




Familiarity increases aggressiveness among clonal fish

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1 *Familiarity increases aggressiveness among clonal fish.*

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6 **Abstract:**

7 Understanding how animal groups form and function is a major goal in behavioural ecology.
8 Both genetic relatedness and familiarity among group mates have been shown to be key
9 mediators of group composition. However, disentangling the two in most species is
10 challenging as the most familiar individuals are often the most related, and vice versa. But
11 whereas the relatedness between individuals is constant, familiarity is not, and so familiarity
12 may be more likely to mediate interactions in dynamic environments. Here, we manipulated
13 the level of familiarity among groups of the naturally clonal, and genetically identical fish,
14 the Amazon molly (*Poecilia formosa*) and monitored group behaviour in an open-field and
15 when given the opportunity to forage. Contrary to our predictions, fish that were the most
16 familiar with each other showed the highest levels of aggression. Additionally, fish that were
17 less familiar with each other exhibited the highest group cohesion and took the longest to
18 begin feeding, compared to the more familiar fish. These results suggest that familiarity may
19 socially buffer individuals from the perception of risk in novel environments, such as is
20 common in most behavioural tests designed to test group behaviour. Increases in aggression
21 that are associated with increasing familiarity as shown here might be a mechanism by

22 which fish maintain a fission-fusion society with important consequences for the patterns of
23 associations in group living animals.

24 **Key words:** Amazon molly, clonal, Genetic relatedness, group behaviour, Familiarity.

25

26 **Introduction**

27 Animals living in groups must balance the potential benefits of group living, such as greater
28 foraging efficiency, against the costs, such as increased aggression and competition
29 (Hamilton, 1971; Krause & Ruxton, 2002). For example, fish in shoals benefit from being able
30 to better sense their local environment and have a higher probability of encountering food,
31 proportional to the size of the group (Berdahl, Torney, Ioannou, Faria, & Couzin, 2013;
32 Magurran & Pitcher, 1987; Peuhkuri, 1997). However group living also incurs costs, such as
33 competition over resources. Thus there is a trade-off between the costs and benefits of
34 group living, particularly when it comes to food exploitation (Conradt & Roper, 2005; Ranta
35 & Lindström, 1990; Reader, 2014). Individuals often mediate this trade-off by preferentially
36 associating with related and/or familiar individuals (Lee-Jenkins & Godin, 2013; Thünken,
37 Hesse, Bakker, & Baldauf, 2016; Ward & Hart, 2003). However, these patterns of social
38 interactions can be complicated by the fact that animal groups often experience high group
39 turnover meaning individuals have to constantly adjust their behaviour to that of new
40 members (Couzin, 2006).

41 Groups are defined by individual animals aggregating and the patterns of interactions
42 among these individuals can vary in response to several factors. Fish, for instance, are known
43 to alter their nearest neighbour distance in response to the presence of a predator or food

44 (Hoare, Krause, Peuhkuri, & Godin, 2000; Sogard, 1997). Furthermore, fish often switch
45 between shoals for any number of reasons, such as phenotypic assortment, differences in
46 swimming speed, or because individuals within the group have conflicting information about
47 the current state of the environment (Croft et al., 2005; Killen, Marras, Nadler, & Domenici,
48 2017; Krause, Reeves, & Hoare, 1998; Merkle, Sigaud, & Fortin, 2015). In these fission-fusion
49 societies, the patterns of social encounters among individuals are very dynamic, with
50 important implications for the transmission of diseases and flow of information (Aureli et al.,
51 2008; Couzin, 2006; Krause & Ruxton, 2002). These repeated associations will in turn affect
52 the degree of familiarity both among and within groups (Griffiths, 2003; Griffiths &
53 Magurran, 1999; Lee-Jenkins & Godin, 2013; Utne-Palm & Hart, 2000; Ward & Hart, 2003).
54 Understanding how and why individuals associate with each other can give insight into how
55 groups may evolve and function.

56 According to kin selection, genetic relatedness amongst individuals should facilitate
57 cooperation and reduce competition thus contributing to the evolution of group living
58 (Hamilton, 1964). Genetic relatedness has been shown to increase cooperation and
59 individual fitness (Gerlach, Hodgins-Davis, MacDonald, & Hannah, 2007; Hesse, Anaya-Rojas,
60 Frommen, & Thünken, 2015; Thünken et al., 2016). Familiarity seems to further contribute
61 to the effects of kinship by further enhancing cooperation among familiar group mates
62 (Griffiths & Magurran, 1999; Lee-Jenkins & Godin, 2013). Several studies show how fish
63 shoaling with familiar conspecifics tend to profit more from social learning, experience less
64 aggression, find food faster and eat more than groups composed of unfamiliar individuals
65 (Berdahl et al., 2013; Swaney, Kendal, Capon, Brown, & Laland, 2001; Utne-Palm & Hart,
66 2000). Associating with familiar individuals also appears to increase the anti-predator and

67 foraging benefits of schooling behaviour (Chivers, Brown, & Smith, 1995; Metcalfe &
68 Thomson, 1995; Pitcher, Magurran, & Edwards, 1985; Utne-Palm & Hart, 2000). However,
69 the ecological context, a group is experiencing at any given moment, will influence whether
70 both familiarity and/or kinship are beneficial or not (Frommen et al., 2012; Kelley, Graves, &
71 Magurran, 1999; West, Pen, & Griffin, 2002). For example, when it comes to mating, male
72 guppies prefer familiar female partners (Kelley et al., 1999), however in a foraging scenario
73 sticking with familiar individuals might render the groups foraging strategy easily
74 recognisable and thus exploitable by outsiders (Ward & Hart, 2005).

75 A major step towards understanding the formation and function of groups relies on
76 the ability to successfully disentangle familiarity and kinship which can be challenging in
77 many species (Lee-Jenkins & Godin, 2013). Importantly, whereas kin relationships are
78 constant, familiarity among group members is not. This suggests that among species with
79 dynamic social interactions, familiarity may play an especially important role in determining
80 social associations and therefore group behaviour. Here we take advantage of a unique
81 species to test the influence of familiarity on group behaviour while holding relatedness
82 constant. We used the naturally clonal fish, the Amazon molly (*Poecilia formosa*), a small
83 live-bearing freshwater fish that produces broods of genetically identical offspring allowing
84 us to isolate and test the role of familiarity on group dynamics. We compared aspects of
85 group behaviour, such as aggressive interactions, group cohesion and resource defence
86 among groups of mollies that differed in their level of familiarity. There is reason to believe
87 that familiarity plays a major role in the group dynamics of the Amazon molly. These social
88 fish form large groups in the wild where they forage and compete for resources together
89 (Ingo Schlupp, Parzefall, & Schartl, 2002). Previous work has already demonstrated that

relatedness among individuals modulates their level of aggression with less related (Makowicz, Tiedemann, Steele, & Schlupp, 2016; I Schlupp, 2009). *P. formosa* are known to be aggressive and form stable dominance hierarchies (Makowicz & Schlupp, 2015) so we predicted that groups that were more familiar with each other would stabilise sooner and show a decrease in aggression and resource defence (i.e. more egalitarian distribution of feeding). Furthermore, we also predicted that this pattern would be accompanied by an increase in group cohesion.

Methods:

The Amazon molly:

This live-bearing, freshwater fish originated through a hybridization event between the Sailfin molly (*Poecilia latipinna*) and the Atlantic molly (*Poecilia mexicana*) an estimated 100,000 years ago (Warren et al., 2018) and now reproduces through gynogenesis (Parzefall, 1989). This means that females require sperm from a male of one of the parental species (*P. latipinna*, *P. mexicana*) in order to induce embryogenesis, but do not incorporate the paternal genetic content (Schartl, Wilde, Schlupp, & Parzefall, 1995).

Fish husbandry:

Prior to the experiment, fish were maintained as part of our laboratory populations in 100L stock tanks and fed ad libitum two times daily on standard flake food with a constant temperature of 25°C. Tanks were cleaned and 50% of the water volume was exchanged weekly. The population of *P. formosa* used for this study were obtained from Manfred Schartl, University of Würzburg. Since Amazon mollies reproduce gynogenetically and females require the sperm from one of their parental species in order to induce

embryogenesis each stock tank also contained several male *P. mexicana*. We used a strain of *P. formosa* that has been kept in captivity since 2002 and regular molecular checks confirm that individuals are clones (M. Scharl 2015, *personal communication*)

Familiarity manipulation & group behaviour assays:

We generated three treatment groups with mollies that had lived together for differing amounts of time. After this familiarity manipulation we measured their behavior in two different contexts: both before and after the addition of a defensible food resource.

Experiments were conducted at the Humboldt University in Berlin in November 2016. We collected 96 individuals from our laboratory population, assembling groups of 4 individuals each in 24 smaller housing tanks (40×20×20 cm; 8 groups/housing tanks per each treatment). We used only adult individuals and size-matched individuals within groups (range: 4.40 - 8.11 cm; mean = 6.14 ± SD = 0.76). Individuals were placed into these housing tanks to standardize social experience prior to having their behaviour observed (see below).

From these housing tanks, we assembled our treatment groups: Low, Mid and High familiarity treatment groups (Figure 1). All fish regardless of treatment spent three weeks in the housing tanks; what differed was the identity of their group mates in the housing tanks and how long they have lived with these group mates prior to behavioural assessment 1, 7 or 21 days respectively (Figure 1). To assemble a treatment group, one individual from four of the assigned housing tanks was haphazardly removed, and these individuals were placed in a new housing tank. For the low familiarity fish, this was done the day before behavioural observations (i.e. 1 day of familiarity prior to testing); for the mid familiarity fish this was done 7 days before observations and the high familiarity fish were kept together in the same

housing tank for the entire 21 days. This experimental design would create differences in the timeline of when the fish are physically handled; i.e. low familiarity fish are handled the day before observations, whereas mid familiarity fish had 7 days to recover from the handling prior to observations. To account for this, all fish were handled at the same time points as the other treatments were being assembled (e.g. low familiarity fish were handled at week 2 to mimic the assembling process being done in the mid familiarity fish, see Figure 1 for schematic details). This involved all fish in the housing tank being netted and held out of the tank for several seconds and then returned to their tank. This process ensured that all groups received the same amount and schedule of handling regardless of treatment; the only difference among treatments should thus be the identity of their group mates.

We started with 24 groups of 4 individuals (8 groups per treatment) but due to a video malfunction we lost the data corresponding to one group (Mid familiarity treatment). Finally due to natural mortality there were 3 groups with 3 individuals (1 in the high familiarity and 2 in the low familiarity treatment). Our final sample size was 20 groups of 4 individuals and 3 groups of 3 individuals.

After this familiarization phase, groups were transferred into a large open-field observation arena (60×30×30 cm) with 5cm deep water and allowed to acclimate overnight. Open-field arenas provide a standardized way to measure behaviour in a novel environment devoid of any obstruction (Walsh & Cummins, 1976). We observed the behaviour of the fish using an overhead webcam (Logitech C920 HD). The following morning, we observed the baseline behaviour of the fish in the observation arena for 5 minutes. After this time, we added a defensible food resource (sinking food tablet) and then observed the group for an additional 5 minutes after at least one individual had started feeding at the tablet. We

counted the total number of aggressive interactions (bites, chases and tail beats) performed among group members (Bierbach et al., 2012; Foran & Ryan, 1994; Heubel & Plath, 2008), and the median inter-individual distance among group members (extracted through video tracking using Ethovision XT12, Noldus Information Technology, Inc) both before and after the addition of the food. After the food was added, we measured a number of foraging behaviours: latency for the first individual to begin feeding, how long each individual spent eating and the distribution of how many individuals were feeding simultaneously (i.e. one, two, three or four individuals at the food tablet at the same time). Additionally, we identified the dominant individual in each group as the individual who performed the majority of aggressive encounters while receiving the fewest (Laskowski, Wolf, & Bierbach, 2016). After having identified the dominant individual, we also scored the amount of time it spent eating. Finally, the collective duration of feeding was obtained by summing the duration of feeding by every individual in the group. At the end of every trial a snapshot from the video was taken to measure the standard length (in cm) of each individual using ImageJ.

Statistics:

We tested how familiarity and context (before and after food) influenced group behaviour using general linear mixed models. We ran separate models with the total number of aggressive behaviours and inter-individual distance as our response variables. Both models included the fixed effects of familiarity (low, mid, high) and context (before/after the food was added) and an interaction between these two factors. We also included median body size and body size variation within the group as a covariate and group as a random effect as we had two measures from each group (across the two contexts).

To investigate how familiarity influenced feeding behaviour, we used three general linear models with latency for the first individual to begin feeding and the total duration of all individuals feeding (i.e. collective feeding), and the duration the dominant individual spent eating as our response variables. These models included familiarity as a fixed effect and both the median and variance of individual size within groups as a covariate; group was not included as a random effect as these response variables only had one observation per group.

We assumed a Gaussian error distribution for all models and we visually confirmed that our residuals met model assumptions (see figures in SM). To test the overall significance of the fixed effects in our mixed models we compared the log-likelihood ratio of a model that contained the effect of interest, to one that did not. In order to obtain the amount of variation explained by each model we additionally estimated both the marginal and conditional R-squared value according to (Nakagawa & Schielzeth, 2013). The marginal R-squared estimates how much variation was explained by the fixed factors whereas the conditional R-squared estimates the variation explained by both fixed and random factors together.

Finally, in order to test for differences in the number of animals feeding simultaneously we ran a chi-square test with the number of seconds there were one, two, three or four, individuals eating (for this test only groups of 4 individuals were counted). All statistics were run in R v3.4.4, using packages nlme and lme4 for linear and mixed models and ggplots and ggplot2 for plotting (Bates, Maechler, Bolker, & Walker, 2015; Pinheiro, Bates, DebRoy, & Sarkar, 2018). The R script of the entire statistical analysis and the raw data can be found in the supplementary material.

202 Ethical Note:

203 This research was conducted in accordance with the ASAB/ABS guidelines for the use of
204 Animals in Research. During the experiment there was always an observer present to
205 determine whether the aggressive interactions resulted in physical damage to the fish.
206 Should that occur experiments would have immediately stopped. Every individual was
207 closely monitored both before and after each trial to ensure there was no indication of
208 physical harm and fish resumed normal feeding and behaviour upon return to their stock
209 tanks. The number of experimental animals used was the minimum necessary for the
210 statistical testing and was based in previously published studies (Griffiths & Magurran, 1997;
211 Kelley et al., 1999; Swaney et al., 2001). Finally, experimental animals were afterwards
212 integrated into our stocks and served for breeding. They have not been used for any further
213 experiments.

214 The reported experiments comply with current German law approved by LaGeSo
215 Berlin (GO124/14 to D.B.).

216 **Results:**

217 Aggression:

218 Contrary to our predictions, we found that aggressive interactions significantly increased
219 with familiarity in a clonal fish (table 1, significant effect of familiarity). We also found that
220 aggression was higher before the food was added compared to after (table 1, significant
221 effect of context). Interestingly, in the mid-familiarity treatment, the decrease in aggression
222 after the food was added appears to be the strongest compared to the other two familiarity
223 treatments (Figure 2), though this effect was not strictly significant (table 1, non-significant

224 interaction.). Finally neither individual size nor size variation within groups significantly
225 affected the level of aggression (Table 1).

226 Feeding behaviour:

227 Again, contrary to our predictions, the collective duration of feeding was not affected by
228 familiarity (Treatment $F_{18,20} = 1.8912$, $P = 0.1797$). However, the time taken for the first
229 individual to start feeding after the addition of food significantly decreased with familiarity
230 (Treatment $F_{18,20} = 5.737$, $P = 0.0118$). That is, in the high familiarity groups, fish began to
231 feed faster. The amount of time the dominant fish of each group spent eating was not
232 affected by treatment (Treatment $F_{18,20} = 0.1988$, $P = 0.8215$). Rather, the distribution of
233 feeding, (i.e. how many individuals were eating at the same time within each group) was
234 significantly different for each of the treatments ($\chi^2 = 38087$, $df = 8$ and $P < 0.001$), with
235 more individuals feeding simultaneously when familiarity was low (Fig. 3). Finally, median
236 body size and body size variation within groups did not significantly influence any measure
237 of feeding behaviour (Collective feeding: Fish Size $F_{18,19} = 1.4077$, $P = 0.2509$; Fish var $F_{18,19} =$
238 2.5045 , $P = 0.1309$; Latency to begin feeding: Fish Size $F_{18,19} = 1.3289$, $P = 0.2641$, Fish var
239 $F_{18,19} = 0.2578$, $P = 0.6178$; Dominant fish feeding duration: Fish Size $F_{18,19} = 0.2531$, $P =$
240 0.621 , Fish Var $F_{18,19} = 1.4243$, $P = 0.2482$)

241 Group behaviour:

242 Finally, also contrary to our initial prediction, increasing familiarity within groups led to
243 decreases in group cohesion as measured by inter-individual distance (table 2, significant
244 effect of treatment). Cohesion increased after food was added (table 2, significant effect of
245 context) and this was independent of the familiarity treatment (non-significant interaction

between treatment and context, Figure 4, Table 2). Median body size had no effect but variation in body size was marginally significant (Table 2, effect of Fish var).

Discussion

A critical step towards understanding the formation of social associations in animal groups relies on the ability to disentangle the roles of familiarity and kinship. Kin selection tells us how genetic relatedness is at the core of the evolution of group living with several studies suggesting how familiarity adds to this effect (Griffiths & Magurran, 1999; Hamilton, 1964; Lee-Jenkins & Godin, 2013). But how does group behaviour differ based on familiarity alone? By exploiting a unique animal system, the clonal Amazon molly (*Poecilia formosa*), we were able to isolate the effects of familiarity on the behaviour of fish groups. We show that, contrary to our predictions, familiarity significantly increased the number of aggressive encounters accompanied by a decrease in group cohesion.

Our study demonstrates how aggression mediated through familiarity may be a potential mechanism driving the maintenance of fission-fusion societies. Social systems characterized by fission-fusion dynamics are wide-spread in the animal kingdom not only in schooling fish, like the Amazon molly, but also in bats, dolphins, elephants, hyenas and several species of primates (Aureli et al., 2008; Connor, 2007; Kerth & König, 1999; Smith, Kolowski, Graham, Dawes, & Holekamp, 2008; Wittemyer, Douglas-Hamilton, & Getz, 2005). Understanding the factors that mediate group formation is therefore at the forefront of studies of animal collective behaviour. For example, in baboons both habitat structure and social interactions significant influence group movement pattern (Strandburg-Peshkin, Farine, Crofoot, & Couzin, 2017). Furthermore, in bison, when individuals possess conflicting

268 information regarding the location of a desired resource, groups often split (Merkle et al.,
269 2015). Finally separating from a group is often necessary due to the existence of high levels
270 of aggression, but this also needs to be carefully managed when joining a new group as
271 conflicts can also arise then (Aureli & Schaffner, 2007). Our study highlights how internal
272 factors such as intra-group conflict may be just as important in mediating group formation
273 and dynamics as external factors such as resource availability and habitat structure.

274 Interestingly, the finding here that more familiar fish are more aggressive contradicts
275 several studies showing that, increasing familiarity within groups often decreases aggression
276 and increases group cohesion and food sharing (Chivers et al., 1995; Höjesjö, Johnsson,
277 Petersson, & Järvi, 1998; Johnsson, 1997). There are a number of potential explanations for
278 this unexpected finding. Due to their unique type of reproduction, the unisexual Amazon
279 molly lives in mixed-species shoals together with their parental species *P. latipinna* and *P.*
280 *mexicana*, and also with different clonal lineages (I. Schlupp & Ryan, 1996). Because these
281 fish require sperm from males of one of the parental species (Ingo Schlupp, Parzefall, &
282 Scharl, 1991), Amazon mollies might display higher aggression as a strategy to increase
283 attractiveness and gain more access to males. Indeed, previous research has already shown
284 that *P. formosa* is more aggressive when compared to the parental species, and this
285 aggression increases over time. Makowicz *et al* (2015) suggest that individuals might have
286 low tolerance for consistent interactions with the same individuals, potentially because in
287 natural conditions, groups are very plastic with high group turnover rates. This increase in
288 aggression with familiarity may therefore provide one reason why so many fish species exist
289 as dynamic fission-fusion societies.

290 Even though familiarity had strong effects on aggression, there was no overall effect
291 of familiarity on the duration of time the group spent feeding as a whole. However, the
292 number of individuals feeding at any given time differed. The dominant individual was
293 always the one who spent the most time feeding, and this was not affected by familiarity.
294 But in less familiar groups, more individuals were able to feed simultaneously, however as
295 familiarity increased, the skew in individual feeding times increased until the dominant
296 individual essentially monopolized the resource. Interestingly, this is contrary to previous
297 work which found greater food sharing in more familiar groups (Utne-Palm & Hart, 2000). It
298 is likely that the pattern of feeding in the Amazon mollies is driven by the increase in
299 aggression in more familiar groups: in low familiarity groups, aggression was lower and
300 group members had more opportunity to reach the food. Thus, individuals may be more
301 motivated to frequently change groups (and decrease familiarity) if this increases their
302 ability to access food resources.

303 Another potential explanation for why less familiar fish show unexpectedly low
304 aggression is because of social buffering. Social buffering is the process by which social
305 groups offer a safer environment in the presence of a perceived threat (Faustino, Tacão-
306 Monteiro, & Oliveira, 2017; Hennessy, Hornschuh, Kaiser, & Sachser, 2006). When familiarity
307 is low, being placed in a novel environment, such as the testing arena used here, might be
308 perceived as risky. Thus, individuals remain cohesive as a group with low levels of
309 aggression. Indeed, after the addition of the food, which required a necessary disturbance,
310 group cohesion in the low familiarity groups increased and individuals took the longest to
311 begin feeding, further suggesting they interpret this disturbance as a potential threat. For
312 example, in rats, the presence of a familiar conspecific is more effective in reducing an

individual's response to a frightening stimulus than an unfamiliar conspecific (Kiyokawa, Honda, Takeuchi, & Mori, 2014). Here too, when individuals are more familiar with one another, as is the case for the mid familiarity treatment, the scenario changes slightly. Then, individuals exhibited lower group cohesion and higher aggression prior to the food being added, but once the group is disturbed by the addition of the food, aggression decreases and cohesion increases similar to what was seen in the low familiarity groups. Finally, three weeks of familiarization in the highly familiarity groups appears to be sufficient to strongly reduce any perceived risk by the introduction to the novel testing arena or the addition of the food as aggression remained high and group cohesion low in these groups. Taken together, our results suggest that familiarity mediates how the group responds to novelty, through social buffering. Groups that are more familiar can cope better both with being in a new environment and with the unexpected food delivery, and thus are able to maintain their high levels of aggression. The fact that aggression increases with familiarity indicates that individuals are no longer seeking safety in numbers but instead asserting their dominance within their groups.

Our study provides new insight into potential mechanisms driving both the formation and maintenance of fission-fusion dynamics. There is a long history investigating how relatedness and familiarity may play a role in these processes. Here, we show that even among genetically identical individuals, familiarity is sufficient to drive patterns of intra-group conflict which could have consequences on group stability and dynamics.

Competing interests:

The authors declare no competing interests

335 **Data statement:**

336 Both the raw data and analysis can be found in the supplementary material

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501

502 Table 1 – Results of the model testing the effects of treatment and context on total number
 503 of aggressive encounters within a group.

Effect*	Estimate	t-value	LLR	P
R²				
Marginal	0.43			
Conditional	0.62			
Fixed effects				
Treatment:Context			4.5936	0.1006
Treatment			16.57263	<0.001
Mid	10.03399	0.796096		
High	48.95636	4.187288		
Context			10.56642	0.0012
After food	-24.82609	-3.581767		
Fish Size	-12.35011	-1.317861	2.118555	0.1455
Fish Var	0.02962	1.109517	1.521526	0.2174
Random effects				
Group variance	16.33983		2.40853	0.1317
Residual variance	23.8325			

504

*base level is TreatLow before food

505

506 Table 2 – Results of the model testing the effects of treatment on median inter-individual
 507 distance within each group.

Effect	Estimate	t-value	LLR	<i>P</i>
<hr/>				
R^2				
Marginal	0.302			
Conditional	0.593			
Fixed effects				
Treatment:context			1.824668	0.4016
Treatment			9.189975	0.0101
Mid	3.625036	1.480117		
High	6.951175	3.024320		
Context			13.90384	>0.001
After food	-5.68	1.3721		
Fish Size	0.623987	0.3898	1.05012	0.3055
Fish Var	0.013100	2.437828	6.380756	0.0115
Random effect				
Group variance	3.211818		2.312011	0.1284
Residual variance	4.624873			
<hr/>				

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509

510 Figure 1 – Detailed schematic of the experimental procedure. Each treatment had a total of
511 eight housing tanks (four are shown here). Low familiarity groups were handled on week 2
512 and assembled the day before testing; Mid familiarity groups were assembled on week 2 and
513 handled once more the day before testing. High familiarity groups were handled twice
514 before being tested.

Figure 2 - Boxplot of the total number of aggressive encounters among all members of a group within each treatment before (dark grey) and after (white) food addition. Dark lines represent medians, the box the inter-quartile range (IQR) and finally the whiskers are 1.5 times the IQR.

Fig 3 – Amount of time one, two, three or four fish were observed feeding together in each treatment. Dark lines represent medians, the box the inter-quartile range (IQR) and finally the whiskers are 1.5 times the IQR.

515 Fig 4 – Median distance within individuals from a group Before Food (dark grey) and After
516 Eating (white) for each treatment. Dark lines represent medians, the box the inter-quartile
517 range (IQR) and finally the whiskers are 1.5 times the IQR.

