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Reproductive behaviour of two tilapia species (*Oreochromis niloticus* , Linné, 1758; *Sarotherodon melanotheron*, Rüppel, 1852) in freshwater intra and interspecific pairing context

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Abstract :

Nile tilapia Oreochromis niloticus (NT) and Black-chinned tilapia *Sarotherodon melanotheron* (BCT) are respectively characterized by fast growth and water salinity tolerance which attract the farmers who could take advantage of both species characteristics by producing hybrids reared in brackish or marine waters. Little information is however available about social interactions between the two species which are distinct by their mode of parental care (oral incubation). The objective of this study was to determine the behaviour of the two species, NT and BCT placed in freshwater intraspecific and interspecific pairing contexts. Females of both species (n = 20 per species) were first isolated for 90 days in a 60 L aquarium to determine the average duration between spawning in the absence of males. With the approach of 4th spawning or after 90 days of isolation, intra and interspecific pairing (fish average weight 200.4 ± 5.1 g) were made 10 times by pairing mode in a 350 L aquarium. The day after fish pairing, 15 min videos were recorded at one hour intervals from 8:00 h to 17:00 h, and courtship and aggressiveness behaviour were quantified. The results showed that isolated NT females had an average duration between two spawning of 15.9 days. No spawning was observed in BCT females. Spawning events were observed in the NT intraspecific pairs, with strictly maternal oral egg incubations. Behavioural differences and some similarities were noted between the two species. NT male was the most dominant in front of females of both species and NT females showed dominance on BCT males. No clear hierarchy was observed in BCT pairs. Courtship behaviours were observed in all pairings with longer durations in NT pairs. Nest building was observed from 8:00 h to 17:00 h in both sexes of BCT and male NT. NT females built nests from 11:00 h with longer durations when paired with males BCT than when paired with their conspecific males. These first results, reflecting behavioural plasticity in both species suggested that other breeding conditions favouring less aggressive behaviour could lead to a natural interspecific hybridization between NT and BCT.

Highlights

► Behaviour of *O. niloticus* and *S. melanotheron* in interspecific pairings has been determined. ► *O. niloticus* (male and female) showed a strong dominance in terms of aggressiveness on *S. melanotheron*. ► Apart from aggressiveness, the two species showed some behavioural similarities.

Keywords : Aggression, hybridization, reproduction, Oreochromis niloticus, Sarotherodon melanotheron, freshwater

1. Introduction

In aquaculture, hybridization of tilapia has been a common practice to obtain male fish and is now used to improve the aquacultural potential of cultivated species (Bartley et al., 2001). Hybridization is possible between species that have very similar reproductive behaviour. This is the case between Nile

tilapia *Oreochromis niloticus* (NT), *Oreochromis aureus* and *Oreochromis mossambicus* (Balcázar et al., 2006; Bakhoum et al., 2009; El-Zaeem et al, 2013; De Verdal et al., 2014). NT and Black-chinned tilapia *Sarotherodon melanotheron* (BCT) belong to two genders of tilapia (Trewavas, 1983) which differ in their reproductive behaviour and in their ecological requirements. Indeed, NT lives only in fresh water, and has a strictly maternal mouthbrooding behaviour while BCT is found in fresh, brackish and saline water, and has a paternal or biparental incubation. Moreover NT is a polygamous species while BCT is monogamous. Although being tilapia, it is not usual to obtain hybrid between these two species (Toguyeni et al., 2009) and this is probably due to behavioural dissimilarity between these two species. In fact specific signals such as visual displays, sex pheromones or sound signals could constitute a premating barrier between these two species (Ptacek, 2000).

Current changes in aquatic environments under anthropogenic management (channel creations, dams…) but also aquaculture activities bring NT and BCT to meet in some rivers in southern Côte d'Ivoire (Gô river of Grand-Lahou and artificial dam of Ayamé) (Koné et al., 2003; Adepo-Gourene and Gourene, 2008). To date, no natural hybrid between NT and BCT were observed or captured by local fishermen or scientists.

Hybridizations between these 2 genders of tilapia were however recently obtained in captivity in brackish environment by Amon et al. (2013a, 2013b). The sex ratio male : female used by these authors 1 : 2.5, corresponded to that used for captive breeding in NT (Adel, 2012), against 1 : 1 used in BCT (Legendre and Trébaol, 1996). Following this work, it appears possible that the two species are not fully reproductively isolated (Gröning and Hochkirch, 2008). So the reproductive behaviour of these species can in some cases be or become similar to obtain natural viable hybrids, fertile and interbreeding (Amon et al., 2013a). Hereby, it can be foreseen a potential agronomic interest for aquaculture (Lazard, 2007), a potential ecological interest (D'Amato et al., 2007) or finally a potential ecological risk (Seiler and Keeley, 2007). To assess hybridization potential between those two species, it is first necessary to learn more about their reproductive behaviour in freshwater confinement context. The objective of this study was to determine the aggressive and reproductive behaviour of males and females of both species (sex ratio male:female 1:1) in intra- and interspecific pairing context.

2. Materials and methods

2.1 Experimental fish

XY males and XX females mature of NT (NT♂ and NT♀ weighing 201.6 ± 4.4 g, 199 ± 6.7 g respectively) and BCT (BCT ♂ and BCT ♀ weighing 204.3 ± 2.6 g, 202.5 ± 3.1 g respectively) were used for the behavioural tests in the experimental farm of the National Polytechnic Institute Félix Houphouët Boigny (INP-HB, Yamoussoukro, 6°49'13"N-5°16'36"W, Côte d'Ivoire). The NT brood stock came from a private fish farm located in Yamoussoukro and was the fourth generation obtained in freshwater ponds by the crossing of parents from the Bouaké synthetic strain (Rognon and Guyomard, 2003). BCT spawners came from brackish water ponds (salinity 2.43) of the Aquaculture Station of the Oceanographic Research Center (Dabou, 5°19'32"N-4°22'36"W, Côte d'Ivoire) and were a fourth generation obtained by the crossing of wild parents from the Ebrié lagoon. Selected breeders were in good condition and showed normal morphology (Condition Factor 3.77 ± 0.55 and absence of abnormalities) while being sexually mature. Males emitted milt and females could easily produce eggs upon stripping. For handling purposes, the fish were anaesthetised in a bath of Benzocaine at 0.1 mL L^{-1} (from a 10% stock solution of Ethyl-p-aminobenzoate (Benzocaine, E1501, Sigma, St Louis, MO, USA) prepared by dissolving 100 g of Benzocaine in 1L of 100% ethanol). Fish were separated by sex and species in four concrete tanks (2.5 m length × 1.5 m width × 1 m height) in an open circuit on the experimental site at INP-HB. Fish were fed ad libitum twice a day (09:00 h and 16:00 h) with food pellets containing 45% of protein (3 mm diameter). Tank water was continuously renewed at a rate of 0.07 L s^{-1}. The dissolved oxygen concentration was 4.02 ± 0.36 mg L^{-1}, with a temperature of 27.60 ± 2.31 °C and a pH of 6.89 ± 1.02.

2.2. Genetic characterization of fish (*DNA extraction, sequencing and microsatellite analysis)*

To ensure that our specimen were clearly from two different species a genetic characterization of two groups of fish was made. Fin clips of the two species (NT and BCT, n = 15 / species) were kept in absolute ethanol before genetic characterization. Genetic analyses were performed at Genindexe Laboratory (France). DNA extraction from fin samples was performed according to a simplified Chelex (5%) method. Fin clip (50 mg) was placed in a micro tube of 200 µL containing 195 µL of Chelex

solution (5% in PBS) and 5 µL of proteinase K. The whole was incubated during one night at 56°C and then denatured at 95°C for 5 min to inactivate the proteins K. Two mix (volume of forward primers and reverse at 100 µM) were carried out for the multiplex PCR with 10 polymorphic microsatellite markers selected from Bezault et al. (2012a), mix 1 (UNH125, UNH 129 UNH154, UNH159, UNH211) and mix 2 (UNH102, UNH131, UNH146, UNH169, UNH197). The reaction mixture was composed of 3 µL of DNA diluted to $1/20^{th}$ with 7 µL of mix (5 µL Master Qiagen, 1 µL of Qiagen solution (enhancer) and 1 µL of primer mix). For each sample, two multiplex PCR were performed (one with the mix primer 1 and the second with the mix primer 2). PCR amplification steps were as follows: 15 min at 95°C to activate the HotStart contained in the master mix, followed by 30 s at 95°C for denaturation of DNA strands, 35 hybridization cycles of primers for 30 s at 50°C or 57°C and for 1 min 30 s at 72°C with the final elongation stage of 30 min at 60°C. PCR products were diluted $1/10^{th}$ and passed through electrophoresis capillary (ABI3130XL) in the presence of a ROX500 size marker. The GeneMapper software was used for the analysis of runs obtained and the allele sizes determination.

2.3. Females acclimatization and control of spawning in aquaria

Before their transfer in aquarium, fish received an antiparasitic treatment in a salt bath (30 g L^{-1} NaCl) for 1 min. NT (n = 20) and BCT females (n = 20) were transferred individually in 60 L glass aquariums (50 cm length × 35 cm width × 35 cm height) installed with a recycled fresh water system with mechanical and biological filtration and temperature of 27-28°C while males remained in the concrete tanks. Females were fed and checked daily for 90 days to note the dates of spawning to determine the average time between two spawns under a photoperiod of 12 hours of light. Females were controlled every morning since in NT, under natural photoperiod, spawning takes place usually in the afternoon until sunset (Baroiller et al., 1997; Tacon et al., 2000; Baroiller and Toguyeni, 2004; Castro et al., 2009). The variable of interest in this test was the average time between spawning in females and was calculated by dividing the number of days between successive spawns by the number of spawn occurrences.

2.4. Fish pairing in the aquarium

For the behavioural study, males of each species (n = 20) were transferred individually (in isolation) in the same 60 L aquarium used for single females two weeks before the pairing. When approaching the fourth spawning or 90 days after female's acclimatization started, one male and one female with the same size were tagged on the dorsal fin with beads of different colours (Bégout et al., 2012) and then paired in another aquarium to rule out the status of resident-intruder on dominance (Nijman and Heuts, 2011). Fish were paired in 350 L aquariums (120 cm length × 60 cm width × 50 cm height) according to 4 pairing modes (A = NT ♂ × NT ♀ ; B = NT ♂ × BCT ♀ ; C = BCT ♂ × NT ♀ ; D = BCT ♂ × BCT ♀). Each aquarium was divided into two identical compartments in size with a movable barrier to prevent the physical and visual contact between the male and the female before the behavioural recording during pairing. Male was placed in the compartment towards the water supply and the female on the discharge side thus allowing chemical contact (Plenderleith et al., 2005). The fish were fed with the same food used in their home concrete tank except on the days of video recording when they were fasted.

2.5. Video recording and fish observation

Video were recorded 2 days after fish were tagged and paired. The movable barrier between male and female was removed 10 min before recording the first video sequence to allow fish to accustom to each other's presence. Each pair was filmed 10 times during 15 min at intervals of one hour from 8:00 to 17:00. The videos were recorded by a camera (Sony HDR-PJ530) positioned at 2 m away from the front of the aquarium. The observation device was hidden behind a black plastic screen and only the camera lens was visible to the fish. After recording the videos, the pair was left in the aquarium with a shelter during three days to observe the reproduction and to note the species and sex of the mouthbreeder. Ten different pairs were filmed to characterize and quantify the reproductive and aggressive behaviours in the four pairing modes (n = 20 males and 20 females per species in total). The behaviours were determined according to the tilapia ethogram (Ros et al., 2006; Taves et al., 2009; Renn et al, 2012; Longrie et al, 2013). It relates to the behaviours observed during reproduction (courtship, chase, flight and nest building corresponding to a friction with the mouth on the bottom of the aquarium because

there was no sand in the aquariums) and also agonistic behaviours (chase and biting). Mouths fighting and tail swipe were not retained after analysing videos because of their low occurrences in the different pairing modes. The variables of interest in this experiment were behaviours occurrences and their durations for each fish in the different interspecific and intraspecific pairing. The Ethovision XT and Observer Module (Noldus, The Netherlands) enabled encoding of the videos. A dominance index was calculated using the sum of the occurrences of aggressive behaviour minus flight occurrences observed for each individual (Renn et al., 2012).

2.6. Data analysis

For each genetic marker, polymorphism and the average number of alleles were calculated. Null allele presence was determined with the software Microchecker (Van Oosterhout et al., 2004). The number of shared alleles between *O. niloticus* and *S. melanotheron* was determined. Intraspecific genetic variability was measured through the observed heterozygosity (Ho), expected heterozygosity under the assumption of Hardy-Weinberg equilibrium (Hn.b) and the inbreeding coefficient (Fis) (Weir and Cockerham 1984) was evaluated with Genepop version 3.4 (Rousset, 2008). These parameters were calculated for each marker and averages for each species were considered. Using the number of groups obtained by the software Structure, the index of differentiation (Fst) was calculated according to Weir and Cockerham (1984) to show the genetic differentiation between the two groups.

Data on fish behaviours were statistically tested using the Statistica 10.0 software (Statsoft, Tulsa, OK, USA). Considering the observation of fish between 8:00 h and 17:00 h, a repeated-measure ANOVA was used to analyze durations and occurrences of the different behaviours between species and sex. When a difference was observed, a post hoc Tukey HSD test was used to determine the level of significance depending on the time of the day. Dominance index was tested by a two-way ANOVA (species and sex as fixed factors) using the post hoc Newman-Keuls test (NK) to assess significant differences. For all statistical tests, the significance threshold retained was $p<0.05$.

3. Results

3.1. Genetic characterization

Nine markers out of the 10 selected markers have been properly amplified (Table 1). The amplified markers were at 100% polymorphic in both species with a number of alleles varying between 3 and 15 per marker. The average number of alleles per marker was greater in BCT (8.3 ± 4.0) compared to that of NT (5.7 ± 1.5). No null allele was detected in BCT group, while a null allele (N) was observed with marker UNH131 in NT. The markers showed a low rate of common alleles (13.4%) between species. The heterozygosity was high in the species with values of 0.7 ± 0.2 in BCT and 0.5 ± 0.3 in NT. Expected heterozygosity (Hn.b) were 0.7 ± 0.2 and 0.7 ± 0.1 respectively in BCT and in NT. The inbreeding coefficient (Fis) values were between -0.2 and 0.2 in BCT and between 0.1 and 1 in NT. Fst value between the two groups was 0.2 and the Neighbours-Joining tree highlighted the dissimilarity between the two species (Fig. 1).

3.2. Average days between spawning in females

During the 90 days of observation in 60 L aquaria, no spawning was recorded in BCT females, in contrast to NT females for which spawning occurred with an average duration between spawning of 15.9 ± 0.6 days.

3.3. Behaviour occurrences and temporal variations

3.3.1. Courtship

There were no significant differences ($p>0.05$) in the courtship behaviour occurrences in NT males paired with their females and with BCT females (Table 2). The same results were observed in females of both species. BCT males showed however significantly more courtship occurrences ($p<0.05$) when paired with females of their species than when they were with NT females. Concerning the temporal parameters, NT and BCT males and females presented varying courtship duration from 8:00 to 17:00 according to the pairing mode (Fig. 2). The longest durations were observed in NT males and females in conspecific pairing ($p< 0.05$).

3.3.2. Chase

NT males showed significantly ($p<0.05$) more occurrences of chase in front of BCT females than when facing conspecific females. BCT males showed significantly ($p<0.05$) more occurrences of chase with their females than when paired with NT females. NT females showed significantly longer chase duration than BCT males (Fig. 3C). BCT males and conspecific females showed chases with significantly different ($p<0.05$) durations between 8:00 and 17:00 (Fig. 3D). Two periods appeared: from 8:00 to 13:00 for the benefit of males and from 14:00 to 17:00 for the benefit of females.

3.3.3. Flight

NT males did not show flight when facing NT or BCT females (Table 2). NT females showed significantly more occurrences of flight in front of a conspecific male than when facing BCT males. BCT males had significantly more flight occurrences in front of NT females than when paired to conspecific females ($p<0.05$). In BCT females there was no significant difference ($p>0.05$) in the occurrence of flight when paired with males of one species of the other. Flight durations in BCT pairs were significantly ($p<0.05$) longer compared with the durations in the other pairing modes (Fig. 4).

3.3.4. Nest building

Nest building occurrences in NT males paired to NT or BCT females showed no significant difference ($p>0.05$). Contrary to males, NT females built their nests with significantly more occurrences ($p<0.05$) when paired to BCT males than when paired with their conspecific males. In BCT, males and females nests building did not show significant differences ($p>0.05$) in occurrences whatever the pairing mode. NT males built nests and spent significantly more time than females of either species ($p<0.05$) (Fig. 5A, 5B). Nest building was nonetheless observed generally in NT females continuously between 11:00 and 17:00 with a significantly longer duration ($p<0.05$) when paired with BCT males (Fig. 5A, 5C). In BCT females nest building was observed between 8:00 and 17:00 without significant differences ($p>0.05$) in the duration regardless the males species with which they were paired (Fig. 5B, 5D).

3.3.5. Bite

NT males had significantly ($p<0.05$) more occurrence of bite to BCT females than when paired to conspecific females (Table 2). In BCT, males showed significantly more occurrences of bite against

their females than when facing NT females ($p<0.05$). NT females had significantly more bite with BCT males than with conspecific males ($p<0.05$).

3.4. Dominance index

NT males were more dominant on NT and BCT females with dominance index significantly higher ($p<0.05$) than that observed in females (Fig 6A, 6B.). BCT males were dominated by NT females (Fig. 6C) and dominant on their conspecific females (significant difference in dominance index, $p<0.05$; Fig. 6D).

4. Discussion

The objectives of this study were to determine the reproductive and agonistic behaviours of NT and BCT in interspecific and intraspecific pairings (sex ratio 1:1) in a freshwater hybridization context. After DNA analysis to validate the genetic differentiation between the two species, the reproductive behaviour and aggressiveness were analysed from video recorded on freely interacting pairs held in aquarium. In brief, the behavioural analysis showed a dominance of NT males on females of both species and dominance of NT females over BCT males. We also noted that these two species had some similarities but also showed differences in the expression of some reproductive behaviour.

Concerning the genetic characterization of the experimental fish, diversity parameters (number of alleles, heterozygosity and Fst) showed that NT and BCT were clearly genetically different. Markers polymorphism's was high in both species. In NT, the number of alleles was different in markers with an average of 5.7 alleles / marker as observed by Bezault et al. (2012a) using these same markers. Such genetic diversity (number of alleles / marker = 4.7 to 6.3; Ho = 0.5 to 0.7, Bezault et al. 2011) was observed in NT specimens from the natural environment in the Sudano-Sahelian zone. In BCT, the number of alleles per marker was high (8.3) compared with the results of previous studies (4.1 and 3.3, Bezault et al., 2012a, 2012b). This difference could be explained by our genotyping protocol in which we used 10 markers against 30 and 32 used by the previous authors. The Fis value observed indicated heterozygote deficiency in both species. The same observation was made by Agnèse et al. (2009) and

Bezault et al. (2011) using different microsatellite markers. This deviation from Hardy-Weinberg equilibrium could be explained by the level of inbreeding between the sampled fish and not by the presence of null allele. Indeed, only one null allele was observed in NT with the marker UHN 131 and could not justify the Fis values that we obtained. Agnèse et al. (2009) showed that in lagoon, populations of BCT are composed of related individuals that breed preferentially among themselves. This reproduction may cause inbred individuals as revealed here by the Fis values. In our study, samples used for genetic characterization are all from fish with parents bred in captivity in ponds and they can reproduce among themselves. With the early maturity of NT in captivity, it is possible that reproduction between mature and closely related conspecifics gives inbred individuals (Thünken et al., 2007).

About reproduction, under a photoperiod of 12 hours, isolated BCT females did not spawn contrary to NT females. The average duration between spawning recorded in this study is similar to that reported in NT by Tacon et al. (1996) and by Campos-Mendoza et al. (2004) with an isolated female in which eggs were removed from the mouth after spawning. In BCT, spawning absence could be explained by the absence of male congener. Indeed, egg layings were obtained from pairs of this species isolated in aquarium which front was covered with a piece of black plastic by Kishida and Specker (2000).

Concerning behavioural data, high-standard deviations indicated a strong intraspecific variability in the behaviour of a fish interacting differently with a partner. Such difference in behaviour observed in the same species and sex could be linked to the genetic variation within each population, the level of domestication of each group of fish used (Mignon-Grasteau et al., 2005; Driscoll et al., 2009) and the physiological status of the fish regarding their reproductive cycle (Tacon et al., 2000) or further, the individual personality (Martins et al., 2011). Indeed, domesticated species exhibit behavioural plasticity in captivity which can differ from the wild strain. In our case, the fish used came from different farms. The number of generation for BCT issued from wild populations is well known (4^{th} generation of brackish water pond rearing). For NT we don't have enough information on the events which occurred through time between the wild populations and the creation of the synthetic Bouaké strain transferred to the supply farm. Indeed, the Bouaké NT strain is a synthetic strain obtained by interbreeding between

two wild populations in 1971, one from the Volta basin (Burkina Faso) and the other from the Nile basin (Uganda) (Rognon and Guyomard, 2003). We used a fourth generation of NT from a NT synthetic Bouaké strain population transferred to the farm, but we cannot say with certainty that fish of both species have the same level of domestication according to Teletchea and Fontaine (2014). Only before the behavioural tests, the fish were stored in similar concrete tank and acclimatized in the aquarium. Even if the fish share a similar 4th generation long pond rearing, we cannot exclude that the difference in the level of domestication could also explain the difference in behaviour between NT and BCT and there were no option to avoid this matter of fact. Moreover, our protocol aimed at avoiding stress to the fish and at following them after the video recordings to see the species and the sex of the mouthbreeder fish. These aims were not compatible with taking a blood sample to analyse sexual steroid concentrations although we recognize that it would have allowed us to analyse a possible relationship between reproductive state and reproductive and aggressive behaviour. In fact, some reproductive behaviours like courtship and nest building in most teleost fish is under the control of hormones according to Munakata and Kobayashi (2010).

However, common traits were observed: male NT always dominated the females in all pairing with more aggression toward BCT females. In NT, juveniles showed a decrease in the number of attack occurrences between the first day of the pairing and the following days (Alvarenga and Volpato, 1995). Such trend in aggression decrease was also observed in our study in NT intraspecific adult pairs with a decrease in the durations of chases and also a decrease in biting occurrences of male to female NT between the first contacts and the following hours. These findings may be related to a sexual recognition between congener male and female known in NT to be based on visual, olfactory or sound stimuli (Plenderleith et al., 2005; Castro et al., 2009) contrary to interspecific pairs where aggression was stable. The conspecific recognition was also seen on courtship with longer duration, as well as on females nests building behaviours in NT conspecific pairs compared to NT females when paired with BCT males. These behaviours suggest that positive feedback can facilitate sexual recognition of the partner and a preference for conspecific in NT exists as it was observed in the guppy *Poecilia reticulata* (Field and Waite, 2004).

Despite the difference in dominance index in BCT pairs, social hierarchy did not seem to be established between male and female in this species. Contrary to NT conspecific pairs in which social dominance hierarchy was clearly in favour of males, males BCT were dominant and sometimes dominated by their females in the analysed pairs. These observations imply that in BCT, social hierarchy cannot be established based on the level of aggression contrary to observations in some species such as *Neolamprologus pulcher* (Fitzpatrick et al., 2008). Females BCT did not respond positively to males in both pairing modes contrary to females NT which showed positive behavioural responses to conspecific males and sometimes with BCT males. It is likely that females BCT were not receptive or not motivated when facing males with which they were paired. Thus, persistence of aggressive behaviour in BCT could be related to the fact that male or female would be very selective in choosing the partner of the opposite sex, as also reported by Balshine-Earn (1996) in *Sarotherodon galilaeus*. In fact, females BCT are known to accept larger male to ensure oral incubation efficiency of offspring (Legendre and Trébaol, 1996), whereas all the fish in our study had equal sizes. Furthermore, the identification and selection of a potential partner for reproduction could be influenced by the social environment in BCT.

Paired with male and female BCT, NT maintained their aggressiveness, suggesting the existence of premating barrier in interspecific fixed pairs, which would reduce the chances of hybridization (Ptacek, 2000; Seiler and Keeley 2007). Changing the sex ratio (Faunce, 2000) and the size of the fish could reduce the aggressiveness in fish and increase the matching opportunities in BCT and thus allow hybridization between BCT and NT. Indeed, the fact that the NT males are polygamous (Adel, 2012) while the BCT males are monogamous (Legendre and Trébaol, 1996) could also explain the difference of behaviour in the two species.

Nest building was observed with different duration in males and females of both species at different time of the day in all the pairing modes. This behaviour started late in the morning (11:00 h) and spanned throughout the afternoon, and egg laying periods were in the afternoon. This observation was also made by several authors (Baroiller et al., 1997; Tacon et al., 2000; Castro et al., 2009). However, when NT females were paired to BCT males, they spent more time in nest building than when they were with NT males. This suggested, first, that NT females are able to recognize their conspecific males from some stimuli and secondly, that in the presence of males, females prefer to use their energy for vitellogenesis

and oocytes final maturation rather than for nest building. Indeed, in natural environment, NT males build nests to attract females during spawning period (Castro et al., 2009). This behaviour was observed in NT conspecific pairs and resulted in egg laying and oral incubation in females, as observed by Tacon et al. (1996). In females BCT, the nest building was not followed by egg laying in interspecies and conspecifics pairs. This could be due to a stress effect in females (Barton, 2002) due to male aggression and / or the absence of the best sexual partner, which could induce stimulation for egg laying.

In conclusion, genetic analyses of the fish that we used confirmed a clear genetic differentiation between our populations of NT and BCT. Behavioural tests showed that in terms of aggressiveness NT always dominated BCT. In addition, recognition of conspecific exists in NT and there were similarities in some behaviours such as courtship and nest building in the two species. Our observations suggested that means to decrease aggressive behaviour under modified breeding conditions could lead to a natural hybridization between NT and BCT. Sex ratio for example could be modified (male:female 1:3, as used in NT (Adel, 2012)) and the reproductive and agonistic behaviours of the two species recorded. Furthermore, brackish water could be used: indeed, if the salinity tolerance in a certain range up to 28 has been demonstrated in NT by several authors (Lemarié et al., 2004 ; Azaza and Kraïem, 2007), the effects on the reproductive and agonistic behaviours have not been evaluated. Finally, in views of the behavioural similarities, the study could focus on NT and BCT reared together in common garden, from the post-larval stage to the adult stage. This would help assessing the impact of familiarity on the reproductive behaviour of each species.

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Figure 1

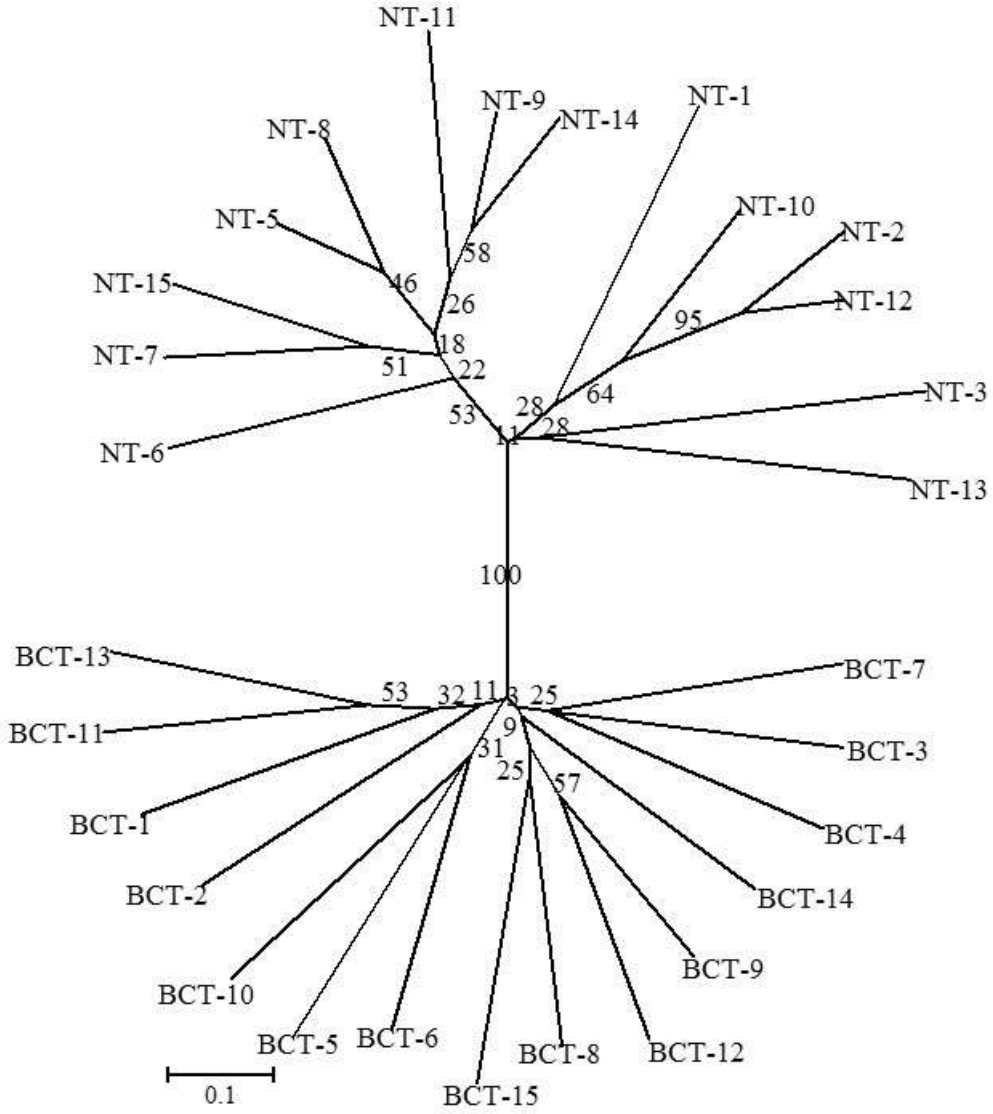

Figure 2

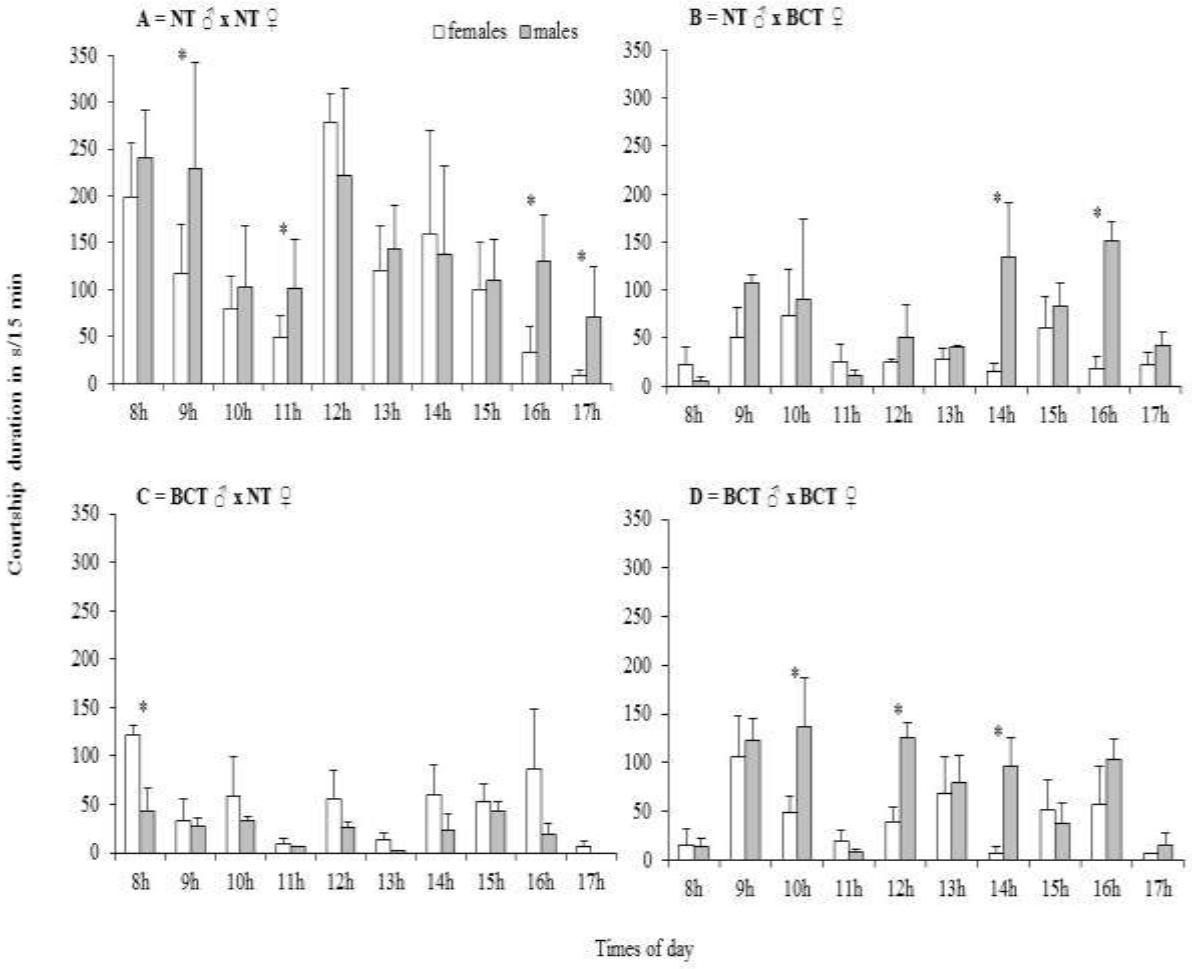

Figure 3

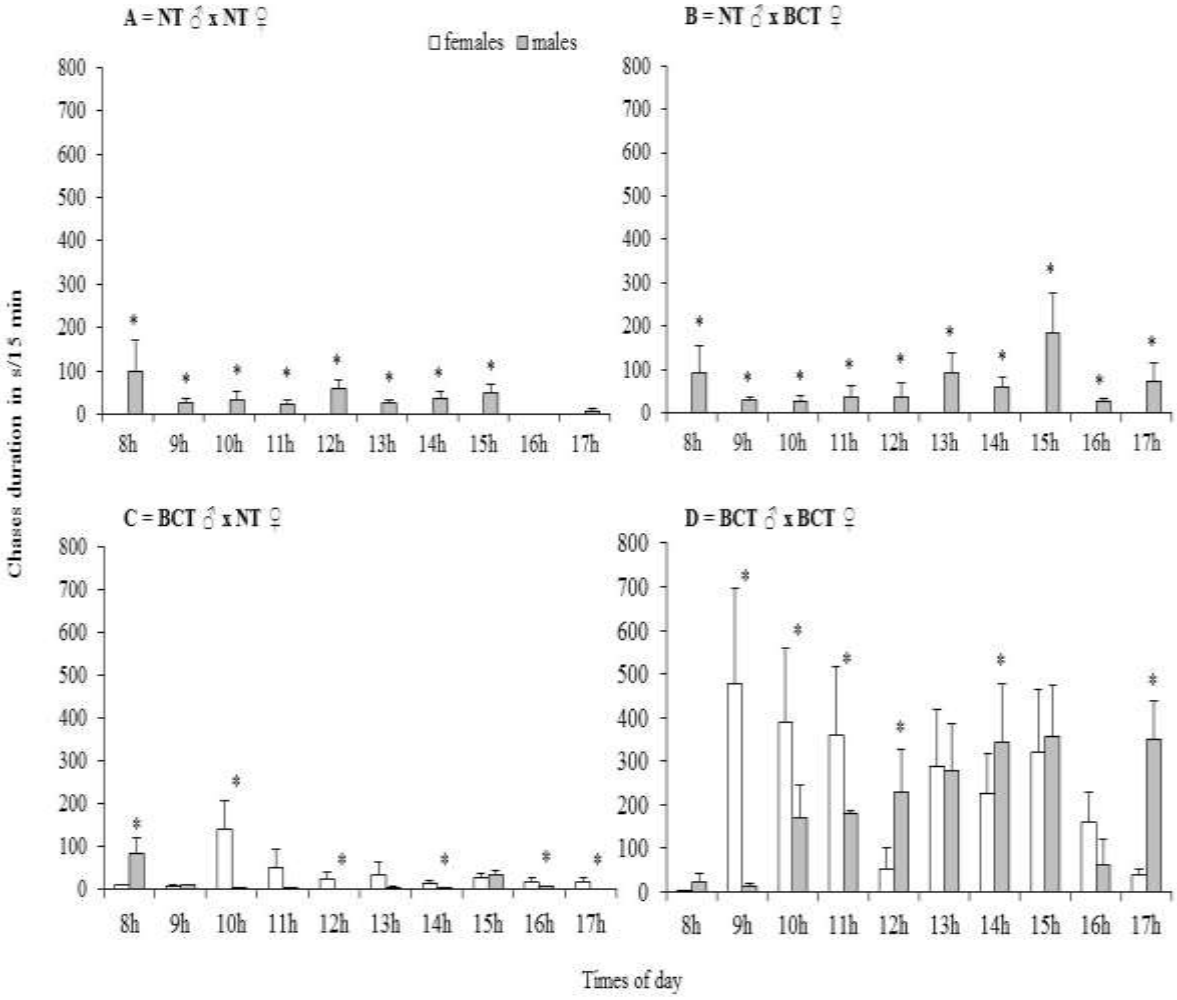

Figure 4

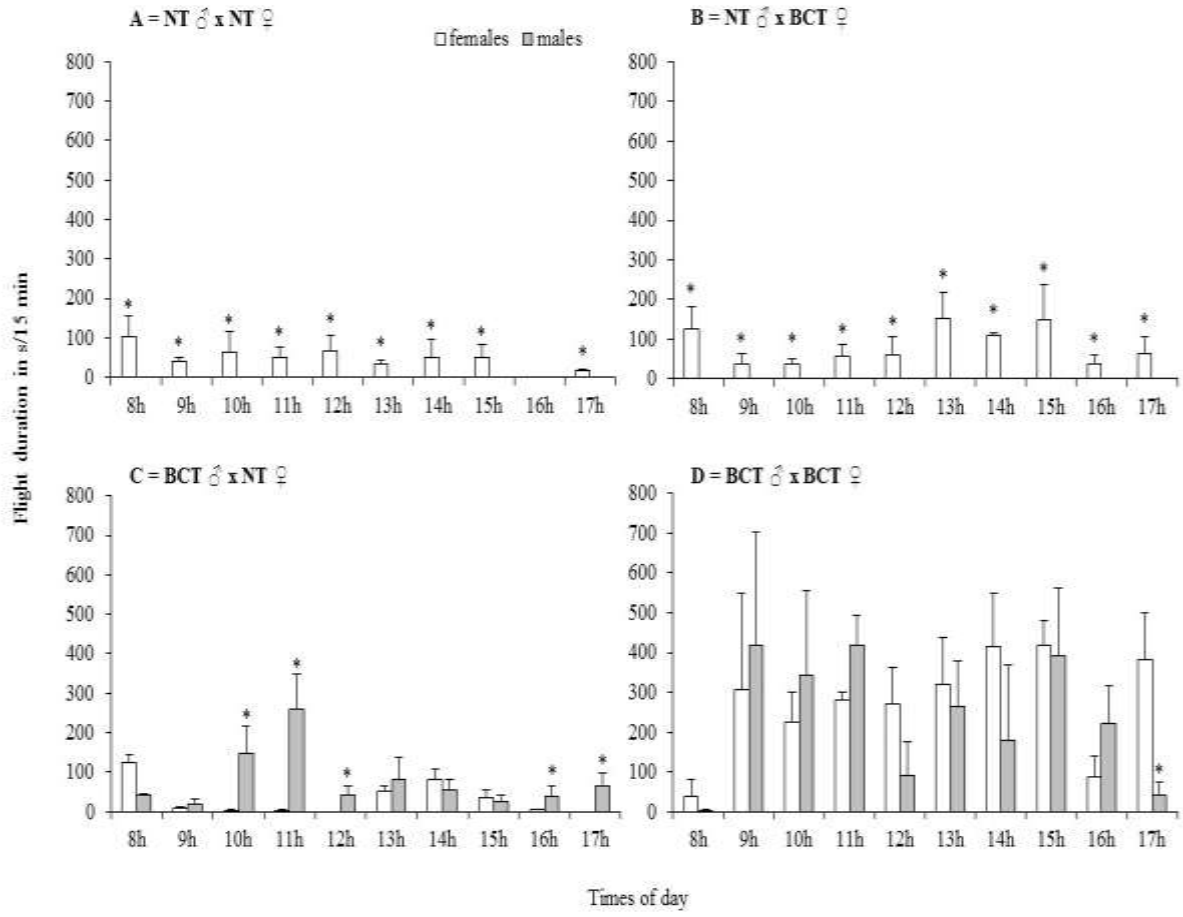

Figure 5

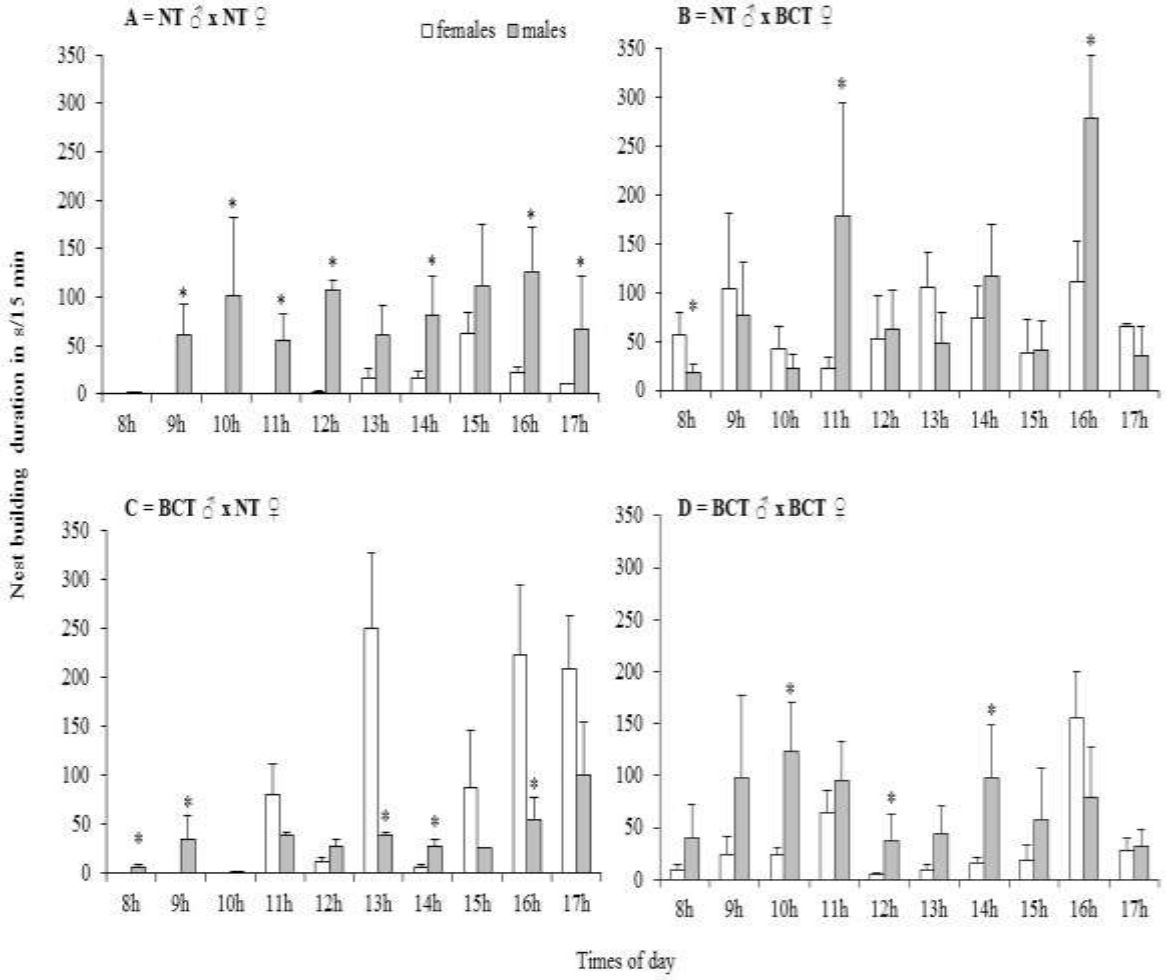

Figure 6

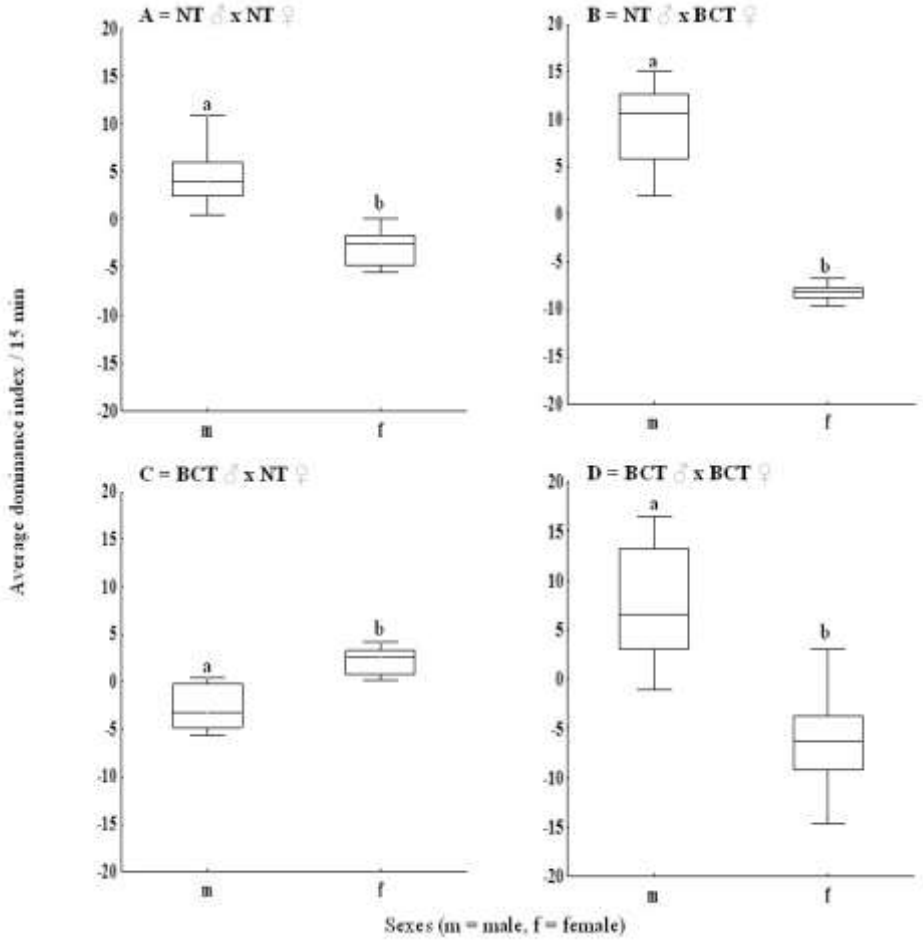

Table 1: Microsatellite markers analysed, sizes of alleles, allelic diversity and shared alleles in BCT and NT.

Markers	GenBank accession	Size of alleles	Allelic diversity		Shared alleles
			BCT	NT	
UNH102	G12255	148-180	6	6	2
UNH129	G12282	181-219	9	6	3
UNH131	G12284	249-301	5	5+N	2
UNH146	G12298	113-131	5	3	0
UNH154	G12306	100-989	10	7	3
UNH159	G12311	207-258	3	7	0
UNH169	G12321	128-182	9	5	2
UNH197	G12348	152-252	13	5	0
UNH211	G12362	118-190	15	8	3
Average allele/locus			8.3	5.7	1.7
Polymorphism P(0.95)			100%	100%	

Table 2: Mean (± sd) of the occurrences of the different behaviours observed during 10 15-min video recording between males and females in intra- and interspecific pairing of NT and BCT (N = 10 pairs by pairing mode).

Pairing mode in aquarium (350 L) (1 male / 1 female)		Courtship	Nest building	Flight	Chase	Bites
NT ♂ × NT ♀	♂	15.9 ± 11.0^{b}	11.2 ± 7.2^{ab}	0.0^{d}	7.0 ± 3.7^{c}	8.1 ± 2.6^{b}
	♀	11.1 ± 7.0^{b}	5.8 ± 3.7^{c}	10.4 ± 4.3^{b}	0.0^{d}	2.5 ± 0.7^{c}
NT ♂ × BCT ♀	♂	10.8 ± 7.6^{b}	8.6 ± 6.1^{bc}	0.0^{d}	12.8 ± 5.2^{b}	13.2 ± 4.0^{a}
	♀	8.0 ± 6.0^{b}	11.9 ± 7.5^{ab}	15.6 ± 4.1^{a}	0.0^{d}	1.5 ± 0.6^{c}
BCT ♂ × NT ♀	♂	8.8 ± 6.5^{b}	6.8 ± 4.7^{c}	16.1 ± 6.3^{a}	6.3 ± 2.8^{c}	5.6 ± 3.6^{bc}
	♀	7.7 ± 5.6^{b}	14.0 ± 9.6^{a}	5.1 ± 3.0^{c}	10.6 ± 4.7^{bc}	12.7 ± 5.1^{a}
BCT ♂ × BCT♀	♂	23.4 ± 15.1^{a}	8.4 ± 5.0^{c}	13.8 ± 6.4^{b}	24.0 ±13.2^{a}	15.1 ± 7.2^{a}
	♀	17.6 ± 11.6^{ab}	6.0 ± 3.7^{c}	24.8 ± 14.0^{a}	18.3 ± 8.5^{b}	7.9 ± 4.5^{b}

In the columns, letters represent significant differences according to HSD Tukey's test ($p<0.05$)