect, and serotonin activity of each compound used in this study**

| Compound | Function | Effect | Serotonin activity |
|---|---|---|---|
| Fluoxetine | Serotonin content-influencing compounds | More serotonin available in the synaptic cleft (selective serotonin reuptake inhibitor) | Facilitates serotonin activity |
| PCPA | | Less serotonin available as it blocks serotonin production (selective tryptophan hydroxylase inhibitor) | Blocks serotonin activity |
| 8-OH-DPAT | Serotonin 1A receptor modulators | Serotonin 1A receptor activated (serotonin 1A receptor agonist) | Facilitates serotonin activity (via a specific receptor) |
| WAY 100.635 | | Serotonin 1A receptor blocked (serotonin 1A receptor antagonist) | Blocks serotonin activity (via a specific receptor) |

measured to the nearest millimeter (total length [TL]). TL of these individuals ranged from 5.5 to 8.0 cm. Fish body weight was estimated from a length-weight regression (Soares MC, unpublished data) and ranged from 1.0 to 4.0 g. The focal female was then given an intramuscular injection of 1 of 5 compounds: saline (0.9 NaCl), fluoxetine (Sigma F132), 8-OH-DPAT (Sigma H8520), PCPA (Sigma C6506), and WAY 100.635 (Sigma W108). Injection volumes were always 15 µL per gram of body weight (gbw) and this process never exceeded 3 min. Because this study was done exclusively in field conditions with limitations of time and number of fish used (collecting permit allowance), dosages chosen were based on previous studies that focused on other model species of teleost fish: a concentration of 5 µL/gbw of fluoxetine (Perreault et al. 2003), 1 µL/gbw of 8-OH-DPAT, and 1.5 µL/gbw of WAY 100.635 (Clotfelter and O'Hare 2007) and PCPA 5 µL/gbw (Clotfelter and O'Hare 2007; Lopez-Mendoza et al. 1998; Lorenzi et al. 2009). The function/effect of each pharmaceutical compound used is given in Table 1. Two of our chosen compounds aimed at the serotonin 1A receptor (but see Table 1). These receptors can be located on the cell body, dendrites, axons, and both presynaptically and postsynaptically in nerve terminals or synapses. Serotonin 1A receptor agonists (in our case 8-OH-DPAT) tend to exert a biphasic mode of action; they decrease serotonin release and postsynaptic serotonin 1A receptor activity in low doses, and further decrease serotonin release but increase postsynaptic serotonin 1A receptor activity at higher doses by directly stimulating the receptors in place of serotonin (Hjorth et al. 2000). Thus, the administration of fluoxetine and PCPA may also serve as a control in relation to the effects produced by both serotonin 1A agonist and antagonist, which will help us to document their effects more clearly in the absence of a previous dosage calibration study. For all treatments, independent cleaner fish were used as all cleaners were caught on different cleaning stations around the island. Once an individual was released, it was then observed and videotaped for the next 45 min using video cameras in waterproof housings (Sony HDR-XR155). Video recordings were made from a distance of between 2 and 3 m.

## Behavioral analysis

During each video analysis, we recorded the following measures: 1) species of each client (estimated visually to the nearest centimeter) visiting the cleaning station; 2) the number of clients that adopted the species-specific immobile pose, which signals the need and availability to be cleaned (Côté et al. 1998), including those that ended up not being inspected by the cleaner wrasse; 3) the number and duration (in seconds) of a cleaner's inspection toward each client 4) the frequency and duration of tactile stimulation provided (where a cleaner touches, with ventral body and fins, the body of the client and no feeding is involved; Bshary and Würth 2001); 5) the number of jolts by clients (cleaners sometimes take bites to which the clients respond with a short body jolt that usually is a behavior associated with cheating by cleaner fish; Bshary and Grutter 2002; Soares et al. 2008); and 6) conspecific-directed behavior, including parallel swimming as a measure of intraspecific social behavior, and antagonistic charges (chases) where one individual rapidly advanced toward the other.

## Statistical analysis

All cleaner fish were caught from different cleaning stations for the cleaning behavior observations following neuroendocrine manipulations and were therefore treated as independent measures. Interspecific cleaner fish behavior was measured for 2 different

behavioral categories: 1) measures of likelihood to interact with clients quantified as the proportion of inspected clients, a measure for seeking cooperative social interactions, and 2) measures of cleaning quality, related to the degree of cooperativeness, quantified as a) duration of inspection by cleaners, b) frequency of jolts per 100 s of inspection, c) proportion of interactions in which tactile stimulation was applied to clients and d) duration of tactile stimulation provided by cleaners. Intraspecific behavior was measured by 1) pair close swimming events as a measure of intraspecific social behavior and 2) frequency of antagonistic chases between the focal female and a conspecific. All data were analyzed using nonparametric tests because the assumptions for parametric testing were not met. We therefore compared each pharmaceutical treatment with the control (saline) group by using Mann–Whitney $U$ tests as is recommended for a small set of planned comparisons (Ruxton and Beauchamp 2008). Bonferroni correction was applied to account for multiple testing, thus reducing the significance level to $\alpha = 0.0125$. All tests were done in SPSS Statistics version 22.

# RESULTS

## Serotonin effect on the likelihood to engage in cleaning behavior

Compared with control treatment (saline), both the fluoxetine and the serotonin 1A receptor agonist 8-OH-DPAT significantly increased the proportion of clients inspected, whereas the tryptophan hydroxylase inhibitor PCPA and the serotonin 1A antagonist WAY 100.635 significantly decreased it (Mann–Whitney $U$ tests, all $n1 = 9$, $n2 = 9$; fluoxetine vs. saline: $U = 3$, $P < 0.001$; 8-OH-DPAT vs. saline: $U = 8$, $P = 0.003$; PCPA vs. saline: $U = 0$; $P < 0.001$; WAY 100.635 vs. saline: $U = 0$; $P < 0.001$, Figure 1).

## Serotonin effect on cleaning quality levels

The antagonist WAY 100.635 significantly decreased cleaner fish's inspection duration, whereas none of the remaining treatments significantly affected inspection duration (all $n1 = 9$, $n2 = 9$; fluoxetine vs. saline: $U = 36$, $P = 0.73$; 8-OH-DPAT vs. saline: $U = 27$, $P = 0.258$; WAY 100.635 vs. saline: $U = 12$, $P = 0.011$; PCPA vs. saline: $U = 38$, $P = 0.863$, Figure 2a). Similarly, WAY 100.635 significantly reduced clients' body jolts, whereas the other substances had no significant effect (all $n1 = 9$, $n2 = 9$; fluoxetine vs. saline: $U = 34$, $P = 0.607$; 8-OH-DPAT vs. saline: $U = 29$, $P = 0.34$; WAY 100.635 vs. saline: $U = 9$, $P = 0.004$; PCPA vs. saline: $U = 31$, $P = 0.436$, Figure 2b).

No difference was found in the proportion of interactions in which cleaners provided tactile stimulation (fluoxetine vs. saline: $U = 33$, $P = 0.546$; 8-OH-DPAT vs. saline: $U = 30$, $P = 0.387$; WAY 100.635 vs. saline: $U = 24.5$, $P = 0.161$; PCPA vs. saline: $U = 20.5$, $P = 0.077$, Figure 2c), However, cleaners treated with the agonist 8-OH-DPAT spent significantly more time providing tactile stimulation to clients (8-OH-DPAT vs. saline: $U = 9.5$, $n1 = 9$, $n2 = 9$, $P = 0.004$), whereas the opposite occurred with the antagonist WAY 100.635 (WAY 100.635 vs. saline: $U = 9$, $n1 = 9$, $n2 = 9$, $P = 0.004$); the other treatments had no effect on time spent providing tactile stimulation (both $n1 = 9$, $n2 = 9$; fluoxetine vs. saline: $U = 28.5$, $P = 0.2973$; PCPA vs. saline: $U = 22.0$, $P = 0.1134$, Figure 2d).

## Serotonin effect on a cleaner fish's likelihood to interact with conspecific partners

There were no significant effects of treatments on the frequency of cleaner parallel swimming (all $n1 = 9$, $n2 = 9$; fluoxetine (median;
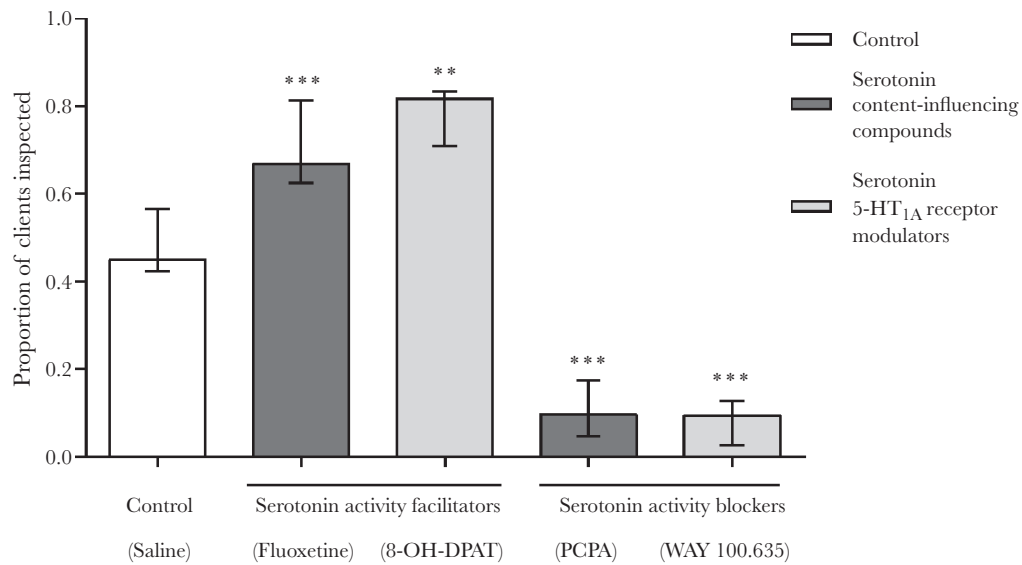
**Figure 1**
The effect of fluoxetine, 8-OH-DPAT, PCPA, and WAY 100.635 compared with a control (saline) on the likelihood to engage in cleaning behavior by cleaner fish *Labroides dimidiatus* of client fish and measured by the proportion of clients that was inspected (number of clients cleaned/total number of visits). Medians are shown ±1 interquartile range. *P* values refer to Mann–Whitney tests of the effect of each treatment group against the reference (saline) group. **\*\*P < 0.005; \*\*\*P < 0.001.**
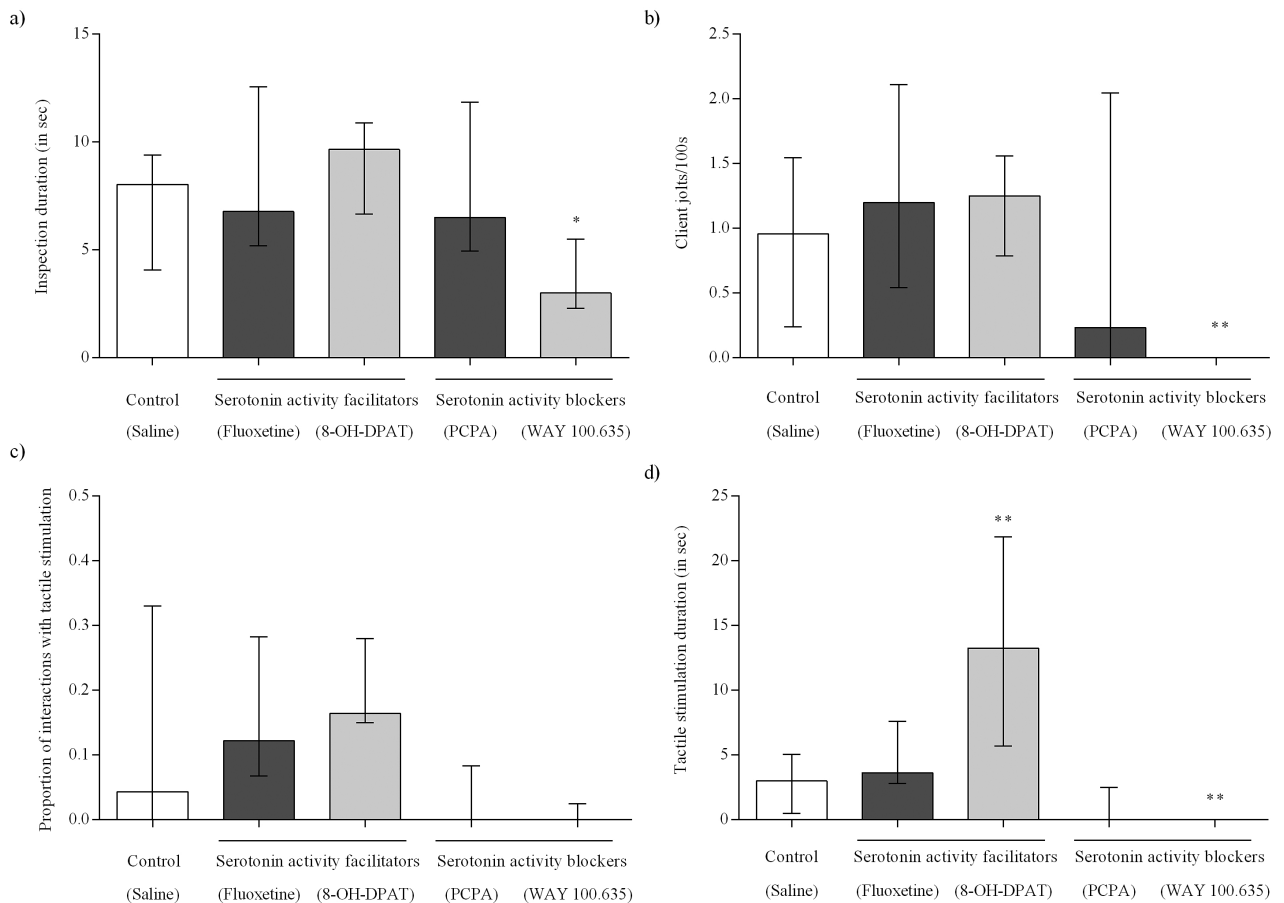


**Figure 2**
The effect of fluoxetine, 8-OH-DPAT, PCPA, and WAY 100.635 compared with a control (saline) on measures of cleaning quality by cleaner fish *L. dimidiatus* to client fish: (a) client inspection duration at cleaning stations (total inspection duration/total number of inspections), (b) number of client jolts per 100 s of inspection, (c) proportion of interactions in which tactile stimulation was applied to clients (number of cleaning events in which cleaner performed tactile stimulation/total number of cleaning events), and (d) tactile stimulation duration (total time of tactile stimulation/total number of inspections where tactile stimulation occurred). Medians are shown ±1 interquartile range. *P* values refer to Mann–Whitney tests of the effect of each treatment group against the reference (saline) group. **\*P < 0.0125; \*\*P < 0.005.**

interquartile range: 2; 0–2) vs. saline (3; 0–4), $U = 35$, $P = 0.666$; 8-OH-DPAT (3; 2–4) vs. saline: $U = 35$, $P = 0.666$; WAY 100.635 (6; 4–8) vs. saline: $U = 16.5$, $P = 0.031$; PCPA (6; 1–8) vs. saline: $U = 22.5$, $P = 0.113$). Individuals treated with WAY 100.635 significantly increased the frequency of chases of smaller conspecifics, whereas no significant effects were found for the remaining treatments (all $n1 = 9$, $n2 = 9$; fluoxetine vs. saline: $U = 27$, $P = 0.258$; 8-OH-DPAT vs. saline: $U = 18$, $P = 0.05$; WAY 100.635 vs. saline: $U = 9$, $P = 0.004$; PCPA vs. saline: $U = 22.5$, $P = 0.113$, Figure 3).

## DISCUSSION

We examined the neuromodulatory role of serotonin in cooperation between unrelated individuals, as well as general social behavior between conspecifics, using a well-known fish model, the cleaner wrasse *L. dimidiatus*. Shifts in serotonin availability and modulation of the serotonin 1A receptor produced subsequent changes of cleaner wrasses' behavioral response (see summary in Table 2). Our findings demonstrate that serotonin generally enhanced individual tendencies to approach clients. Below we further discuss our results.

### Serotonin modulation in seeking to interact

Serotonin is probably one of the main neuromodulators mediating the basic drive to be social (Young 2013). To date, human and other primates research has shown that altering serotonin levels can directly influence individuals' social perception and mood, namely that lower amounts of serotonin can induce a state in which isolation is preferred and prosocial contacts are undesired (Bartz 2011; Bilderbeck et al. 2011; Higley and Linnoila 1997; Higley et al. 1996). Moreover, in locusts, a rise in serotonin level is involved in their transformation from being solitary to gregariousness (Anstey et al. 2009). Our results on the proportion of clients inspected are consistent with the latter studies; the administration of the SSRI fluoxetine and the serotonin 1A receptor agonist 8-OH-DPAT lead to a general increase in the motivation to engage in cleaning interactions, whereas the blocking of serotonin-mediated effects by the tryptophan hydroxylase inhibitor PCPA and the serotonin 1A receptor antagonist WAY 100.635 had the opposite effect. One possible
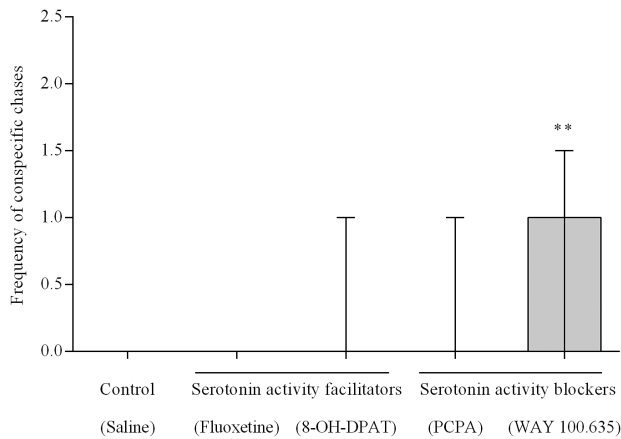
**Figure 3**
The effect of fluoxetine, 8-OH-DPAT, PCPA, and WAY 100.635 compared with a control (saline) on the cleaner fish *L. dimidiatus* frequency to chase other smaller conspecifics. Medians are shown ±1 interquartile range. *P* values refer to Mann–Whitney tests of the effect of each treatment group against the reference (saline) group. **$P < 0.005$.

**Table 2**
**Summary of the effect of fluoxetine, 8-OH-DPAT, PCPA, and WAY 100.635 on cleaner fish *Labroides dimidiatus* behavior compared with saline (control)**

| Behavioral response | Subjects analyzed | Fluoxetine | 8-OH-DPAT | PCPA | WAY 100.635 |
|---|---|---|---|---|---|
| **Interspecific** | | | | | |
| Likelihood to engage in cleaning behavior | | | | | |
| Proportion of clients inspected (Figure 1) | Cleaner | ↑ | ↑ | ↓ | ↓ |
| Cleaning service quality | | | | | |
| Inspection duration (Figure 2a) | Cleaner/client | NS | NS | NS | ↓ |
| Frequency of client jolts in response to cleaner bites/100 s (Figure 2b) | Client | NS | NS | NS | ↓ |
| Proportion of tactile stimulation provided (Figure 2c) | Cleaner | NS | NS | NS | NS |
| Duration of tactile stimulation provided to clients (Figure 2d) | Cleaner | NS | ↑ | NS | ↓ |
| **Intraspecific** | | | | | |
| Frequency of smaller conspecific chases (Figure 3) | Cleaner | NS | NS | NS | ← |
| | Cleaner | NS | NS | NS | ← |

Arrows indicate the effect of each treatment, relative to saline, on each behavior: "↑" denotes a significant increase, "↓" a significant decrease, and NS indicates that no significant effect was detected.

mechanism by which altered serotonin activity might work to change cleaner wrasses' motivation to interact is through the modulation of the reinforcement and incentive-motivational processes, which happens in interaction with the dopamine system (Baumgarten and Grozdanovic 1995) and via the regulatory roles of other serotonin receptor subtypes (Walsh and Cunningham 1997). Evidence has, for instance, been found in rats where increased serotonin levels not only facilitated reward-related behavior but also affected general arousal and motor tone (Sasaki-Adams and Kelley 2001). Another possibility for the lack of motivation for engaging in cleaning by cleaners under the effects of PCPA and WAY 100.635 is that lowering serotonin might enhance a form of detection and potential prediction of aversive signals in visiting clients, findings that have been reported following manipulations that reduce serotonin function in humans (Crockett et al. 2009). Athough we may assume that the serotonin 1A receptor plays a major role in modulating cleaners' motivation to interact with clients, other members of serotonin receptors family might also be involved. The overall effect observed may be related to serotonin additionally binding to other receptors, such as serotonin 2A, 6, and 7, which are also involved in social behavior, for example, by inhibiting anxiety effects in social interactions and by producing changes in social recognition (Barnes and Sharp 1999; King et al. 2008).

The serotoninergic system is also known to have an important role in the mediation of the hypothalamic-pituitary-interrenal (HPI) tissue stress axis. For instance, fish studies involving the administration of serotonin receptor agonists have provided evidence that serotonin can affect circulating cortisol levels (Höglund et al. 2002; Winberg et al. 1997). However, these effects may depend on the state of the fish and the dosage used. In the cleaning mutualisms, the rise of stress levels elicited by predatory clients is known to induce cleaning gobies *Elacatinus evelynae* to become more proactive and to reduce the elapsed time between client approach and the start of the interaction (Soares et al. 2012). In the case of the cleaner wrasse *L. dimidiatus*, the rise of cortisol levels seem to similarly relate to shifts in behavioral patterns (Soares et al. 2014). Thus, an additional potential explanation for the increase of cleaner wrasse motivation levels in this study may also occur via serotonin receptor agonists' stimulation of the HPI axis.

## Serotonin modulation of cooperative service quality

Serotonin may be one of the neuromodulators directly influencing the quality of cooperative interactions. For instance, in vervet monkeys, increasing serotonin via pharmacological treatment results in investing more in grooming (Raleigh et al. 1980, 1985). Our results partially matched these findings as cleaners spent more time providing tactile stimulation to their clients. A possible neuromodulation mechanism may occur via the interaction between the serotonergic and the dopaminergic neuronal system: 8-OH-DPAT has been referred to facilitate dopamine transmission and promote nonspecific motivational arousal (Montgomery et al. 1991). Indeed, previous studies have shown that 8-OH-DPAT affects dopamine neuronal activity (dopamine synthesis and turnover) in the limbic system (Ahlenius et al. 1989; Arborelius et al. 1993), a system critical for the attribution of valence and salience to social stimuli (Rilling et al. 2008). In this case, the putative change of rewarding value involved in the provision of tactile stimulation (as it decreases client stress; Soares et al. 2011) would be coded by the circuitry underlying the serotonin receptor 1A (located mainly in limbic areas; Pompeiano et al. 1992) influencing the mesolimbic dopaminergic pathways.

The blocking of serotonin activity effects is predicted to cause inhibition to approach stimuli and a rise in impulsivity and aggression (Young 2013). Here, the blocking of serotonin-mediated effects did not lead to a rise in cleaners' dishonesty levels, measured as client jolts. On the contrary, blocking serotonin action with the 1A receptor antagonist WAY 100.635 seems to induce a decrease in cheating frequencies. However, this result may also be a product of the general decrease of proportion of clients inspected and of the average duration of interactions, so it should be interpreted with caution. Interestingly, WAY 100.635 caused treated individuals to be more frequently aggressive toward conspecifics. Taken together, these results may imply that on a mechanistic level, cheating by feeding on client mucus is not linked to aggressive motivations. The results on intraspecific interactions fit many studies in vertebrates showing that highly aggressive individuals (which are usually dominant males) have lower serotonergic activity than less aggressive males (Blanchard et al. 1991; Raleigh et al. 1991; Summers et al. 1997; Winberg et al. 1993). As cleaner wrasse are protogynous hermaphrodites living in harems, it is possible that changes of social status and social dominance are also built on shifts of serotonin levels during cleaner wrasse ontogenetic development. Future work should be done to analyze differences of serotonin concentrations in the brain during different stages and transitions of individual life histories (i.e., small females, large females, and males).

## Cooperating or cheating: implication of the serotonin system via mediation of cortisol levels

As briefly mentioned above, serotonin can affect the HPI axis in fish. Previous studies done on fish have focussed on how these 2 systems work together during social interactions (Clotfelter et al. 2010; Larson et al. 2003; McDonald et al. 2011; Winberg and Nilsson 1993). Namely, social stressors seem to result in a rise of brain serotonin turnover (Winberg and Nilsson 1993), whereas socially subordinate fish display elevated cortisol levels (Ejike and Schreck 1980). Moreover, exogenous treatment with the serotonin agonist 8-OH-DPAT has been demonstrated to result in an increase of cortisol level in the goldfish *Carassius auratus* (Lim et al 2013). In the cleaning mutualism, the exogenous increase of cortisol affects *L. dimidiatus* cooperative levels but in a conditional manner: treated cleaners were observed to provide more tactile stimulation to the smaller clientele just to attract larger clients that are then bitten to obtain mucus (Bshary and Grutter 2002, Soares et al. 2014). In the current study, we have not categorized clients according to size; however, we did find an overall effect of the 8-OH-DPAT on tactile stimulation duration. The potential rise in cortisol levels (mediated by the treatment with 8-OH-DPAT) could be influencing the duration of tactile stimulation provision, similar to the prolongation of the interaction duration in gobies when cleaning predators (2012). However, the rise of cortisol levels did not influence the number of events in which tactile stimulation was given, as previously reported by Soares et al. (2014). The levels of clients' jolts were also relatively low after treatment with serotonin agonists, when compared with those demonstrated by Soares et al. (Soares et al. 2014). The absence of significant results in the proportion of tactile stimulated provided and in the outcome jolting behavior of clients could be due to the use of a smaller dosage or a question of treatment effect duration: for instance, in goldfish (mentioned above), plasma cortisol samples were collected 1.5 h following treatment, whereas in our case, data were solely collected during the first 45 min following treatment. Future work should include a wider collection of behavioral effects in relation to time (up to 8 h, Lim et al. 2013).

## Serotonin link to neuropeptide systems

A neurohormone that was first identified to be involved in the regulation of cooperative behavior in the cleaner wrasse is AVT (Soares et al. 2012, Cardoso et al. 2015). Earlier research, mostly done in rats, has confirmed that serotonin (and its agonists and SSRIs) plays a key role in altering the secretion and release of AVP and oxytocin (Jørgensen et al. 2003). According to Jørgensen et al. (2003), the treatment with the agonist 8-OH-DPAT induces significant oxytocin release, which is inhibited by the serotonin 1A receptor antagonist. It is thus possible that the effect 8-OH-DPAT had on tactile stimulation duration can also occur via an effective release of isotocin (the homolog of oxytocin in fish), which might also be involved in the rise of cleaner wrasses' prosocial behavior resulting from the treatment with both of the agonists (8-OH-DPAT and fluoxetine) used. The lack of effects found by Soares et al. (2012) regarding isotocin, in this system, is not necessarily a demonstration of absence of influence but otherwise might be directly linked to the dosages used or to individuals' previous social context (Cardoso et al. 2015b). As for the role of serotonergic activity on the AVT system, studies on hamsters are consistent with those done on teleost fish (Semsar et al. 2004), with the use of fluoxetine being responsible for a decrease in brain AVP levels (in hamsters) and lowering mRNA AVT levels in fish (Ferris 1996; Semsar et al. 2004). If indeed the rise of serotonin activity levels potentiates a decrease in brain AVT production and release, then cleaner wrasses' behavioral response (which increased in their motivation to engage in cleaning) is in line with 1 previous study, which identified the involvement of the AVT pathways in cleaner wrasse behavior (Soares et al. 2012). Our results thus support the evidence for a connection between the serotonin and the AVT system.

## CONCLUDING REMARKS

In the wild, cleaner wrasses' behavioral responses need to adapt in accordance with a dynamic social context, which provides constant fluctuations in stimuli sources (both interspecific and conspecific). Our results provide first evidence that serotonin is a neuromodulatory driver for both social and cooperative activities and contribute to the understanding of neural pathways of cooperation. Unravelling the potential interactions with other neurohormonal systems will also be a key challenge for future research. For instance, future work should focus on the relation between the serotoninergic system and other neurohormones, namely cortisol, AVT but mostly dopamine. Moreover, further testing should aim at finding out whether serotonin is actively involved in conditional cooperative transitions, similar to those produced by cortisol level shifts (Soares et al. 2014).

## FUNDING

**Handling editor:** John Fitzpatrick

## REFERENCES

Ahlenius S, Hillegaart V, Wijkström A. 1989. Evidence for selective inhibition of limbic forebrain dopamine synthesis by 8-OH-DPAT in the rat. Naunyn Schmiedebergs Arch Pharmacol. 339:551–556.

Albert PR, Zhou QY, Van Tol HH, Bunzow JR, Civelli O. 1990. Cloning, functional expression, and mRNA tissue distribution of the rat 5-hydroxytryptamine1A receptor gene. J Biol Chem. 265:5825–5832.

Anstey ML, Rogers SM, Ott SR, Burrows M, Simpson SJ. 2009. Serotonin mediates behavioral gregarization underlying swarm formation in desert locusts. Science. 323:627–630.

Arborelius L, Chergui K, Murase S. 1993. The 5-HT1A receptor selective ligands,(R)-8-OH-DPAT and (S)-UH-301, differentially affect the activity of midbrain dopamine neurons. Naunyn Schmiedebergs Arch Pharmacol. 347:353–362.

Axelrod R, Hamilton WD. 1981. The evolution of cooperation. Science. 211:1390–1396.

Barnes NM, Sharp T. 1999. A review of central 5-HT receptors and their function. Neuropharmacology. 38:1083–1152.

Bartz JA. 2011. Serotonin and the neurochemistry of intimacy. Biol Psychiatry. 69:716–717.

Baumgarten HG, Grozdanovic Z. 1995. Psychopharmacology of central serotonergic systems. Pharmacopsychiatry. 28(Suppl 2):73–79.

Beulig A, Fowler J. 2008. Fish on Prozac: effect of serotonin reuptake inhibitors on cognition in goldfish. Behav Neurosci. 122:426–432.

Bilderbeck AC, McCabe C, Wakeley J, McGlone F, Harris T, Cowen PJ, Rogers RD. 2011. Serotonergic activity influences the cognitive appraisal of close intimate relationships in healthy adults. Biol Psychiatry. 69:720–725.

Blanchard D, Cholvanich P, Blanchard R. 1991. Serotonin, but not dopamine, metabolites are increased in selected brain regions of subordinate male rats in a colony environment. Brain Res. 568:61–66.

Booij L, Tremblay RE, Leyton M, Séguin JR, Vitaro F, Gravel P, Perreau-Linck E, Lévesque ML, Durand F, Diksic M, et al. 2010. Brain serotonin synthesis in adult males characterized by physical aggression during childhood: a 21-year longitudinal study. PLoS One. 5:e11255.

Bshary R, Grutter AS. 2002. Asymmetric cheating opportunities and partner control in a cleaner fish mutualism. Anim Behav. 63:547–555.

Bshary R, Würth M. 2001. Cleaner fish Labroides dimidiatus manipulate client reef fish by providing tactile stimulation. Proc Biol Sci. 268:1495–1501.

Bshary R. 2011. Machiavellian intelligence in fishes. In: Brown C, Laland K, Krause J, editors. Fish cognition and behavior. Oxford, UK: Wiley-Blackwell

Cardoso SC, Paitio JR, Oliveira RF, Bshary R, Soares MC. 2015a. Arginine vasotocin reduces levels of cooperative behaviour in a cleaner fish. Physiol Behav. 139:314–320.

Cardoso SC, Grutter AS, Paula JR, André GI, Messias JP, Gozdowska M, Kulczykowska E, Soares MC. 2015b. Forebrain neuropeptide regulation of pair association and behaviour in cooperating cleaner fish. Physiol Behav. 145:1–7.

Clotfelter E, O'Hare E. 2007. Serotonin decreases aggression via 5-HT 1A receptors in the fighting fish Betta splendens. Pharmacol Biochem Behav. 87:222–231.

Coccaro EF, Lee R, Kavoussi RJ. 2010. Aggression, suicidality, and intermittent explosive disorder: serotonergic correlates in personality disorder and healthy control subjects. Neuropsychopharmacology. 35:435–444.

Côté IM, Arnal C, Reynolds JD. 1998. Variation in posing behaviour among fish species visiting cleaning stations. J Fish Biol. 53:256–266.

Côté IM. 2000. Evolution and ecology of cleaning symbioses in the sea. Oceanogr Mar Biol Annu Rev. 38:311–355.

Crockett MJ, Clark L, Robbins TW. 2009. Reconciling the role of serotonin in behavioral inhibition and aversion: acute tryptophan depletion abolishes punishment-induced inhibition in humans. J Neurosci. 29:11993–11999.

Crockett MJ, Clark L, Tabibnia G, Lieberman MD. 2008. Serotonin modulates behavioral reactions to unfairness. Science. 320:11–13.

De Dreu CKW, Shalvi S, Greer LL, Van Kleef G, Handgraaf MJJ. 2012. Oxytocin motivates non-cooperation in intergroup conflict to protect vulnerable in-group members. PLoS One. 7:e46751

Ejike C, Schreck CB. 1980. Stress and social hierarchy rank in coho salmon. Trans Am Fish Soc 109:423–426.

Ferris CF, Melloni RH Jr, Koppel G, Perry KW, Fuller RW, Delville Y. 1997. Vasopressin/serotonin interactions in the anterior hypothalamus control aggressive behavior in golden hamsters. J Neurosci. 17:4331–4340.

Ferris CF. 1996. Serotonin diminishes aggression by suppressing the activity of the vasopressin system. Ann NY Acad Sci. 794:98–103.

Fox E, Ridgewell A, Ashwin C. 2009. Looking on the bright side: biased attention and the human serotonin transporter gene. Proc R Soc B. 1663:1747–1751.

Grutter A. 2004. Cleaner fish use tactile dancing behavior as a preconflict management strategy. Curr Biol. 14:4–7.

Grutter AS, Bshary R. 2003. Cleaner wrasse prefer client mucus: support for partner control mechanisms in cleaning interactions. Proc Biol Sci. 270(Suppl 2)S242–S244.

Higley JD, Linnoila M. 1997. Low central nervous system serotonergic activity is traitlike and correlates with impulsive behavior. Ann NY Acad Sci. 836:39–56.

Higley JD, Mehlman PT, Poland RE, Taub DM, Vickers J, Suomi SJ, Linnoila M. 1996. CSF testosterone and 5-HIAA correlate with different types of aggressive behaviors. Biol Psychiatry. 40:1067–1082.

Hjort S. 2000 Serotonin autoreceptor function and antidepressent drug action. J Psychopharmacol. 14:177–185.

Höglund E, Balm PH, Winberg S. 2002. Stimulatory and inhibitory effects of 5-HT(1A) receptors on adrenocorticotropic hormone and cortisol secretion in a teleost fish, the Arctic charr (Salvelinus alpinus). Neurosci Lett. 324:193–196.

Johnstone R, Bshary R. 2002. From parasitism to mutualism: partner control in asymmetric interactions. Ecol Lett. 5:634–639.

Jørgensen H, Riis M, Knigge U, Kjaer A, Warberg J. 2003. Serotonin receptors involved in vasopressin and oxytocin secretion. J Neuroendocrinol. 15:242–249.

King MV, Marsden CA, Fone KC. 2008. A role for the 5-HT(1A), 5-HT4 and 5-HT6 receptors in learning and memory. Trends Pharmacol Sci. 29:482–492.

Knutson B, Wolkowitz OM, Cole SW, Chan T, Moore EA, Johnson RC, Terpstra J, Turner RA, Reus VI. 1998. Selective alteration of personality and social behavior by serotonergic intervention. Am J Psychiatry. 155:373–379.

Laaris N, Haj-Dahmane S, Hamon M, Lanfumey L. 1995. Glucocorticoid receptor-mediated inhibition by corticosterone of 5-HT1A autoreceptor functioning in the rat dorsal raphe nucleus. Neuropharmacology. 34:1201–1210.

Larson ET, Norris DO, Summers CH. 2003. Monoaminergic changes associated with socially induced sex reversal in the saddleback wrasse. Neuroscience. 119:251–263.

Lesch KP, Mössner R. 1998. Genetically driven variation in serotonin uptake: is there a link to affective spectrum, neurodevelopmental, and neurodegenerative disorders? Biol Psychiatry. 44:179–192.

Lim JE, Porteus CS, Bernier NJ. 2013. Serotonin directly stimulates cortisol secretion from the interrenals in goldfish. Gen Comp Endocrinol. 192:246–255.

Lopez-Mendoza D, Aguilar-Bravo H, Swanson HH. 1998. Combined effects of Gepirone and (+)WAY 100135 on territorial aggression in mice. Pharmacol Biochem Behav. 61:1–8.

Lorenzi V, Carpenter RE, Summers CH, Earley RL, Grober MS. 2009. Serotonin, social status and sex change in the bluebanded goby Lythrypnus dalli. Physiol Behav. 97:476–483.

Marazziti D, Baroni S, Giannaccini G, Betti L, Massimetti G, Carmassi C, Catena-Dell'Osso M. 2012. A link between oxytocin and serotonin in humans: supporting evidence from peripheral markers. Eur Neuropsychopharmacol. 22:578–583.

McDonald MD, Gonzalez A, Sloman KA. 2011. Higher levels of aggression are observed in socially dominant toadfish treated with the selective serotonin reuptake inhibitor, fluoxetine. Comp Biochem Physiol C. 153:107–112.

Medeiros LR, McDonald MD. 2013. Cortisol-mediated downregulation of the serotonin 1A receptor subtype in the Gulf toadfish, Opsanus beta. Comp Biochem Physiol Part A. 164:612–621.

Montgomery A, Rose I, Herberg L. 1991. 5-HT1A agonists and dopamine: the effects of 8-OH-DPAT and buspirone on brain-stimulation reward. J Neural Transm. 83:139–148.

Neumann ID. 2008. Brain oxytocin: a key regulator of emotional and social behaviours in both females and males. J Neuroendocrinol. 20:858–865.

Perreault HA, Semsar K, Godwin J. 2003. Fluoxetine treatment decreases territorial aggression in a coral reef fish. Physiol Behav. 79:719–724.

Pompeiano M, Palacios JM, Mengod G. 1992. Distribution and cellular localization of mRNA coding for 5-HT1A receptor in the rat brain: correlation with receptor binding. J Neurosci. 12:440–453.

Raihani NJ, Bshary R. 2011. Resolving the iterated prisoner's dilemma: theory and reality. J Evol Biol. 24:1628–1639.

Raleigh MJ, Brammer GL, McGuire MT, Yuwiler A. 1985. Dominant social status facilitates the behavioral effects of serotonergic agonists. Brain Res. 348:274–282.

Raleigh MJ, Brammer GL, Yuwiler A, Flannery JW, McGuire MT, Geller E. 1980. Serotonergic influences on the social behavior of vervet monkeys (Cercopithecus aethiops sabaeus). Exp Neurol. 68:322–334.

Raleigh MJ, McGuire MT, Brammer GL, Pollack DB, Yuwiler A. 1991. Serotonergic mechanisms promote dominance acquisition in adult male vervet monkeys. Brain Res. 559:181–190.

Rilling JK, Goldsmith DR, Glenn AL, Jairam MR, Elfenbein HA, Dagenais JE, Murdock CD, Pagnoni G. 2008. The neural correlates of the affective response to unreciprocated cooperation. Neuropsychologia. 46:1256–1266.

Ros AF, Vullioud P, Bshary R. 2012. Treatment with the glucocorticoid antagonist RU486 reduces cooperative cleaning visits of a common reef fish, the lined bristletooth. Horm Behav. 61:37–43.

Ruxton GD, Beauchamp G. 2008. Time for some a priori thinking about post hoc testing. Behav Ecol. 19(3):690–693.

Sasaki-Adams DM, Kelley AE. 2001. Serotonin-dopamine interactions in the control of conditioned reinforcement and motor behavior. Neuropsychopharmacology. 25:440–452.

Semsar K, Perreault HA, Godwin J. 2004. Fluoxetine-treated male wrasses exhibit low AVT expression. Brain Res. 1029:141–147.

Soares M, Bshary R, Mendonça R, Grutter A, Oliveira R. 2012. Arginine vasotocin regulation of interspecific cooperative behaviour in a cleaner fish. PLoS One. 7:1–10.

Soares M, Cardoso S, Grutter A. 2014. Cortisol mediates cleaner wrasse switch from cooperation to cheating and tactical deception. Horm Behav. 66:346–50.

Soares MC, Bshary R, Cardoso SC, Côté IM. 2008. The meaning of jolts by fish clients of cleaning bobies. Ethology. 114:209–214.

Soares MC, Oliveira RF, Ros AFH, Grutter AS, Bshary R. 2011. Tactile stimulation lowers stress in fish. Nat Commun. 2:534.

Soares MC, Bshary R, Cardoso SC, Côté IM, Oliveira RF. 2012. Face your fears: cleanerfish inspect predators despite being stressed by them. PLoS One. 7(6):e39781.

Summers CH, Matter JM, Ronan PJ, Summers CH. 1997. Central monoamines in free-ranging lizards: differences associated with social roles and territoriality. Brain Behav Evol. 51:23–32.

Trivers R. 1971. The evolution of reciprocal altruism. Q Rev Biol. 46:35–57.

Tse WS, Bond AJ. 2002. Serotonergic intervention affects both social dominance and affiliative behaviour. Psychopharmacology (Berl). 161:324–330.

Walsh SL, Cunningham KA. 1997. Serotonergic mechanisms involved in the discriminative stimulus, reinforcing and subjective effects of cocaine. Psychopharmacology (Berl). 130:41–58.

Winberg S, Carter C, McCarthy I, Nilsson GE, Houlihan DF. 1993. Feeding rank and brain serotonergic activity in rainbow trout Oncorhynchus mykiss. J Exp Biol. 211:197–211.

Winberg S, Nilsson A, Hylland P, Söderstöm V, Nilsson GE. 1997. Serotonin as a regulator of hypothalamic-pituitary-interrenal activity in teleost fish. Neurosci Lett. 230:113–116.

Winberg S, Nilsson GE. 1993. Roles of brain monoamine neurotransmitters in agonistic behaviour and stress reactions, with particular reference to fish. Comp Biochem Physiol. 106:597–614.

Wood RM, Rilling JK, Sanfey AG, Bhagwagar Z, Rogers RD. 2006. Effects of tryptophan depletion on the performance of an iterated Prisoner's Dilemma game in healthy adults. Neuropsychopharmacology. 31:1075–1084.

Young LJ, Wang Z. 2004. The neurobiology of pair bonding. Nat Neurosci. 7:1048–1054.

Young S. 2013. The effect of raising and lowering tryptophan levels on human mood and social behaviour. Philos. Trans R Soc B Biol Sci. 368:20110375.