Profiles of cooperative brains: A discriminant analysis of cleaner and client fish monoaminergic responses to different social contexts

Caio Maximino[1*], Ana Cristina R. Gomes[2*], Murilo S. Abreu[3], Sónia C Cardoso[2], Monica Lima-Maximino[4], Svante Winberg[5], Marta C. Soares[2]

*shared first authorship

[1] IESB, Instituto de Estudos em Saúde e Biológicas, Universidade Federal do Sul e Sudeste do Pará, Unidade III, Marabá, Brazil

[2] CIBIO, Centro de Investigação em Biodiversidade e Recursos Genéticos, Universidade do Porto, Campus Agrário de Vairão, 4485-661 Vairão, Portugal.

[3] Bioscience Institute, University of Passo Fundo (UPF), Passo Fundo, RS, Brazil.

[4] DMCF, Departamento de Morfologia e Ciências Fisiológicas, Universidade do Estado do Pará, Marabá, Brazil

[5] Department of Neuroscience, Uppsala University, Box 593, Husargatan 3, 75124, Uppsala, Sweden

Abstract

Vertebrate cognitive function requires a dynamic coordination of multiple specialized areas of the brain. The challenge here is to understand how these brain areas respond in dependence to the neurophysiological mechanisms in place, as to enable the successful processing of information. For instance, social and cooperative behaviour has been linked to the activation of some specific brain areas, mostly associated with reward processing, however mechanisms could in principle change, in accordance to species social demands and taxon involved. Here we chose a classic model system of cooperation between species of fish and compared the brain monoaminergic response of cleaners *Labroides dimidiatus* exposed to several social-related treatments to the response of one client species (*Naso elegans)* introduced to similar contexts. We demonstrate that the variable appraisal of each social challenge contributes to brain dopaminergic and serotonergic changes, in cleaners and clients, with both showing the diencephalon and optic tectum as main areas of metabolite response. However, the role of the serotoninergic system activation was mostly demonstrated at the diencephalon and cerebellum of cleaners, a response that was driven by the exposure to clients. Our current results are the first to jointly demonstrate the level of selective similarity in brain monoaminergic mechanisms that underlie fish mutualistic and social engagement, for both sides of these partnerships.

Key-words:

1. Introduction

The neural substrate of cooperative behaviour is gaining increasing attention in the latter years, due to the appearance of novel techniques to study neurochemistry and neurophysiology as well as to the recognition that cooperation is widespread in nature [1]. In recent years, the focus on fish species as model organisms to study cooperative behaviour increased [2].

Cleaner wrasses (*Labroides dimidiatus*) are mutualistic fish that engage in cooperative behaviour, inspecting the surface, gills and sometimes the mouth of 'client' reef fish, eating ectoparasites, mucus, scales and dead or infected tissue [3]. These cleaning mutualisms involve repeated iterations with multiple partners (referred to as clients) that have asymmetrical options of behaving cooperatively or to defect [2]. Cleaners display flexibility in adjusting their cleaning services in accordance with clients, varying between "cooperating" (removing ectoparasites) or "defecting" (eating fish mucus) [3]. Cleaners adjust their behaviour towards clients by changing cheating frequency and/or providing a form of physical contact with clients ("tactile stimulation"), which is thought to "appease" the client after a cheating event, to prolong interactions with clients about to leave the cleaning station, or to act as a pre-conflict management strategy with predators [4–6].

There is limited evidence for a role of monoamines on cooperative behaviour. In healthy humans, tryptophan depletion reduced the level of cooperation shown by participants in the first day (but not the second day) of an iterared Prisoner's dillema game, and a diminished probability of cooperative responding given previous mutually cooperative behaviour [7]. In cleaner wrasses, fluoxetine or the serotonin (5-HT) 1A receptor agonist 8-OH-DPAT increased motivation to engage in cleaning behaviour and the frequency of tactile stimulation, while blocking serotonin synthesis with pCPA or

antagonizing the 5-HT_{1A} receptor with WAY 100,635 decreased cleaner's cheating levels [8]. Blocking dopamine (DA) D1 receptors increases tactile stimulation and induces cleaners to initiate more interactions with clients [9]; these effects appear to be mediated by the degree of familiarity with the client, with D1 antagonists increasing tactile stimulation only when interacting with novel clients [10]. It has been suggested [2] that dopamine is tracking predicted outcomes, with reductions in DA signalling worse outcomes than predicted (e.g., lower probability of getting food during an interaction, or higher likelihood of being punished). Interestingly, blocking D1 receptors in cleaners which were trained to wait for a larger reward improves learning [11], while blocking 5-HT_{1A} receptors impair learning of the task [12], suggesting that 5-HT and DA have opponent roles in tracking outcomes.

A relevant question regarding the role of monoamines in this cooperative behaviour regards the importance of physical contact. Monoaminergic signalling has been proposed to mediate social behaviour in fish, with increases in dopamine after visual contact with conspecifics in zebrafish [13]. For a cleaning mutualism to take place in fish, however, physical contact is needed. Monoamines could act at various points of the cleaning sequence: client evaluation, motivation to approach the client, maintenance of cleaning behaviour after initial contact, and during inspection, choosing whether to clean or to cheat. There is some evidence that both DA and 5-HT participate in client evaluation [12]. Dopamine, for example, is the main neurotransmitter in the mesolimbic reward system [14], which interacts with the social behaviour network as to reinforce responses to salient social stimuli [15], which could include the motivation to cooperate. Here we test the hypothesis that monoamines are also associated with the motivation to approach the client by presenting cleaner wrasse *Labroides dimidiatus* with either a novel, non-living stimulus, a conspecific with only visual access, a

conspecific with both visual and tactile access, a client with visual (but not tactile) access, and a client with both visual and tactile access, and measuring levels of DA and 5-HT, as well as their metabolites, in the forebrain, diencephalon, optic tectum, cerebellum, and brain stem. We predict that, if monoamines are related to the motivation to approach the client, their increase should be related to presentation of heterospecifics vs. conspecifics, but not visual vs. tactile access.

A secondary aim refers to neurochemical changes in the client brain. Clients are not passive receivers in a cleaning mutualism, and indeed may react to cheating by cheating in return (if they are piscivores), switch between cleaner stations, or by punishing the cheating cleaner [16–19]. Indeed, cleaners can establish mutualisms with herbivore fishes which can reciprocate by chasing cheaters or swimming away, or with piscivores which can, in addition to those strategies, "cheat back" by eating the cleaner in a subsequent encounter [3]. In an encounter with herbivore clients, then, the cooperation is asymmetric [17]. If monoamines participate in this relationship, it is to be expected that these neurotransmitters also modulate client behaviour. In that case, monoamines could act at the approach motivation or as a "reward signal" as well, or they could be produced in response to the reception of tactile stimulation (which decreases cortisol in clients [6]). Our second hypothesis is that monoamines are also associated with the motivation to approach the cleaner by presenting Naso tang *Naso elegans* with either a novel, non-living stimulus, a conspecific with both visual and tactile access, and a client with both visual and tactile access, and measuring monoamines in the same brain regions as in cleaners. If monoamines are thus related to the motivation to approach the client, their increase should be related to presentation of heterospecifics vs. conspecifics, but not to a novel stimulus.

Material and Methods

2.1. Experimental conditions

Experiments were conducted at the fish housing facilities of the Oceanário de Lisboa (Lisbon, Portugal). In total, 53 Indo-Pacific bluestreak cleaner wrasses, *Labroides dimidiatus* and 18 adult blond naso tang *Naso elegans* (family Acanthuridae; from now on referred to as "clients") were used. For similar methodologies please see [20–22].

A) Experiment 1: cleaners

Cleaners were exposed to five different contextual treatments. Indeed, on each experimental day, one of the five following treatments was randomly allocated to each subject cleaner wrasse aquarium: a) conspecific (*L. dimidiatus*, n = 10), b) client (*N. elegans*, n = 11), c) conspecific inside another smaller aquarium (n = 12), d) client inside another smaller aquarium (n = 10) and e) ball (white ball, about 5 cm in diameter, which stayed at the bottom, completely sessile, n = 10), see Fig 1. Cleaners were exposed to treatments for the next 60 minutes. At the end of experiments, each subject cleaner wrasse was captured and immediately sacrificed with an overdose of tricaine solution (MS222, Pharmaq; 500–1000 mg/L) and the spinal cord was sectioned, the period between anaesthesia and the spinal cord section does not exceed 2 min, no change of monoamines levels. The brain was immediately dissected under a stereoscope (Zeiss; Stemi 2000) into five macro-areas: forebrain (FB - which included the olfactory bulbs and the telencephalon), diencephalon (DL), optic tectum (OT), cerebellum (CB) and brain stem (BS) (for a drawing of the major brain areas, please see [20–22]. Major brain areas were frozen with dry ice and the stored at - 80°C.

B) Experiment 2: Clients

Three different contextual treatments were allocated to our subject clients (*N. elegans*): a) conspecific (*N. elegans*, n =7), b) a cleaner (*L. dimidiatus*, n = 7) and c) ball (same as above, n = 4) see Fig 1. Clients were distributed across experimental treatments as follows: 7 clients for the sympatric cleaner group, 7 clients for the conspecific group and 4 for the ball group. Each client was introduced inside the experimental tank and left for at least 2-5 min until normal behaviour was restored. Cleaners were exposed to treatments for the next 60 minutes. At the end of experiments, each subject client was captured and immediately sacrificed and brain micro-dissected as explained above (experiment 1).

2.2. Quantification of monoamines by high performance liquid chromatography with electrochemical detection (HPLC-EC)

High performance liquid chromatography with electrochemical detection (HPLC-EC) was used to analyze the monoamines dopamine (DA) and serotonin (5-HT, 5-hydroxytryptamine), the DA metabolite DOPAC (3,4-dihydroxyphenylacetic acid), the 5-HT metabolite 5-HIAA (5-hydroxy indole acetic acid), as described by [23] (for the complete protocol used in both experiments please see [21,22]). The ratios of 5-HIAA/5-HT and DOPAC/DA were calculated and used as an index of serotonergic and dopaminergic activity, respectively. For normalization of brain monoamine levels, brain protein weights were determined with Bicinchoninic acid protein determination (Sigma–Aldrich, Sweden) according to the manufacturer's instructions. The assay was read on Labsystems multiskan 352 plate reader (Labsystems, Thermo Fisher Scientific) wavelength of 570 nm.

2.3. Statistical analysis

We used multivariate discriminant analysis (MDA) to test for differences in the pattern of metabolite expression among the 5 social contexts for cleaners (with

conspecific, with client, conspecific inside another aquarium, client inside another aquarium, and control), or the 3 social contexts for clients (with conspecific, with cleaner, and control). We focused our measures to the dopaminergic system and measure levels of dopamine and those of DOPAC (3,4- Dihydroxyphenylacetic acid), a dopamine's metabolite and also levels of serotonin (5-hydroxytryptamine, 5-HT), and serotonin's metabolite 5HIAA (5-Hydroxyindoleacetic acid). One set of MDA uses as data the concentrations of each monoamine (DA and 5-HT) and metabolite (DOPAC and 5-HIAA) at each brain area. This allows us to find the combination of metabolites and brain areas that discriminate the most the different social contexts. Another set of MDA uses as data the ratios of DOPAC/DA and 5-HT/5-HIAA as these will enable us to interpret the relation between monoaminergic production and its subsequent metabolic use. We interpret the two principal discriminant functions (DF1 and DF2) of each MDA based on trait loadings higher than 0.40 [24]. We assessed the significance of the discrimination within the entire set of social contexts using Wilk's Lambda, and we used ANOVAs with post-hoc Tukey HSD tests to find which pairs of social context differ significantly in DF1 or DF2. All values were standardized using script available [25] and normality was checked through q-q plots. All analyses were run with R (version 3.3.3; R Core Team, 2017) package car (version 2.1-4; [26] and MASS (version 7.3-47; [27]).

2.4. Ethical note

The protocols were carried out in accordance to the approved guidelines by the Oceanário de Lisboa (fish housing facilities), where the experiments were then developed. Animal procedures used in this study were also approved by the Portuguese Veterinary Office (Direccao Geral de Veterinaria, license # 0420/000/000/2009).

2.5. Open science practices

The analyses performed were not formally preregistered. Data for this manuscript can be found at our GitHub repository (https://github.com/lanec-unifesspa/cleaners).

3. **Results**

3.1) Experiment 1: cleaners

Overall, social contexts differed in the levels of metabolite expression (Wilk's lambda = 0.03, p = 0.001; Fig.2A) and in metabolite ratios (Wilk's lambda = 0.23, p = 0.02; Fig.2 B) across the brain tissues of cleaners.

For metabolite levels, DF1 and DF2 explained 83.9% of the variation between all the contexts. DF1 differed significantly between most pairs of contexts, except for the comparison between client vs. conspecific inside another aquarium, which instead were found to differ in DF2 (Fig. 2A, Table 1). DF1 was characterized mostly by strong negative loadings of DOPAC at the brain stem, and of DA, 5-HIAA and 5-HT at the diencephalon of cleaners (loadings >-0.40; all other loadings <|0.29|; Fig. 2C and Table 2). DF2 was characterized mostly by a strong positive loading of DA at the optic tectum (loading = 0.58; all other loadings <|0.36|; Fig. 2C and Table 2).

When using instead ratios of metabolite expression in the brain tissues of cleaners, DF1 and DF2 together explained 75% of the variation among contexts. DF1 differed significantly between cleaners that were prevented to interact with clients (inside a smaller aquarium) from those that could engage freely with clients (Fig 1B; Table 1). DF1 also differed between fully interactive contexts (i.e. interaction with clients) and either controls or interaction with conspecifics, and between clients in

separate aquaria and conspecifics in separate aquaria (Table 1). DF2 differed only between interaction with clients and either conspecifics in separate aquaria or conspecifics (Table 1). DF1 was characterized mostly by loadings of 5-HIAA/5-HT ratios (loadings = -0.51 at the diencephalon and 0.45 at the cerebellum; all other loadings <|0.33|; Fig. 2D and Table 3). DF2 was characterized mostly by a strong negative loading of 5-HIAA/5-HT ratio at the diencephalon and cerebelum (loading = -0.50 and -0.41, respectively; all other loadings <|0.27|; Fig. 2D and Table 3).

3.2.) Experiment 2: clients

Regarding clients, the exposure to different social contextual treatments was solely found to differ significantly in the levels of metabolite expression (Wilk's lambda = 0.01, p = 0.02), showing a clear separation between the individuals exposed to different contexts (Fig. 3 A) but not for metabolite ratios (Wilk's lambda = 0.42 , p = 0.51 , Fig 3B; Table 4).

For monoaminergic metabolite production, DF1 and DF2 explained 99% of the variation and differ significantly among all pairs of contexts, except for the context of a cleaner with a client, which was not significant for DF2. DF1 seems to be explained by two areas of client fish *N. elegans*: the diencephalon (varying in terms of DOPAC and 5-HIAA, in opposite directions) and the optic tectum (solely for DOPAC; Fig. 3, Table 2), while DF2 seems to be explained by the DOPAC on the TC (Fig. 3, Table 2). Surprisingly, no differences were found in the overall ratio patterns (monoaminergic ratios DOPAC/DA and 5-HIAA/5-HT; Wilk's lambda = 0.42, p = 0.51).

4. Discussion

Discriminant analysis is a powerful statistical tool that enabled us to determine the contribution of social and mutualistic behaviour to the modulation of brain monoaminergic levels of cleaners and clients, and specifically those linked to the activation of some localized brain areas. We found that brain monoaminergic response to social challenges shared interesting similarities for cleaners and clients, distinguishing broadly between stimuli that fish could physically interact with, from all those that were either physically inaccessible (stimuli inside aquaria) or not able to respond (sessile, non-living stimulus), with most relevant alterations occurring at the diencephalon and optic tectum, in reference to DAergic and 5-HTergic monoaminergic change. And while client fish clearly responded to differences posed by conspecifics compared to heterospecifics, we discovered that it was cleaners' 5-HT system that mostly contributed to separate mutualistic activities from all others, changing crucially whenever these individuals were given the possibility to physically engage in cleaning (occurring at the cerebellum) or were prevented to interact (occurring at the diencephalon) (but see also [22]).

Broad visual analysis of cleaners' monoaminergic response in respect to all different treatments suggests the distinction of 3 groups: a first with inaccessible clients and control, a second composed by inaccessible conspecifics and a third that included conspecifics and heterospecifics that were fully accessible. Specifically, the DF1 axis represented most of the variation observed, being negatively associated with DA, 5-HT and 5-HIAA at the diencephalon and DOPAC at the brain stem; while the DF2 mostly distinguished those stimuli that cleaners could physically access from those they could not and was positively associated with DA levels at the optic tectum. It is usually considered that monoaminergic increase (in this case, DA or 5-HT) means an increment in production while the elevation of metabolite levels (such as DOPAC or 5-HIAA)

signals for the actual system activation and monoamine usage (see [13]). One potential explanation for the positive response of the DAergic system at the optic tectum is cleaners' visual stimulation, while the DOPAC at the brain stem should be related to an increase of motor activity. The overall decrease of monoamine levels, particularly those concerning the 5-HT system, may mostly occur in response to the inability to contact clients, as the opposite occurs following the exogenous treatment with serotonin activity antagonists (PCPA and WAY) which produced a general decrease in interspecific activities (but not the conspecific ones, see [8]). Interestingly, the exogenous infusion of DAergic antagonists is also known to produce a decrease to the actual foraging (aka cleaning) of cleaners [9], with these behaving as if cleaning was not secured, which would be similar to the inability to interact produced in two of our treatment groups, being that correlated to a decrease of DA and DOPAC. And while there was a clear distinction between the cleaners that could freely contact with stimuli and those that were unable (increment of DA levels at the optic tectum), it was solely by looking at the metabolite/neurotransmitter ratios that the differences between being able to clean or not, were finally disclosed: the DF2 axis distinguishing between mutualistic and non-mutualistic activities and the DF1 discriminating between the actual physical contact, of those that were able to fully inspect a client (positive association with 5-HIAA/5-HT at the cerebellum) and those that could not engage in cleaning (negative association with 5-HIAA/5-HT at the diencephalon). These points for an extra expenditure of 5-HTergic metabolites at the diencephalon whenever cleaners fail to achieve their needs and an increase of availability at the cerebellum during mutualistic activities.

Clients brain monoaminergic levels was also tested in respect to 3 main social challenges, revealing interesting similarities but also some relevant dissimilarities with cleaners' overall response; for instance, all 3 groups clearly discriminated, with the DF1

axis again representing the majority of the variation observed, being negatively associated with 5-HIAA at the diencephalon and DOPAC at the optic tectum but positively with DOPAC at the diencephalon; while the DF2 mostly distinguishing living from non-living stimuli and positively associated with DA levels at the diencephalon. Thus, clients seem to experience a general stimulation of the diencephalic DAergic system at the diencephalon contrasting with the overall decrease function at the optic tectum or brain stem in cleaners. One possible explanation is that clients' increase in DA and DOPAC levels at the diencephalon is associated with positive social engagement (when introduced with cleaners and other conspecifics), contrary to negative values reported for diencephalons of zebrafish and matrinxã (*Brycon amazonicus)* when subject to agonistic contexts [28,29]. The diencephalon is also associated with the modulation of the posterior tuberculum/hypothalamic 5-HT neuronal populations, but however, usually give emphasis to rises of 5-HT activity, normally correlated with overt aggression, for instance, in fish introduced to a real opponent situation [28] or following the loss of a fight [28], while the event of winning a fight seems to contribute to a reduction of 5-HIAA / 5-HT [29,30].

Teleost fish (and other vertebrate groups) cognitive function requires a dynamic coordination of multiple specialized areas of the brain. Here we aimed to understand how these brain areas responded in dependence to the monoaminergic mechanisms in place, as to enable the successful processing of social and mutualistic information. We demonstrate that the variable appraisal of each social challenge contributes to brain dopaminergic and serotonergic changes, in cleaners and clients, with both showing the diencephalon and optic tectum as main areas of metabolite response. However, the role of the serotoninergic system activation was mostly demonstrated at the diencephalon and cerebellum of cleaners, a response that was driven by the exposure to mutualistic

stimuli. Our current results are the first to jointly demonstrate the level of selective similarity in brain monoaminergic mechanisms that underlie fish mutualistic and social engagement, for both sides of these partnerships.

Author contributions

M.C.S. conceived the study. A.C.R.G. performed statistical analysis. All authors participated in writing and provided comments and criticism and gave final approval for publication. The authors declare no competing interests.

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Figures

Figure 1. Experimental Schematics

Figure 2. Cleaner's discriminant analysis plot of DF1 versus DF2 to visualize how the discriminant functions discriminate between contextual treatments, for both monoaminergic metabolites and monoaminergic ratios, synthesized at each brain area. Bigger symbols with dotted drop line in the linear discriminant plot indicate centroid of each group. DF1 and DF2 explained variation is, respectively, for monoaminergic metabolites analysis (A) 63.8% and 20.1% and for monoaminergic ratios (B), 50.8% and 25.1%. (C) and (D), Trait loadings of each variable on DF1 and DF2; the most relevant metabolites to interpret DF1 or DF2 (trait loadings > 0.40) are shown in green. See the methods section for acronyms.

Figure 3. Clients' discriminant analysis plot of DF1 versus DF2 to visualize how the discriminant functions discriminate between contextual treatments, for both monoaminergic metabolites and monoaminergic ratios, synthesized at each brain area. Bigger symbols with dotted drop line in the linear discriminant plot indicate centroid of each group. DF1 and DF2 explained variation is, respectively, for monoaminergic metabolites analysis (A), 87.4% and 12.6% and for monoaminergic ratios (B), 70.2% and 29.8%. In (C) and (D), relevant metabolites (correlational coefficient > 0.40) are shown in green. See the methods section for acronyms.

Tables

Table 1. Discriminant model significance and pairwise combinations of contextual groups' differences that are significantly explained by each of the discriminant functions, for cleaners.

	Monoamine		**Monoamine ratio**	
	DF1	**DF2**	**DF1**	**DF2**
Conspecific vs. Control	0.02*	6.85E-06*	1.00	0.75
Conspecific inside aquarium vs. Control	5.07E-11*	0.98	0.63	0.34
Heterospecific vs. Control	2.92E-07*	<0.01*	<0.01*	0.42
Heterospecific inside aquarium vs. Control	0.02*	0.37	0.11	0.51
Conspecific inside aquarium vs. Conspecific	2.47E-05*	5.55E-07*	0.58	0.99
Heterospecific vs. Conspecific	0.03*	0.65	<0.01*	0.05*
Heterospecific inside aquarium vs. Conspecific	1.48E-06*	<0.01*	0.21	0.07
Heterospecific vs. Conspecific inside aquarium	0.16	2.23E-05*	0.08	<0.01*
Heterospecific inside aquarium vs. Conspecific inside aquarium	2.49E-13*	0.12	<0.01*	0.01
Heterospecific inside	7.95E-12*	0.05	8.49E-07*	1.00

aquarium vs. Heterospecific				

Table 2. Coefficients of each monoaminergic metabolites, synthesized at each brain area, for both clients and cleaners. DF1 and DF2 explained variation is, respectively, 87.4% and 12.6% for clients, and 63.8% and 20.1% for cleaners. * important metabolites (correlational coefficient > 0.40).

	Cleaners		**Clients**	
	DF1	**DF2**	**DF1**	**DF2**
BS_DOPAC	*-0.419	0.137	-0.321	0.220
BS_5-HIAA	0.185	-0.110	-0.147	0.011
BS_DA	0.196	0.364	-0.219	0.116
BS_5-HT	0.233	0.102	0.053	0.180
DL_DOPAC	-0.087	-0.257	*0.427	0.062
DL_5-HIAA	*-0.536	0.116	*-0.401	0.085
DL_DA	*-0.503	0.022	-0.214	*0.503
DL_5-HT	*-0.588	0.167	-0.154	0.396
FB_DOPAC	-0.153	-0.305	-0.363	-0.107
FB_5-HIAA	0.167	0.212	-0.228	-0.329
FB_DA	-0.068	-0.272	-0.267	-0.081
FB_5-HT	-0.276	0.190	0.166	-0.302
OT_DOPAC	0.045	-0.260	*-0.444	0.117
OT_5-HIAA	0.187	-0.012	-0.041	0.007
OT_DA	-0.165	*0.582	-0.214	0.051

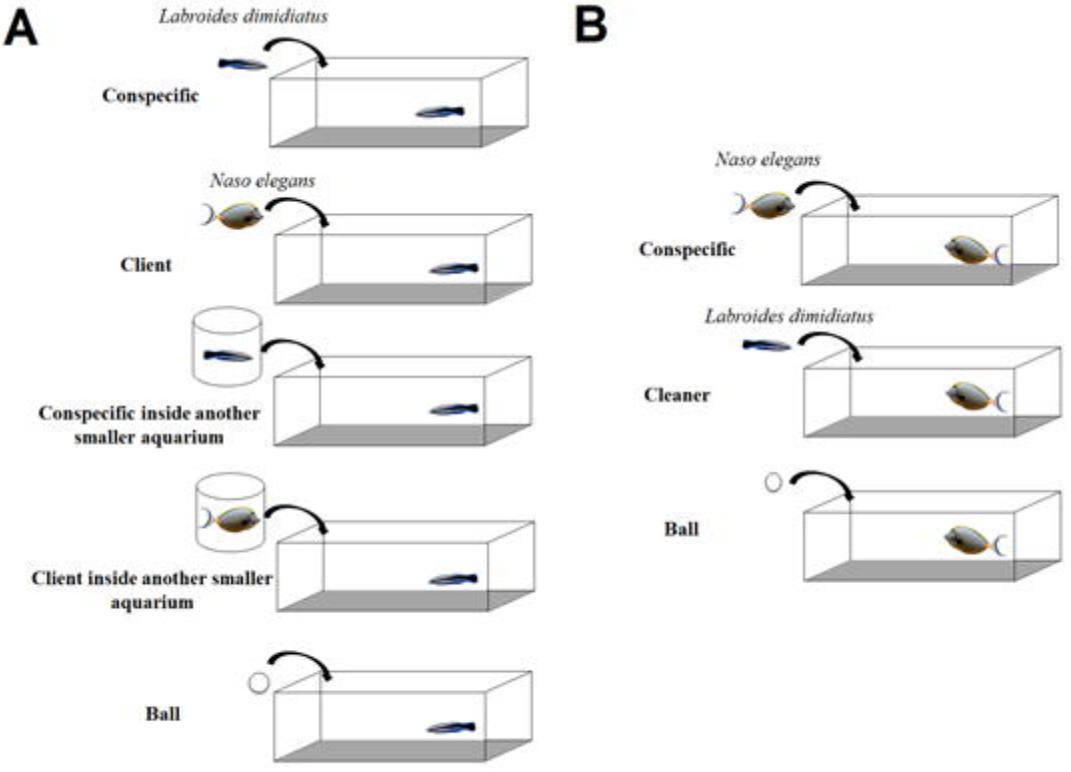
OT_5-HT	0.075	0.233	0.011	-0.211
CB_DOPAC	0.067	-0.316	-	-
CB_5-HIAA	-0.201	-0.235	-	-
CB_DA	0.050	-0.151	-	-
CB_5-HT	-0.292	-0.183	-	-

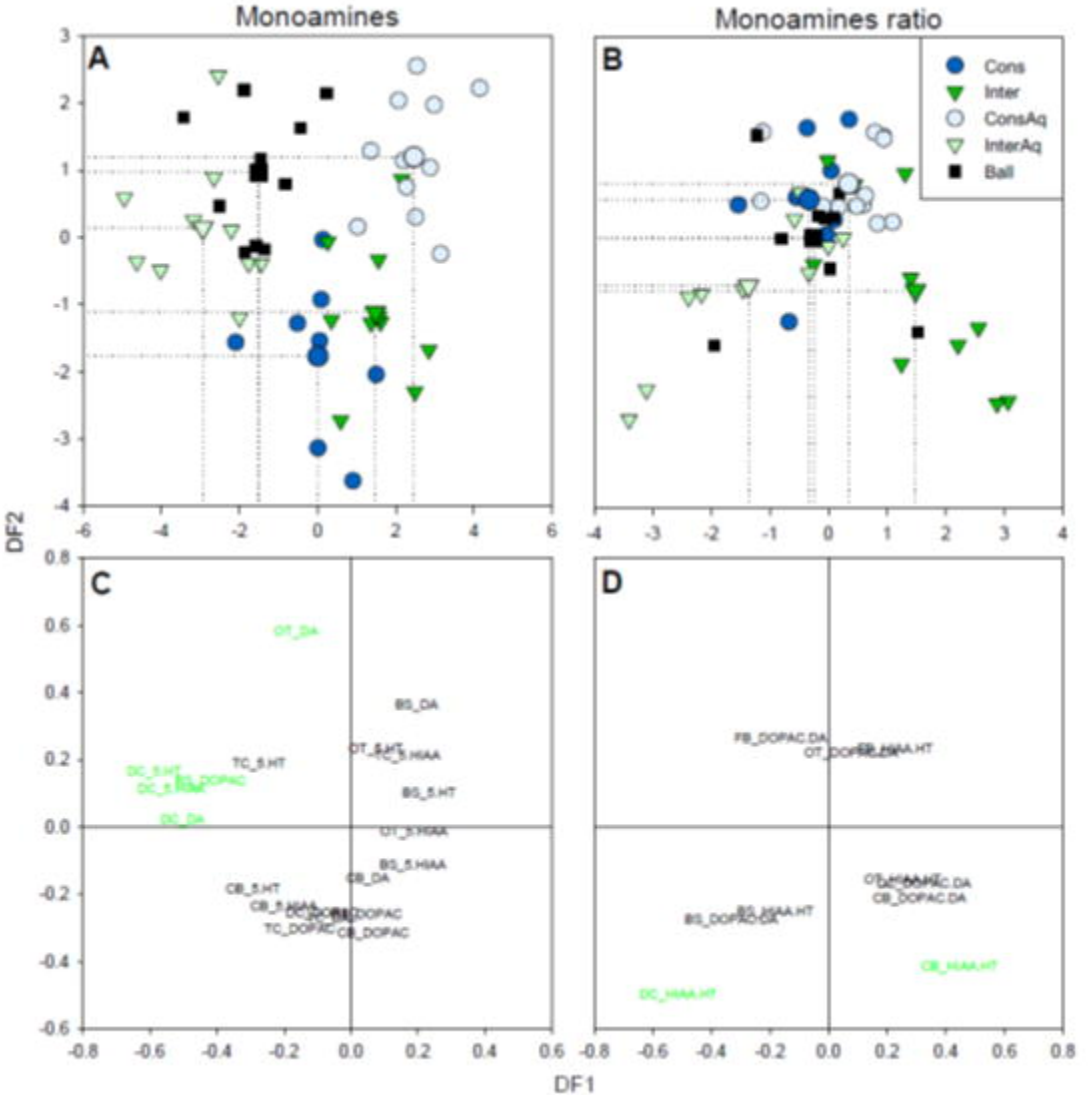
Table 3. Coefficients of each monoaminergic ratios, synthesized at each brain area, for both clients and cleaners. DF1 and DF2 explained variation is, respectively, 70.2% and 29.8% for clients, and 50.8% and 25.1% for cleaners. * important metabolites (correlational coefficient > 0.40).

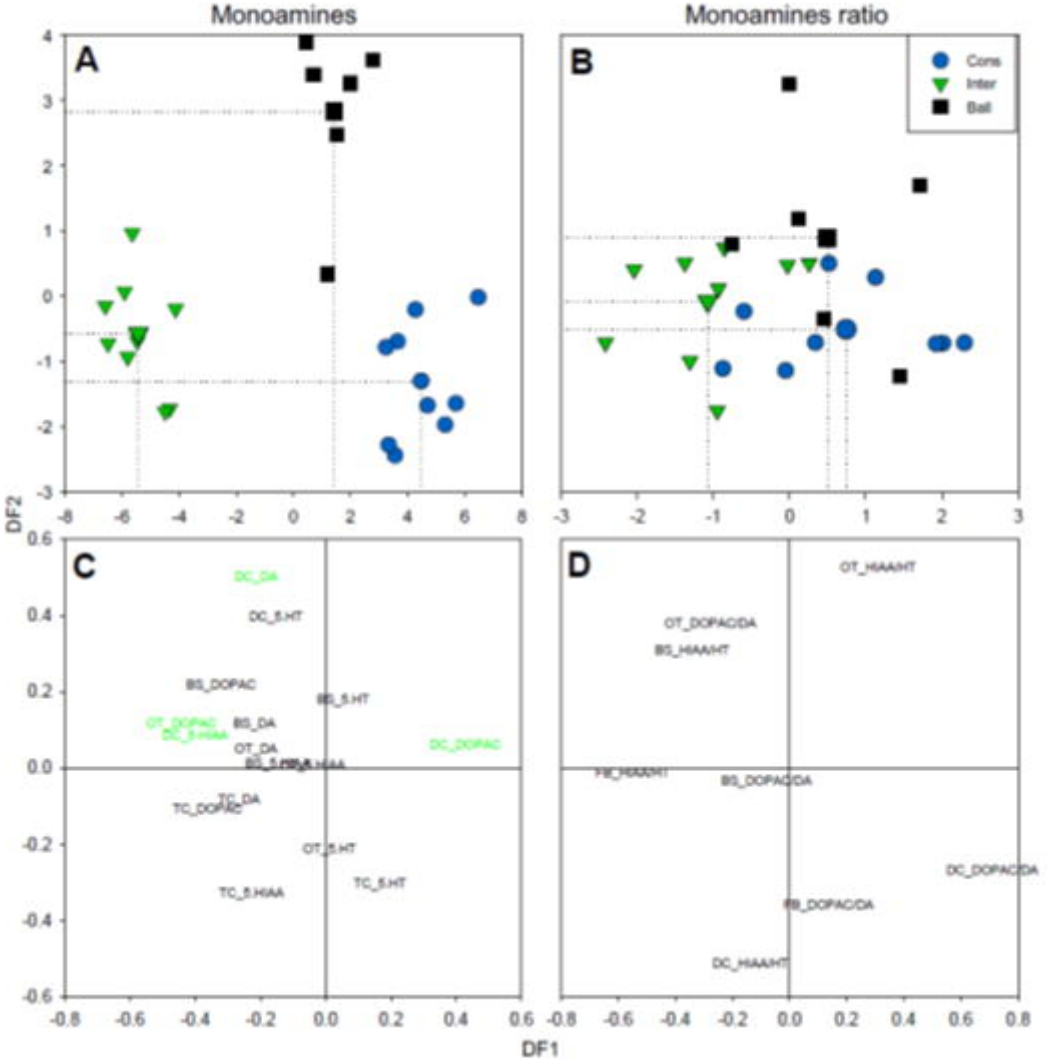
	Cleaners		**Clients**	
	DF1	**DF2**	**DF1**	**DF2**
BS_DOPAC/DA	-0.332	-0.276	-0.081	-0.033
BS_5-HIAA/5-HT	-0.182	-0.252	-0.341	0.310
DL_DOPAC/DA	0.324	-0.166	0.710	-0.269
DL_5-HIAA/5-HT	*-0.514	*-0.495	-0.138	-0.514
FB_DOPAC/DA	-0.165	0.265	0.136	-0.357
FB_5-HIAA/5-HT	0.227	0.233	-0.551	-0.012
OT_DOPAC/DA	0.077	0.220	-0.278	0.380
OT_5-HIAA/5-HT	0.252	-0.156	0.309	0.526
CB_DOPAC/DA	0.309	-0.210	-	-
CB_5-HIAA/5-HT	*0.446	*-0.413	-	-

Table 4. Discriminant model significance and pairwise combinations of contextual groups' differences that are significantly explained by each of the discriminant functions, for clients.

	Monoamine		**Monoamine ratio**
	DF1	**DF2**	**DF1 and DF2**
Heterospecific vs. Control	4.67E-11*	6.26E-06*	-
Conspecific vs. Control	2.96E-05*	3.38E-07*	-
Conspecific vs. Heterospecific	4.72E-14*	0.29	-


A
Labroides dimidiatus
Conspecific
Naso elegans
Client
Conspecific inside another smaller aquarium
Client inside another smaller aquarium
Ball
B
Naso elegans
Conspecific
Labroides dimidiatus
Cleaner
Ball


Monoamines
Monoamines ratio
A
B
C
D
Cons
Inter
ConsAq
InterAq
Ball
DF2
DF1
OT_DA
BS_DA
TC_5.HT
BS_5.HT
DC_DA
CB_5.HT
CB_DA
TC_DOPAC
CB_DOPAC
FB_DOPAC.DA
CB_DOPAC.DA
CB_HIAA.HT
DC_HIAA.HT


Monoamines
Monoamines ratio
A
B
C
D
Cons
Inter
Bail
DF1
DF2
DC_DA
DC_5.HT
BS_DOPAC
BS_5.HT
OT_DOPAC
DC_5.HIAA
BS_DA
OT_DA
DC_DOPAC
TC_DA
TC_DOPAC
OT_5.HT
TC_5.HIAA
TC_5.HT
OT_HIAA/HT
OT_DOPAC/DA
BS_HIAA/HT
BS_DOPAC/DA
DC_DOPAC/DA
BS_DOPAC/DA
DC_HIAA/HT