NTNU

Norwegian University of
Science and Technology

The effect of body size on intrasexual competition in the coral reef fish *Chrysiptera cyanea*

Miriam Horstad Ness

Acknowledgement

Abstract

Intrasexual competition is considered important in driving selection on morphological traits, and the role of body size in male-male competition is well studied in a wide range of animals. In coral reef fishes however, there are still aspects left relatively unexplored, such as the competitive dynamics between different sized males. The effects of body size on intrasexual competition in the damselfish *Chrysiptera cyanea* were studied, investigating both the nature and outcome of the competitions. Dyads of male *Chrysiptera cyanea* were allowed to interact using a standardized experimental set up, and agonistic behaviours, resource monopolization and area use were recorded. Larger males performed considerably more chases than smaller males, were more successful in monopolizing the available resources and spent more time in the lower area of the aquarium, closer to the potential resources. Theory for conflict resolution predicts intrasexual conflicts to start with low-risk behaviours and follow a certain behavioural pattern. In addition, a more escalated conflict and a less predictable outcome are expected when size asymmetries are small. As predicted, both males showed a high frequency of visual fin displays, and more escalated behaviour was done increasingly towards the end and almost exclusively by the larger male. Despite an overall small difference in within pair body size (larger male < 10% longer than smaller male in total length), conflict outcome was clear with the larger male showing competitive superiority. These results thus emphasize the importance of size advantage in intrasexual competition between male *C. cyanea*, and suggest that male-male competition is likely to be involved in creating selection on body size in the wild.

Sammendrag

Intraseksuell konkurranse er ansett som en viktig faktor i seleksjon av morfologiske trekk, og kroppsstørrelsens rolle i hann-hann konkurranse er godt studert i et bredt spekter av dyr. I korallrevsfisk er det likevel aspekter som fortsatt er relativt lite utforsket, eksempelvis selve konkurransedynamikken hos hannfisker av ulik størrelse. Effekten av kroppsstørrelse på intrasexuell konkurranse hos jomfrufisken *Chrysiptera cyanea* ble studert, hvor både konkurransens natur og utfall ble undersøkt. To og to *C. cyanea* hanner fikk interagere i et standardisert forsøksoppsett hvor aggressive adferd, ressursmonopolisering og arealbruk ble registrert. Den større hannen i paret jaget konkurrenten sin vesentlig mer, hadde større suksess i å monopolisere de tilgjengelige ressursene, og tilbrakte mer tid i den nedre delen av akvariet (der de potensielle ressursene befant seg), sammenlignet med den mindre hannen. Konfliktløsningsteori forutser at intraseksuell konflikt vil starte med lavrisikoadferd og vil følge et bestemt adferdsmønster. Videre, en eskalert konflikt og et mer uforutsigbart utfall er forventet når størrelsesforskjellen er liten. Som forutsett så hadde begge hannene høy frekvens av "halefinne-visning", og forekomsten av mer eskalert adferd økte mot slutten, og ble utført nesten utelukkende kun av den større hannen. Til tross for generelt liten forskjell i kroppsstørrelse (større hanner hadde <10% lengre total lengde enn den mindre hannen i paret) mellom hannene i hvert par, var konkurranseutfallet tydelig, hvor den større hannen viste overlegen konkurransedyktighet. Disse resultatene understreker viktigheten av størrelse i intraseksuell konkurranse hos *C. cyanea* og indikerer at hann-hann konkurranse sannsynligvis er med å skape seleksjon på kroppsstørrelse i naturen.

Table of Contents

Introduction

Intrasexual competition occurs in situations where resources needed by more than one individual of the same sex are limited. The winner of a conflict may gain access to resources needed to ensure a higher reproductive success, for example nest sites and mates (Andersson 1994; Maynard Smith & Price 1973). This way, competition shapes the distribution of resources among individuals and is an important driving force behind selection (Darwin 1871; Andersson 1994).

Studies investigating the ecology of territoriality and territorial behaviour exist for a wide range of animals (e.g. Davies 1978; Ostfeld 1990; Marler et al. 1995; Johnsson et al. 1999; Hau et al. 2000). Theory predicts the formation of territories when resources are spatially and temporally predictable, and the benefits of defending an area exceeds the related costs of defence, for example loss of time, energy and opportunities and risk of injury (Noble 1939; Brown 1964; Grant 1993; Maher & Lott 2000). The defended territory may be an area of food resources, breeding grounds or a combination of both (Noble 1939). In territorial species, individuals compete for dominance to get exclusive access to the territories with the highest quality resources (Kaufmann 1983).

Direct confrontation in form of fights can lead to serious injury to the contestants, or even death. However, animals often use non-escalated conflict behaviours (Maynard Smith & Price 1973), where the opponents appear to assess each other's fighting ability by sequences of various displays and thus avoid the costs of fighting (Maynard Smith & Price 1973; Parker 1974). An example of this kind of conflict strategy is red deer stags (*Cervus elaphus*), where competing stags will avoid costly contest with individuals they are unlikely to beat (Clutton-Brock & Albon 1979). They assess each other through roaring, and parallel walks, sizing each other up (Clutton-Brock & Albon 1979). Another type of visual signal used as non-escalated conflict behaviour is the dewlap display of male *Anolis Segrei* (Evans 1936), used in combination with "head bobs" in intrasexual aggressive encounters. Based on information gained in this assessment phase, the contestants may decide whether they are the stronger competitor and can benefit from escalating the contest or if they are inferior and should avoid costly escalation by retreating (Parker 1974; Enquist & Leimar 1983).

Concepts often used in relation to intrasexual competition are resource holding potential (RHP; Parker 1974) and resource value. RHP is a measure of an individual's fighting ability, which itself is affected by characteristics such as body size and weaponry (Parker 1974). The motivation, prior experience, or residency of the contestants may cause asymmetries in individual resource value,

leading to differences in the effort they put into the conflict (Parker 1974). Thus, theory predicts that the outcome of intrasexual conflicts can be determined from the asymmetries in both RHP and resource value (Parker 1974; Maynard Smith & Parker 1976; Enquist & Leimar 1983). However, the contestants may match in RHP, or the increased effort caused by a higher resource value for one of the competitors may compensate for a lesser RHP, and the outcome becomes less predictable (Parker 1974). In such situations, the ritualized conflict behaviour alone may not be sufficient to decide who would be the superior competitor, and one could predict a fight that include more escalated but accurate means of assessment (Parker 1974). For example, the intrasexual conflicts of the red deer (*Cervus elaphus*) mentioned above, may escalate beyond the roaring and parallel walk to a phase where they interlock antlers and push against each other (Clutton-Brock & Albon 1979).

An important characteristic affecting RHP is as mentioned, body size (Parker 1974). Body size or body mass correlates naturally with strength, and may thus be the most important determinant of the fighting ability of an animal and of its chance to win an escalated conflict (Parker 1974; Enquist & Leimar 1983; Archer 1988). That body size has a role in determining the outcome of intrasexual competition has been supported by empirical studies in a wide range of taxa including mammals (Celliah & Sukumar 2013), birds (Hagelin 2002; Bolund et al. 2007), reptiles (Tokarz 1985; Madsen et al. 1993), insects (Peterson & Hardy 1996) and fish (Rowland 1989; Moretz 2003; Wacker et al. 2012).

Fishes have indeterminate growth resulting in adults varying much in size and thus more scope for size to matter (Sebens 1987). Despite the vast amount of literature on body size in fishes, the under-representation in studies on behaviour using fish as a model is striking considering their species diversity (33200 species listed by FishBase in 2015), and when compared to birds (Amundsen 2003). In addition, contrary to what one might expect from the relative ease of working in warm, shallow waters where one could get very close to the focal individuals, there is surprisingly little data on the behaviour of coral reef fishes (Amundsen 2003; Harborne 2013). However, among the existing studies on reef fish behaviour, there are quite many related to the effect of body size on male mating success (e.g. Clarke 1970; Hoelzer 1990; Karino 1999; Wong et al. 2007). The structuring of a coral reef allows numerous microhabitats, which makes it possible for a high number of individuals to live in close proximity (Sale 1977; Connell 1978). This high density, particularly of fishes associated with the reef, facilitates frequent interactions both within and between species (Connell 1978). Intraspecific conflicts regarding resource acquisition and individual territories are likely in such an environment (Sale 1977), and coral reef fish thus provide excellent subjects for studies on competitive behaviour.

The purpose of this study was to investigate the effect of body size on male-male competition in the territorial damselfish *Chrysiptera cyanea*. Territoriality is common in several reef fish families, and has for example been described in species of butterflyfishes (fam. *Chaetodontidae*; Tricas 1985), parrotfishes (fam. *Scaridae*; Robertson & Warner 1978), surgeonfishes (fam. Acanthuridae; Morgan & Kramer 2004) and damselfishes (fam. *Pomacentridae*; Clarke 1977). A territory may be actively defended against conspecifics and even heterospecifics if resources of interest overlap or there is a risk of egg predation (e.g. Low 1971; Itzkowitz 1990). Observations of male *C. cyanea* suggests that they are doing both (Thresher & Moyer 1983; Gronell 1989; T. Amundsen, unpubl. observations; personal observations). The *C. cyanea* is a demersal spawner with paternal care of the eggs and inhabits coral reefs in the Central Indo-Pacific (Gronell 1989). Demersal spawning fish with paternal care of the eggs in pre-prepared nests may especially benefit from investing in territorial defence and competing for territories with high quality nest sites (e.g. Unger 1983; Jaroensutasinee & Jaroensutasinee 2003). The quality of a nest may be determined by factors such as its size, location and defendability, and has been found to influence the female choice and thus the male mating success (Sargent 1982; Jones & Reynolds 1999; Östlund-Nilsson 2000). Previous work on the species by Thresher and Moyer (1983) and Gronell (1989) found the male reproductive success in the wild to be highly skewed towards larger males. Thresher & Moyer (1983) observed vigorous fights between males where the result could be displacement of the losing male and in some cases nest take-overs. The males also have an orange caudal fin that they display to other males in competitive encounters (Gronell 1989). They can engage in prolonged display sessions, before any chases or fights occur (T. Amundsen, unpubl. data).

In the present study, I aimed to test the effect of difference in body size on the nature and outcome of competition in the coral reef fish *C. cyanea*. Males of different size were allowed to compete in small, standardized aquaria in the laboratory. It seems as the majority of research regarding intraspecific competition is often focused on the motivation behind and outcome of the conflict, and not on the nature of the conflict itself. The approach in this study allowed me to investigate the nature of the conflict by analysing the behaviours that leads to the outcome. Based on descriptions of male-male interactions from Thresher & Moyer (1983) and the theories for conflict resolution, I predicted to see the males engaging in competitive interactions with an assessment phase in the start of the trial. As mentioned, theory predicts asymmetries in RHP (including body size) to be an important determinant of conflict outcome. Given that all else were kept equal from the standardized set up, I thus predicted that the larger male would be competitively superior. The

superiority of the larger male was predicted to be reflected in the area use in the aquarium, with more association with the lower area, that contained possible resources (e.g. shelter, nest site, food from substrate) and was less exposed compared to near the surface. Where the males were successful in assessing each other's fighting ability, I predicted a unilateral escalation of behaviours from the larger male in an attempt to chase away the competitor. In addition, manifestation of competitive superiority would be seen by resource monopolization. With variable size asymmetry between the trials, I also expected to see conflicts of varying intensity, with a more escalated conflict in pairs of smaller size differences and a clearer outcome when size differences were bigger.

Methods

Experimental trials were carried out during December 28, 2010 to January 2, 2011 (except for one trial November 22, 2010) at Lizard Island Research Station, Great Barrier Reef, Australia (14° 40' S, 145° 27' E).

Study species

Chrysiptera cyanea, is a small, planktivorous damselfish (family Pomacentridae). It lives on shallow reefs (often less than 3 m) and is common on the Great Barrier Reef and throughout the Western Indo-Pacific (Gronell 1989). Their size distributions range from 49 to 73 mm total length (TL) for males and from 38 to 54 mm TL for females (Gronell, 1989). Both sexes have a bright blue colour and black markings on the head. Males have a bright orange coloured tail fin (Figure 1, 2, 3) that the females lack (Thresher and Moyer 1983; Gronell 1989). The almost separated size distributions of males and females, together with a female biased sex ratio, suggest that the species is a protogynous hermaphrodite (Thresher & Moyer 1983; Gronell 1988). Thresher & Moyer (1983) found a mean population density of 0.2 individuals/m^2 within their study area (approximately 25 x 50 m) and a mean distance between neighbouring males of 2.6 metres. During the main breeding season (October-January), some of the males take up nests typically in shells of dead clams (*Tridacna* ssp.) embedded in coral (Gronell 1989). A male can receive eggs from several females, and care for the eggs by guarding, cleaning and fanning them (Gopakumar et al. 2009). Their spawning period usually starts at first light and females can spawn approximately every four days (Thresher & Moyer 1983; Gronell 1989). Agonistic interactions including mutual fin displays, chases and even vigorous fights resulting in nest take-overs have been observed between males of this species (Thresher & Moyer 1983; Gronell 1989). Thresher & Moyer (1983) reports that during the spawning period, several non-nest holding males aggregate around the nest site of nest holding males. In such situations, aggressive interactions are almost continuous, with the nest holding male defending his nest against the intruders (Thresher & Moyer 1983). Gronell (1989) found a significant positive relationship between the number of eggs in the nest and attack rates towards other males. Aggressive interactions can be short or long-lasting (> 30 minutes)(T. Amundsen, unpublished observations), and appears to happen at all times of day during the main breeding season.

Collection and husbandry

Fish were caught by SCUBA diving in the lagoon outside Lizard Island. It was sedated with clove oil (Munday & Wilson 1997), captured with a dip net and held in catch bags before they were put in buckets in the boat. In the lab, the fish were weighed on a digital scale (wet weight, to the nearest 0.01 g), measured for total length (TL) on a measuring board (to the nearest 0.5 mm) and photographed in a standard photo aquarium. The fish were held individually in 5-10 L aquaria visually separated from other fish, for four to 25 days before being used in the trials. During individual housing, the fish were fed ad lib twice a day with live brine shrimp (*Artemia* sp.), marine fish flakes, and a mixture (previously frozen) of brine shrimp, bloodworms and mysids. Aquaria were placed in an outdoor lab area under a semi-transparent roof, providing natural daylight and shelter. All aquaria were provided with a PVC tube for shelter, air stones and a flow of surface seawater at ambient temperature (28-30 °C).

Experimental setup

To study competitive interactions between different-sized males, pairs of males were simultaneously introduced into experimental aquaria of 46x35x40 cm (approx. 40 L; Fig. 1). The bottom of the aquaria was covered with a layer of sand, and a PVC-tube (4.2 cm diameter, 10 cm long) was placed on the bottom, in the middle of the aquarium, acting as a potential nest and/or shelter. Aquaria were covered with grey, matte PVC on three sides to prevent mirror effects and visual contacts among fish in neighbouring aquaria. An opaque plexiglass divider separated the aquaria into one compartment for each male before trial start. They were kept in the experimental aquaria for 2-4 days prior to the trial to allow acclimation. The two males were allowed to interact for 30 minutes, during which a Canon HD video camera, positioned approximately 50 cm away from the aquarium, recorded the trial. This was repeated for 25 trials. The males used in this study had been subject to a mate choice experiment earlier the same day, in which they were allowed visual interaction to a female and to the other male through a transparent plexiglass divider. The same pairs were used in both experiments. Between the end of the mate choice trial and the start of the competitive trial, there was a minimum pause of 30 minutes where the female was removed and the opaque plexiglass divider inserted to visually separate the males. An observer visually inspected the two fish at regular intervals during the trials of this experiment to make sure that none of them were being physically harmed, in which case the experiment would have been stopped. Fortunately, this was not necessary as none of the males showed signs of physical damage. Occasional disturbance caused both fishes to take shelter but this was infrequent and affected both fish similarly.

Pairs of males to be used in the trials were selected to be different in length with otherwise similar characteristics (tail colouration). Matching was based on TL as measured on the measuring board (Table 1) and subjective judgement of tail coloration. Large and small males did not differ significantly in time spent in lab before trial (Wilcoxon rank sum test, W = 316.5, p = 0.95; Table 1). Whenever possible, the two males in a trial were collected from different reefs, to avoid possible familiarity. This was achieved in 12 out of 25 trials used for analysis. The initial position of the large and small male in left or right compartment was balanced among trials. The tail coloration was judged using a 5-graded nominal scale that ranged from the lowest score of two to the highest score of 3+. The scale was transformed to be numerical for the analysis, in a way that for example 2+ became 2.33, thus a difference of 0.33 between each step. The median (IQR) colour measure was 3.00 (0.33) for the larger male, ranging from 2.00 to 3.33, and 2.67 (0.67) for the smaller male, ranging from 2.00 to 3.00. There was a trend for larger males to have brighter tails, but the median difference in colour between the large and small male was very small (0.33 (0.67); Fig. 2), and statistically they did not differ significantly in tail colouration (Wilcoxon rank sum test, W = 398.5, p = 0.07).

Behavioural recordings

Male agonistic behaviours and positions of the two contestants in the tank were recorded by focal observations of the video recordings. Behaviours, tube entries and corner entries were continuously recorded whereas positions in the aquarium were recorded by scan sampling every 20 seconds. The behaviours were recorded for both males and the two males were identified by their size (larger or smaller male), which was unambiguous. Recorded behaviours included (1) display: fanning of the tail fin in a visual display (Fig. 3), (2) approach: one fish swimming towards the other fish, and (3) chase: one fish swimming rapidly towards or after the other fish. The displays were recorded as either (1) mutual displays: one male starts displaying and the other responds with a similar display before the first stops displaying (taper its tail), (2) unilateral displays: only one male displaying at a time with no overlap in display from the other male, or (3) simultaneous display: both males displaying at the same time (undistinguishable with resolution of 25 frames per second). Mutual displays were registered as either "large male initiating" or "small male initiating". This means one event includes two displays, one from each male. Thus, for the purpose of giving an impression of the overall frequency, the mutual displays were added twice when calculating total amount of displays for each male. *C. cyanea* males fan their tails very conspicuously in competitive interactions. Any less clear tail

displays were conservatively excluded. The duration of a fin display varies from just a fraction of a second, to several second, but this was not timed.

Other actions continuously recorded included (1) tube entries: one of the males entering the PVC tube and (2) corner entries: one of the males swimming into the crevice beneath one of the aquarium outlets (see Fig. 1). Tube entries were included in the continuous recording because the PVC tube could be considered a potential resource that may be monopolized by the more dominant male. The crevices beneath the outlets in each corner were not intended as a part of the design, but were included in analysis as they possibly could resemble potential nests or simply shelters, and the males were clearly using them. It was noted when analysing the recordings whether tube and corner entries were related to outside disturbance.

Horizontal and vertical positions of the two fish in the aquarium were recorded by scan sampling every 20 seconds. For the analysis of difference in positioning within the aquarium between the large and small fish, the aquarium was divided into a lower, middle and upper part. The lower part included scans from the corners and tube as well. The motivation behind scan sampling positions, and the division of the aquarium into three parts, is that physical displacement of one fish by the other would be indicative of competitive superiority and dominance. It can be predicted that the larger male will more frequently be positioned at the bottom part of the aquarium because of the occurrence of possible resources such as the outlets, the tube and the substrate (possible food source) in this area. The bottom part would in addition provide more cover from potential predators compared to the more exposed upper area near the surface. It was predicted that over the course of the trial, the behaviour of the two fish could change as a result of increased knowledge of each other and an emerging dominance relationship. Thus, the 30 minutes of the trial were divided into six five-minute periods to enable comparison of behaviours across time intervals within trials.

Statistical analysis

The statistical analyses were carried out in the software R version 0.98.1103 (R Core Team 2015) and IBM SPSS Statistics 21.

Size-related differences in competitive behaviour were analysed using non-parametric tests when requirements for parametric tests were not met. Size difference were used as a dichotomous variable (larger vs. smaller male), except when effect of relative within pair size difference was

tested, where size difference was entered as a continuous variable. In order to test for behavioural asymmetry and possible resource monopolization, the proportion of agonistic behaviours, tube entries and corner entries performed by the large male within each trial was calculated and tested against the expectation of 0.5 (equal proportions).

The within pair size difference was measured as difference between large and small male in TL relative to the absolute TL of the smaller male. Generalized linear models (GLM) were used in order to test for an effect of within pair size difference on any of the behaviours, including tube entries and corner entries. Both the proportion of behaviours done by the larger male and the combined large and small frequency of behaviours were tested. The models were fitted with a quasibinomial error distribution for the proportions, and a quasipoisson error distribution for the frequencies, to correct for overdispersion.

In order to test for asymmetry in area use, the proportion of scans registered in the lower area was calculated for both large and small male. It was then tested if the large and small male proportions differed significantly from each other. The test was repeated for the proportion of scans in the upper area.

In order to test for changes in behaviours over the course of the trials, repeated measures ANOVAs were separately carried out on all the behavioural variables, tube entries end corner entries. The effect of time-intervals within trials was tested on both the proportion of behaviours, tube entries and corner entries done by the large male and the frequency of behaviours, tube entries and corner entries done by both fish combined. To satisfy the assumption of normality of the residuals, the proportion values derived from count data were arcsine transformed and the count variables were square root transformed. Where the Mauchley's test of sphericity indicated that the assumption of sphericity had been violated, the Greenhouse-Geisser correction was applied (Greenhouse & Geisser, 1959).

Results

Competitive behaviour during trials

Both males were residing under the outlets when the recording started in all but two trials. In these two trials, the larger was out swimming before the start in one and the smaller male in the other. The larger male started swimming after a mean of 119 ± 86 seconds (49 – 421) after the start, and was the first to swim in 13 of 25 trials. The smaller male started swimming after a mean of 127 ± 102 seconds (56 – 427) and was the first to swim in 11 of the 25 trials. The fish started swimming simultaneously 100 seconds into the recording (undistinguishable with resolution of 25 frames per second) in one of the 25 trials. The first male to swim would often approach the other male still under the outlet. In this first encounter, the approaching male would typically vigorously beat his tail towards the male under the outlet and fan his tail fin in a visual display just under the outlet. This would typically cause a response by fin displays from the male under the outlet and he would be driven out from the corner. When both males were out from under the outlets, several bouts of parallel swimming and mutual displaying usually happened. New bouts of rapid displaying, both mutual and unilateral, could be initiated, and displays from both males would typically continue throughout the trial. One male approaching the other would usually lead to the approached male retreating, the approached male displaying its tail fin towards the approaching male, or the initiation of a new bout of fin displays including both males. A chase would lead to the chased male retreating, or quickly dodging to avoid the chasing male.

Distribution and effect of size difference on agonistic behaviours

Fin displays

Fin displays were performed by both males in all trials (frequencies per trial in Table 2). Both mutual fin displays and unilateral fin displays could happen within bouts of displaying, where the males typically performed rapid displays back and forth. In all but one of the trials, all periods within the trial included fin displays from one or both males. The exception was a trial where the males did not interact at all during the first period (5 min). The majority (83%) of fin displays were unilateral. Of the 25 trials, there was one where unilateral displays (from both males) were the only display variable being performed. The proportion of unilateral displays performed by the larger male was tested against the equal-proportions expectation of 0.5, with the result of no significant difference from the expectation (Wilcoxon Signed-Rank test, V = 224, p = 0.10; Fig. 4). The proportion of mutual fin displays initiated by the larger male did not differ significantly from the expectation of 0.5 either

10

(Wilcoxon Signed-Rank test, V = 135, p = 0.51; Fig. 4). Because of the minimal difference in who most often initiated the mutual fin displays (Fig. 4), the mutual fin displays were not included when analysing the effect of within pair size difference. The fin displays recorded as simultaneous were only included when calculating median frequency of total fin displays per trial (Table 2).

Approaches

Both males performed approaches in all trials (frequencies per trial in Table 2). The proportion of approaches performed by the larger male was significantly different from the expected proportion of 0.5 (Wilcoxon Signed-Rank test, V = 226, p = 0.03; Fig. 4).

Chases

Chases were done exclusively by the large male in 17 of 25 trials, exclusively by the small male in two of 25 trials and by both males in four trials (frequencies per trial in Table 2). Two trials had no chases by either male. The proportion of chases performed by the larger male was highly significantly different from the expected proportion of 0.5 (Wilcoxon Signed-Rank test, V = 207, p < 0.001; Fig. 4).

Distribution and effect of size difference on continuously recorded positions

Tube entries

Two trials had no tube entries at all. In the 23 trials where tube entries occurred, they were done exclusively by the large male in 16 of 25 trials, and exclusively by the small male in five of 25 trials (frequencies per trials in Table 2). Tube entries were done by both males in the same trial in only two of the 25 trials. The proportion of tube entries done by the large male was significantly different from the expected proportion of 0.5 (V = 211, p = 0.02, Fig. 4). No tube entries were recorded in relation to disturbance. An example to illustrate this is a situation where the larger male were repeatedly swimming in and out of the tube, but swam in under the outlet when movement outside the aquaria was registered. He would then continue entering the tube when the movement had stopped.

Corner entries

The corner entries were done exclusively by the large male in seven trials, in two trials they were done exclusively by the small male and one trial had no corner entries at all (frequencies per trial in Table 2). The proportion on corner entries done by the large male was significantly different from the expectation of 0.5 (Wilcoxon Signed-Rank test; V = 225, p = 0.03; Fig. 4). When the fish swam back in under the outlets during the trial, it was done both in relation to disturbance from outside the aquarium and when it appeared to be no disturbance.

Effect of relative size difference on behaviour

The effect of within pair size difference (relative to absolute body size of the smaller male) on any of the behaviours, including tube entries and corner entries, was tested using generalized linear models (GLM). Both the proportion of behaviours done by the larger male and the combined large and small frequency of behaviours was tested. The within pair size difference did not have any significant effects on the proportion of agonistic behaviours, tube entries and corner entries done by the large male (Table 3, Fig. 5). Neither did the within pair size difference have any significant effects on the frequency of agonistic behaviours, tube entries and corner entries done by both males combined (Table 4, Fig. 6).

Effect of size difference on area use in aquarium

Analysis of the scan samples for the two males indicated the predicted occurrence of higher association with the lower area for the larger male (Fig. 7). The difference between the large and small male's proportion of scans was tested for both the lower and upper area of the aquarium. There was a significant difference between large and small male in proportion of scans in lower area (Wilcoxon Signed-rank test for paired samples; $V = 307$, $p < 0.001$). There was also a significant difference between the large and small male in proportion of scans in the upper area of the aquarium (Wilcoxon Signed-rank test for paired samples; $V = 57$, $p < 0.005$).

Effect of period on frequency and proportion of behaviours

Repeated measures ANOVA was used to test for differences in frequency and proportion of behaviours between the six time intervals within the trials (from here on referred to as periods). No significant effect of period was found on the proportion of behaviours or corner entries done by the large male (Table 4; Fig. 8). Chases and tube entries were excluded from the analysis of effect of period on proportions done by the large male because of the highly skewed data towards the large male, resulting in very little variation within trials. There was however, a significant effect of period on the frequency of mutual displays, chases, tube entries and corner entries for large and small male combined (Table 5, Fig. 9). There was a clear increase in frequency of chases and tube entries over time, and a decrease in corner entries (Fig. 9 d, e, f). The pattern was not as clear for the frequency of

mutual displays, but there appeared to be a decrease over time (Fig. 9 b). No significant effect of period was found on the frequency of unilateral display or approaches (Table 5, Fig. 9 a, c).

Discussion

In this study, the effect of body size on intrasexual competitive behaviour of male *Chrysiptera cyanea* was tested through a dyadic experimental set up. Both males showed a high overall frequency of visual fin displays, and the frequency did not differ between the males. Frequency of mutual fin displays decreased towards the end of the trial and frequency of chases increased. Larger males performed considerably more chases than smaller males, leading to a one sided escalation and the displacement of the smaller male. Larger males were more successful in monopolizing the available resources and spent more time in the lower area of the aquarium, closer to the potential resources.

There are numerous studies on coral reef fishes relating to effects behind differences in male mating success (e.g Hoelzer 1990; Petersen 1995; Thresher 1983; Kuwamura et al. 2000; Johnson & Hixon 2011). These studies usually focus on observations in the wild, manipulating the availability of nest sites or standardizing nest quality by providing artificial nests. Detailed descriptions of specific behaviours and their development during intrasexual conflicts are rarely included in studies like these, but can however frequently be found for fresh water fish (e.g. Enquist & Jakobsson 1986; Maan et al. 2001; Ladich & Myrberg 2006). This study thus stands out from studies particularly on reef fish as it gives detailed information about behavioural patterns and dynamics during male-male conflict resolution in the damselfish *C. cyanea*.

Relationship between body size and competitive superiority

The results supported the prediction of size-related behavioural asymmetry between competing males and a competitive advantage for the male with the highest RHP (Parker 1974). The strength of an animal is often related to body mass (Parker 1974), while in this study, the measure of body size was total length. Despite using length alone, conflict outcome was clear with the larger male showing competitive superiority. That chases were almost exclusively done by the larger males, could suggest either that the information in the fin displays or the difference in body size (TL) were sufficient to settle the conflict. Thus, this study illustrates the importance of size advantages and display signals when determining success in intrasexual conflicts in the coral reef fish *C. cyanea* and emphasizes the role of size in male-male competition found in fishes in general (Bisazza 1988; Lindström 1988; Bisazza et al. 1996; Terleph 2004; Prenter 2008; Wacker et al. 2012).

Stages of conflict resolution and the effect of time-intervals within trials

The males in this study were performing visual fin displays at a considerably higher frequency than chases. This is in line with theory of conflict resolution (Maynard Smith & Price 1973; Parker 1974), which predicts competitors to use low-risk behaviours to assess the competitor's fighting ability and avoid the risk of injury. The phase of low-risk behaviours is predicted to be followed by more escalated behaviours if the first phase is not sufficient to resolve the conflict (Parker 1974). Ladich and Myrberg (2006) describe in details the development of conflicts in a dyadic lab set up for fresh water fish in their review of agonistic behaviour and acoustic signals. The conflicts start with a phase of low-risk vocal signals, followed by phases of increasingly escalated behaviours more likely to incur injury (Ladich & Myrberg 2006). Escalation could happen from both contestants, or from one of them, leading to the retreat of the other (Ladich & Myrberg 2006). In the present study, the pattern of decrease in frequency of mutual displays (low-cost behaviour) and increase in frequency of chases (more escalated behaviour) over the duration of the trial is in line with the predicted development of the conflict and similar to the descriptions of Ladich & Myrberg (2006). This dynamic with the larger male escalating to chase away the competitor, is what could be predicted to happen in a natural context, based on previous observation of this fish in the wild (Thresher & Moyer 1983; Gronell 1989; personal observations).

Effect of relative size difference on conflict resolution

According to theory, the outcome of a conflict is more predictable when differences in RHP is large (Parker 1974). In such a situation, smaller males would benefit from entering low-risk competition only and withdraw instead of retaliate if escalation occurs, due to their lower RHP and ability to pay the related costs (Enquist & Leimar 1983). This pattern predicted with large differences in RHP was reflected in the results of this study as both males engaged in low-risk fin displays in all trials, while chases were almost exclusively performed by the larger males. In line with this, the results from the present study illustrates that the males managed to resolve the conflict without any escalated physical fight involving both males. However, the mean relative size difference in the present study was less than 10% of the mean overall total length. More importantly, there was no significant relationship between the relative size differences and the degree of competitive advantage for the larger male. This suggests that when other variables are kept equal, a size advantage of less than 10% was enough to cause a considerable increase in the competitive superiority of the larger male, and to settle the conflict without any costly, high-risk fighting.

The effect of relative size difference has been studied in several fish species, as for example the cichlid *Nannacara anomala* (Jakobsson & Enquist 1986), the green swordtail *(Xiphophorus helleri;* Beaugrand 1996) and reef fish species such as the damselfish *Pomacentrus amboinensis* (Poulos & McCormick 2014). In the study *N. anomala*, fight duration and the probability of escalation increased when the weight difference between the contestants decreased (Jakobsson & Enquist 1986). In the green swordtail, size only overruled effects of prior social experience or residency when the asymmetry was larger than 20% (Beaugrand 1996). Both of these studies thus represent effects of relative size differences that were not found in the present study. The study on *P. amboinensis* however, showed that size had a strong influence on the outcome of intrasexual interactions with a size advantage of only 7% promoting success (Poulos & McCormick 2014). Notably, the subjects of the conflicts in this study was in an early life stage (newly metamorphosed from larvae), unlike the fish in the present study. Still, the study on the damselfish *P. amboinensis* represent an effect of relative size difference similar to that of the present study.

Monopolization of resources and displacement

The larger male was predicted to monopolize the available resource and displace the smaller male to the extent possible within the confined space of the aquarium. The monopolization of the resource by the larger male was clearly reflected in his proportion of tube entries compared to the smaller male. The frequency of tube entries also increased towards the end of the trial, which suggest that the utilization of the tube was a result of the established competitive relationship. A study on competition for territories in threespine sticklebacks (*Gasterosteus aculeatus*; Rowland 1989) bears several similarities with the present study. Male threespine sticklebacks differing in size were paired against each other in an experimental aquarium (Rowland 1989). The larger male (weight) in each contest generally dominated and nested, while the smaller male was hiding or hovering at the water surface (Rowland 1989). It is unclear if the PVC-tube in the present study were assessed as a potential nest by the males or not, but the patterns in both studies still follow the same line.

Unlike the tube, the space beneath an outlet (corner) was apparently used predominantly as shelter when any disturbance occurred. Interestingly, the larger male had a significantly higher proportion of corner entries as well and thus seemed to monopolize this space also. This was not predicted, as the function of the outlet as a shelter did not become apparent before observing them in the experimental aquarium. However, a larger male could be expected to monopolize a shelter as well,

which makes this result particularly interesting considering there were two outlets providing shelter, and they were in addition spaced some distance apart. The frequency of the corner entries for both males combined decreased throughout the trial. However, this might not necessarily provide any information about the competitive relationship, but may rather have reflected a habituation to repeated disturbance.

The results from difference in area use between the two males supported the prediction of the larger male associating more with the area including the potential resources (tube as nest site or shelter and substrate for possibility of finding food). In addition to the larger male spending more time in the lower part of the aquarium compared to the smaller male, he also spent significantly less time in the upper area compared to the smaller male. The results suggest that the larger male has the ability to displace the smaller male and stay safe closer to the bottom. The unilateral escalation in behaviour indicated this attempt from the larger male to completely displace the smaller male from the area. Because of the confined area of the experimental tank, the smaller male had limited options of retreating in this situation. However, the difference in area use showed an asymmetry between the males in which part of the aquarium they spent the most time. This may indicate the smaller male attempting to avoid the larger male. In addition, some chases from the larger male resulted in the smaller male actually breaking the water. The *C. cyanea* is rarely seen near the surface in the wild (personal observations), probably because this would make them more vulnerable to predation. These results thus suggest the larger male were successful in controlling an area of resources, and the successful displacement of the smaller male indicates the threat posed to the smaller fish by an eventual escalation from the larger male.

Possible motivation behind the conflict

Thresher & Moyer (1983) suggested from their results, including strong correlation between body size and mating success, observations of vigorous male-male competition and female *C. cyanea* spawning with locally dominant males only, that as a consequence, their sexual selection occurs entirely through the agency of inter-male competition for dominance. Thus, they suggest that males compete for dominance. Gronell (1989) reported anecdotal evidence for female nest preference which may suggest that the males are competing for high quality nests. Evidence of male-male competition for spawning sites preferred by females has been found in other coral reef fish species as well, for example the blue-headed wrasse (Warner & Schultz 1992). Females of this species preferred to spawn at particular sites (Warner & Schultz 1992). The males would compete for

territories at these sites, with the largest males defending the best sites and so gaining most mates (Warner & Schultz 1992). The skew in mating success towards larger males found in blue-headed wrasse was similar to that found in the *C. cyanea* by Thresher & Moyer (1983) and Gronell (1989). This suggests that males may be competing for specific nest sites. One could argue, however, that in territorial species like *C. cyanea*, with several males living as neighbours, a dominance hierarchy is established and kept relatively stable through frequent interaction and competition. This would mean that the competition for dominance and the competition for specific nest sites might be inter-related, as the dominant male would get priority access to the resource. Hoelzer (1990) found a relationship in the Cortez damselfish (*Stegastes rectifraenum*) between body size and reproductive success when nest sites were not manipulated. This relationship disappeared however, when the quality of nest sites were standardized, suggesting the correlation between male reproductive success and body size is produced indirectly by female choice for high quality nest sites in combination with male competition for high quality territories (Hoelzer 1990). However, both Gronell (1989) and Thresher & Moyer (1983) provided the C. cyanea males in their studies with artificial nests, and still got the results of considerable skew in mating success towards the larger males. This suggest that there are different mechanisms behind the relationship between body size and mating success in C. cyanea.

Conclusion

The results of this study emphasize that body size affects the outcome of an intrasexual conflict in the coral reef fish *Chrysiptera cyanea*. The behavioural pattern within a trial supported the theory of animals avoiding unnecessary risks when competing, by using ritualized conflict behaviour to assess the opponent's fighting ability. Behavioural asymmetries are usually predicted to vary with the degree of size asymmetry, but were in this study rather consistent, despite some variation in within pair size differences and an overall small within pair size difference. This study contributes to the understanding of conflict dynamics and the importance of body size in a coral reef fish. For further studies, I would suggest observations of *C. cyanea* in the wild focusing on competitive behaviour in relation to their social context, morphological traits and resources obtained. Building on the results of these experimental trials, this could provide a greater understanding of the causes and consequences of intrasexual competition in this species.

References

Amundsen, T. (2003). Fishes as models in studies of sexual selection and parental care. *Journal of Fish Biology*, *63*(s1), 17-52.

Archer, J. (1988). The behavioural biology of aggression (Vol. 1). CUP Archive.

Beaugrand, J. P., Payette, D., & Goulet, C. (1996). Conflict outcome in male green swordtail fish dyads (*Xiphophorus helleri*): interaction of body size, prior dominance/subordination experience, and prior residency. *Behaviour*, *133*(3), 303-319.

Beaugrand, J. P. (1997). Resolution of agonistic conflicts in dyads of acquainted green swordtails (Xiphophorus helleri): a game with perfect information. *Behavioural processes*, *41*(1), 79-96.

Bisazza, A., & Marconato, A. (1988). Female mate choice, male-male competition and parental care in the river bullhead, *Cottus gobio* L. (Pisces, Cottidae). *Animal Behaviour*, *36*(5), 1352-1360.

Bisazza, A., Novarini, N., & Pilastro, A. (1996). Male body size and male-male competition: Interspecific variation in poeciliid fishes. *Italian Journal of Zoology*, *63*(4), 365-369.

Bolund, E., Schielzeth, H., & Forstmeier, W. (2007). Intrasexual competition in zebra finches, the role of beak colour and body size. *Animal Behaviour*, *74*(4), 715-724.

Brown, J. L. (1964). The evolution of diversity in avian territorial systems. *The Wilson Bulletin*, 160-169.

Caldwell, R. L., & Dingle, J. (1979). The influence of size differential on agonistic encounters in the mantis shrimp, *Gonodactylus viridis*. *Behaviour*,*69*(3), 255-264.

Chelliah, K., & Sukumar, R. (2013). The role of tusks, musth and body size in male–male competition among Asian elephants, *Elephas maximus*. *Animal Behaviour*, *86*(6), 1207-1214.

Clarke, R. D. (1977). Habitat distribution and species diversity of chaetodontid and pomacentrid fishes near Bimini, Bahamas. *Marine Biology*, *40*(3), 277-289.

Clarke, T. A. (1970). Territorial behavior and population dynamics of a pomacentrid fish, the garibaldi, *Hypsypops rubicunda*. *Ecological monographs*, 189-212.

Clutton-Brock, T. H., & Albon, S. D. (1979). The roaring of red deer and the evolution of honest advertisement. *Behaviour*, *69*(3), 145-170.

Connell, J. H. (1978). Diversity in tropical rain forests and coral reefs. *Science*, *199*(4335), 1302-1310.

Darwin, C. (1871). The descent of man and selection in relation to sex. *London: Murray*.

Davies, N. B. (1978). Territorial defence in the speckled wood butterfly (*Pararge aegeria*): the resident always wins. *Animal Behaviour*, *26*, 138-147.

Enquist, M., & Jakobsson, S. (1986). Decision making and assessment in the fighting behaviour of *Nannacara anomala* (Cichlidae, Pisces). *Ethology*, *72*(2), 143-153.

Enquist, M., & Leimar, O. (1983). Evolution of fighting behaviour: decision rules and assessment of relative strength. *Journal of theoretical Biology*, *102*(3), 387-410.

Evans, L. T. (1936). A study of a social hierarchy in the lizard, *Anolis carolinensis*. *The Pedagogical Seminary and Journal of Genetic Psychology*, *48*(1), 88-111.

Grant, J. W. (1993). Whether or not to defend? The influence of resource distribution. *Marine & Freshwater Behaviour & Physiology*, *23*(1-4), 137-153.

Greenhouse, S. W., & Geisser, S. (1959). On methods in the analysis of profile data. *Psychometrika*, *24*(2), 95-112.

Gronell, A. M. (1989). Visiting behaviour by females of the sexually dichromatic damselfish, Chrysiptera cyanea (Teleostei: Pomacentridae): a probable method of assessing male quality. *Ethology*, *81*(2), 89-122.

Harborne, A. R. (2013). The ecology, behaviour and physiology of fishes on coral reef flats, and the potential impacts of climate change. *Journal of fish biology*, *83*(3), 417-447.

Hagelin, J. C. (2002). The kinds of traits involved in male—male competition: a comparison of plumage, behavior, and body size in quail. *Behavioral Ecology*, *13*(1), 32-41.

Higham, J. P., Heistermann, M., & Maestripieri, D. (2011). The energetics of male–male endurance rivalry in free-ranging rhesus macaques, *Macaca mulatta*. *Animal Behaviour*, *81*(5), 1001-1007.

Hau, M., Wikelski, M., Soma, K. K., & Wingfield, J. C. (2000). Testosterone and year-round territorial aggression in a tropical bird. *General and Comparative Endocrinology*, *117*(1), 20-33.

Hoelzer, G. A. (1990). Male-male competition and female choice in the Cortez damselfish, *Stegastes rectifraenum*. *Animal Behaviour*, *40*(2), 339-349.

Itzkowitz, M. (1990). Heterospecific intruders, territorial defence and reproductive success in the beaugregory damselfish. *Journal of Experimental Marine Biology and Ecology*, *140*(1), 49-59.

Jaroensutasinee, M., & Jaroensutasinee, K. (2003). Type of intruder and reproductive phase influence male territorial defence in wild-caught Siamese fighting fish. *Behavioural processes*, *64*(1), 23-29.

Johnson, D. W., & Hixon, M. A. (2011). Sexual and lifetime selection on body size in a marine fish: the importance of life-history trade-offs. *Journal of evolutionary biology*, *24*(8), 1653-1663.

Johnsson, J. I., Nöbbelin, F., & Bohlin, T. (1999). Territorial competition among wild brown trout fry: effects of ownership and body size. *Journal of Fish Biology*, *54*(2), 469-472.

Jones, J. C., & Reynolds, J. D. (1999). The influence of oxygen stress on female choice for male nest structure in the common goby. *Animal Behaviour*, *57*(1), 189-196.

Karino, K. (1999). Growth or reproduction: Intrasexual competition in a colonial damselfish *Stegastes nigricans*. *Journal of Ethology*, *17*(1), 57-62.

Kaufmann, J. H. (1983). On the definitions and functions of dominance and territoriality. *Biological Reviews*, *58*(1), 1-20.

Kuwamura, T., Karino, K., & Nakashima, Y. (2000). Male morphological characteristics and mating success in a protogynous coral reef fish, Halichoeres melanurus. *Journal of Ethology*, *18*(1), 17-23.

Ladich, F., & Myrberg, A. A. (2006). Agonistic behavior and acoustic communication. *Communication in fishes*, *1*, 121-148.

Lindström, K. (1988). Male-male competition for nest sites in the sand goby, *Pomatoschistus minutus*. *Oikos*, 67-73.

Low, R. M. (1971). Interspecific territoriality in a pomacentrid reef fish, *Pomacentrus flavicauda* Whitley. *Ecology*, 648-654.

Maan, M. E., Groothuis, T. G., & Wittenberg, J. (2001). Escalated fighting despite predictors of conflict outcome: solving the paradox in a South American cichlid fish. *Animal Behaviour*, *62*(4), 623-634.

Madsen, T., Shine, R., Loman, J., & Håkansson, T. (1993). Determinants of mating success in male adders, *Vipera berus*. *Animal Behaviour*, *45*(3), 491-499.

Maher, C. R., & Lott, D. F. (2000). A review of ecological determinants of territoriality within vertebrate species. *The American Midland Naturalist*, *143*(1), 1-29.

Marler, C. A., Walsberg, G., White, M. L., Moore, M., & Marler, C. A. (1995). Increased energy expenditure due to increased territorial defense in male lizards after phenotypic manipulation. *Behavioral Ecology and Sociobiology*, *37*(4), 225-231.

Maynard Smith, J., & Price, G. R. (1973). The Logic of Animal Conflict. *Nature*, *246*, 15.

Moretz, J. A. (2003). Aggression and RHP in the northern swordtail fish, *Xiphophorus cortezi*: the relationship between size and contest dynamics in male–male competition. *Ethology*, *109*(12), 995-1008.

Morgan, I., & Kramer, D. (2004). The social organization of adult blue tangs, *Acanthurus coeruleus*, on a fringing reef, Barbados, West Indies. *Environmental Biology of Fishes*, *71*(3), 261-273.

Munday, P. L., & Wilson, S. K. (1997). Comparative efficacy of clove oil and other chemicals in anaesthetization of *Pomacentrus amboinensis*, a coral reef fish. *Journal of Fish Biology*, *51*(5), 931-938.

Noble, G. K. (1939). The experimental animal from the naturalist's point of view. *American Naturalist*, 113-126.

Ostfeld, R. S. (1990). The ecology of territoriality in small mammals. *Trends in Ecology & Evolution*, *5*(12), 411-415.

Panhuis, T. M., & Wilkinson, G. S. (1999). Exaggerated male eye span influences contest outcome in stalk-eyed flies (Diopsidae). *Behavioral Ecology and Sociobiology*, *46*(4), 221-227.

Parker, G. A. (1974). Assessment strategy and the evolution of fighting behaviour. *Journal of theoretical Biology*, *47*(1), 223-243.

Petersen, C. W. (1995). Male mating success and female choice in permanently territorial damselfishes. *Bulletin of marine Science*, *57*(3), 690-704.

Peterson, G., & Hardy, I. C. (1996). The importance of being larger: parasitoid intruder–owner contests and their implications for clutch size. *Animal Behaviour*, *51*(6), 1363-1373.

Poulos, D. E., & McCormick, M. I. (2014). Who wins in the battle for space? The importance of priority, behavioural history and size. *Animal Behaviour*, *90*, 305-314.

Prenter, J., Taylor, P. W., & Elwood, R. W. (2008). Large body size for winning and large swords for winning quickly in swordtail males, *Xiphophorus helleri*. *Animal Behaviour*, *75*(6), 1981-1987.

Robertson, D. R., & Warner, R. R. Sexual Patterns in the Labroid Fishes of the Western Caribbean, II: The Parrotfishes (Scaridae). *Smithsonian Contributions to Zoology*; no 255.

Rowland, W. J. (1989). The effects of body size, aggression and nuptial coloration on competition for territories in male threespine sticklebacks, *Gasterosteus aculeatus*. *Animal Behaviour*, *37*, 282-289.

Sale, P. F. (1977). Maintenance of high diversity in coral reef fish communities. *American Naturalist*, 337-359.

Sargent, R. C. (1982). Territory quality, male quality, courtship intrusions, and female nest-choice in the threespine stickleback, *Gasterosteus aculeatus*. *Animal Behaviour*, *30*(2), 364-374.

Sebens, K. P. (1987). The ecology of indeterminate growth in animals. *Annual Review of Ecology and Systematics*, 371-407.

Smith, J. M., & Parker, G. A. (1976). The logic of asymmetric contests. *Animal behaviour*, *24*(1), 159-175.

Terleph, T. A. (2004). The function of agonistic display behaviours in *Gnathonemus petersii*. *Journal of fish biology*, *64*(5), 1373-1385.

Thresher, R. E. (1983). Habitat effects on reproductive success in the coral reef fish, *Acanthochromis polyacanthus* (Pomacentridae). *Ecology*, 1184-1199.

Thresher, R.E. and J.T. Moyer (1983). Male success, courtship complexity and patterns of sexual selection in three congeneric species of sexually monochromatic and dichromatic damselfishes (Pisces: Pomacentridae). *Animal Behaviour*, *31*, 113-127.

Tokarz, R. R. (1985). Body size as a factor determining dominance in staged agonistic encounters between male brown anoles (*Anolis sagrei*). *Animal Behaviour*, *33*(3), 746-753.

Tricas, T. C. (1985). The economics of foraging in coral-feeding butterflyfishes of Hawaii. In *Proc. 5th Int. Symp. Coral Reefs* (Vol. 5, pp. 409-414).

Unger, L. M. (1983). Nest defence by deceit in the fathead minnow, *Pimephales promelas*. *Behavioral Ecology and Sociobiology*, *13*(2), 125-130.

Wacker, S., de Jong, K., Forsgren, E., & Amundsen, T. (2012). Large males fight and court more across a range of social environments: an experiment on the two spotted goby *Gobiusculus flavescens*. *Journal of fish biology*, *81*(1), 21-34.

Warner, R. R., & Schultz, E. T. (1992). Sexual selection and male characteristics in the bluehead wrasse, *Thalassoma bifasciatum*: mating site acquisition, mating site defense, and female choice. *Evolution*, 1421-1442.

Wong, M. Y., Buston, P. M., Munday, P. L., & Jones, G. P. (2007). The threat of punishment enforces peaceful cooperation and stabilizes queues in a coral-reef fish. *Proceedings of the Royal Society of London B: Biological Sciences*, *274*(1613), 1093-1099.

Östlund-Nilsson, S. (2000). Are nest characters of importance when choosing a male in the fifteen-spined stickleback (*Spinachia spinachia*)? *Behavioral Ecology and Sociobiology*, *48*(3), 229-235.

Tables

Table 1. Length (TL), weight and days spent in lab before trial of male *Chrysiptera cyanea* in a study of size-related intraspecific competition. Males were selected to differ in size.

	Large male		Small male		Within trial contrast	
	mean ± SD	range	mean ± SD	range	mean ± SD	range
Length (TL) (mm)	63.3 ± 2.72	60.5 – 71	58.4 ± 3.21	45 – 64	4.88 ± 3.98	3 – 8 (23)ɫ
Weight (g)	4.25 ± 0.69	3.41 – 5.83	3.59 ± 0.43	2.68 – 4.41	0.67 ± 0.52	-0.34 – 1.97
Days in lab	8.52 ± 5.31	4 – 25	8.68 ± 5.67	4 – 25	-0.16 ± 5.05	-15 - 15

ɫ Outlier in parenthesis included in calculations of mean ± SD and the analysis.

Table 2. Frequency of behaviours, tube entries and corner entries per trial (30 min) reported as median and interquartile range (IQR) for the large and small male in a study of size-related intrasexual competition in the *Chrysiptera cyanea.* The sum of unilateral, mutual and simultaneous fin displays was included in to give an impression of the overall frequency of the different behaviours.

	Fin displays (total)		**Approaches**		**Chases**		**Tube entries**		**Corner entries**	
	Median	IQR	Median	IQR	Median	IQR	Median	IQR	Median	IQR
Large male	119	115	41	51	4	14	5	25	8	19
Small male	62	122	13	21	0	0	0	1	3	7

Table 3. Effect of within pair size difference on the proportion done by the large male out of the total number of each of the agonistic behaviours, tube entries and corner entries in a study of size-related intrasexual competition in the *Chrysiptera cyanea.* The within pair size difference was measured as difference between large and small male in TL relative to absolute TL of the smaller male. Estimates from separate GLMs per behaviour.

	Intercept			Slope		
	Estimate ± SE	t	p	Estimate ± SE	t	p
Unilateral fin displays	0.08 ± 0.45	0.19	0.85	3.58 ± 5.53	0.65	0.52
Approaches	0.52 ± 0.44	1.20	0.25	2.37 ± 4.44	0.53	0.60
Chases	-4.13 ± 3.71	-1.11	0.28	92.26 ± 61.20	1.51	0.15
Tube entries	-4.22 ± 3.57	-1.18	0.25	87.96 ± 58.38	1.51	0.15
Corner entries	0.82 ± 0.46	1.80	0.09	0.50 ± 3.76	0.13	0.90

Table 4. Effect of within pair size difference on frequency of agonistic behaviours, tube entries and corner entries for large and small male combined in a study of size-related intrasexual competition in the *Chrysiptera cyanea.* The within pair size difference was measured as difference between large and small male in TL relative to absolute TL of the smaller male. Estimates from separate GLMs per behaviour.

	Intercept			Relative size		
	Estimate ± SE	t	P	Estimate ± SE	t	P
Unilateral fin displays	5.27 ± 0.22	24.04	< 0.001	-3.19 ± 2.51	-1.27	0.22
Approaches	4.26 ± 0.10	41.34	< 0.001	-0.83 ± 0.93	-0.89	0.38
Chases	2.89 ± 0.44	6.56	< 0.001	-3.16 ± 5.03	-0.63	0.54
Tube entries	2.50 ± 0.24	10.52	<0.001	1.93 ± 1.41	1.36	0.19
Corner entries	2.87 ± 0.24	11.92	<0.001	0.61 ± 1.77	0.35	0.73

Table 5. Effect of period within trial on the proportion done by the large male out of the total number of agonistic behaviours, corner entries and tube entries in a study of size-related intrasexual competition in *Chrysiptera cyanea.* Test statistics after applied Greenhouse-Geisser correction are shown where Mauchley's test of sphericity indicated violation of the assumption of sphericity (see appendix for test statistics from Mauchley's test of sphericity and epsilon values from the Greenhouse-Geisser correction).

	N	df	F	P
Mutual displays	7	5, 6	1.26	0.31
Unilateral displays ɫ	24	3.63, 23	1.22	0.31
Approaches ɫ	24	3.23, 23	1.25	0.30
Corner entries	7	5, 6	1.31	0.29

ɫ Values with Greenhouse-Geisser correction

Table 6. Effect of period within trial on frequency of behaviours, corner entries and tube entries done by large and small male combined in a study of size-related intrasexual competition in *Chrysiptera cyanea.* Test statistics after applied Greenhouse-Geisser correction are shown where Mauchley's test of sphericity indicated violation of the assumption of sphericity (see appendix for test statistics from Mauchley's test of sphericity and epsilon values from the Greenhouse-Geisser correction).

	N	df	F	P
Mutual displays	25	5	3.02	**0.01**
Unilateral displays ⱦ	25	3.34, 24	2.24	0.08
Approaches ⱦ	25	3.52, 24	2.25	0.08
Chases ⱦ	25	2.99, 24	5.95	**< 0.05**
Tube entries ⱦ	25	3.31, 24	14.94	**< 0.001**
Corner entries ⱦ	25	3.41, 24	10.56	**< 0.001**

ⱦ Values with Greenhouse-Geisser correction

Figures
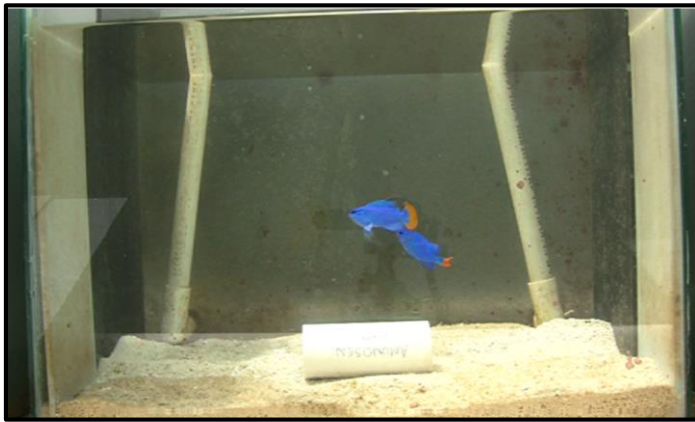
Figure 1. The front view of the experimental aquarium set-up in a study of size-related intrasexual competition in *Chrysiptera cyanea*. The aquarium was equipped with a PVC-tube in the middle, two outlets on each side and sand as substrate.
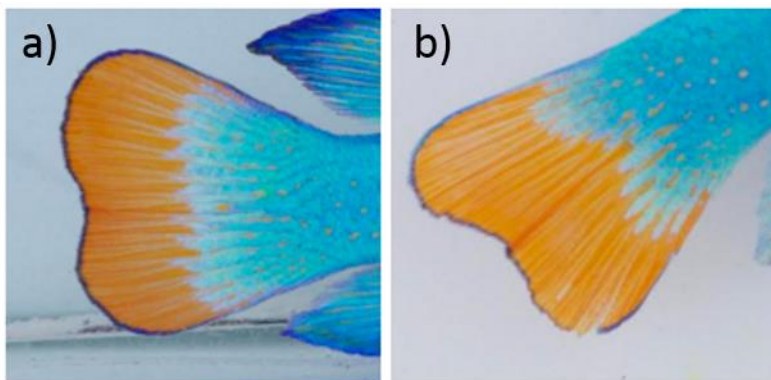

Figure 2. Photos of tail colouration subjectively measured as a) 3 and b) 3- taken in a standard photo aquarium in a study of size-related intrasexual competition in *Chrysiptera cyanea*. The difference between these two measures is one step on the 5-graded scale for subjective measurement, and translates to a difference of 0.33.

Figure 3. Photo of two males in the wild where one (right) is performing a fully fanned tail fin display in a study of size-related intrasexual competition in *Chrysiptera cyanea.*
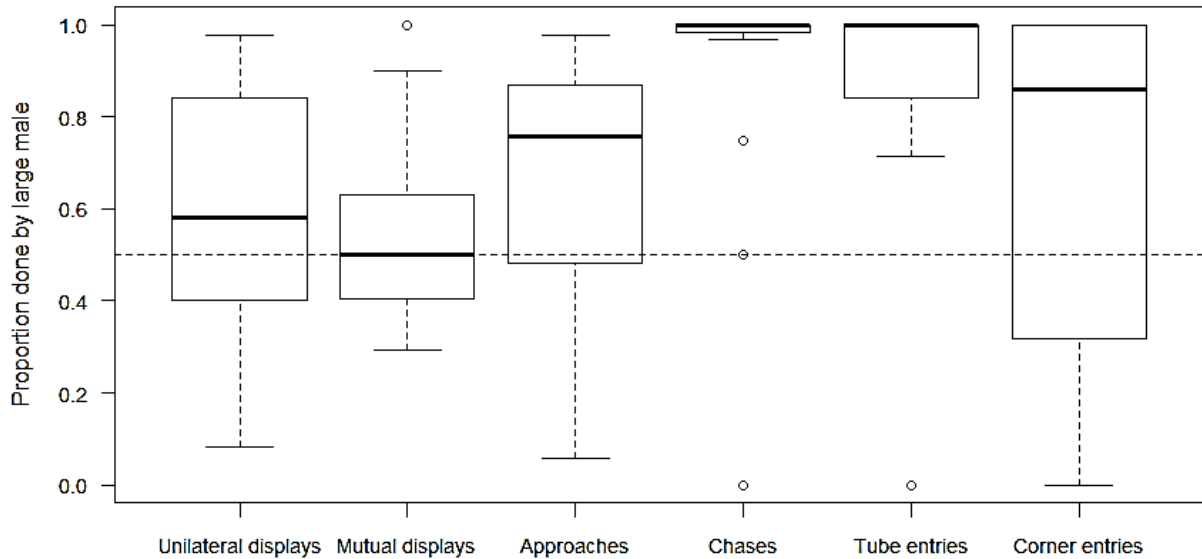
Figure 4. Box plots for the proportion done by the large male out of the total number of each of the agonistic behaviours, tube entries and corner entries in a study of size-related intrasexual competition in the *Chrysiptera cyanea*. The black line within each box represents the median, boxes represent the interquartile range, whiskers represent the maximum and minimum values and open circles show the outliers. The dashed line represent the expected proportion for the large male when there is no difference between the large and small male proportion (0.5).
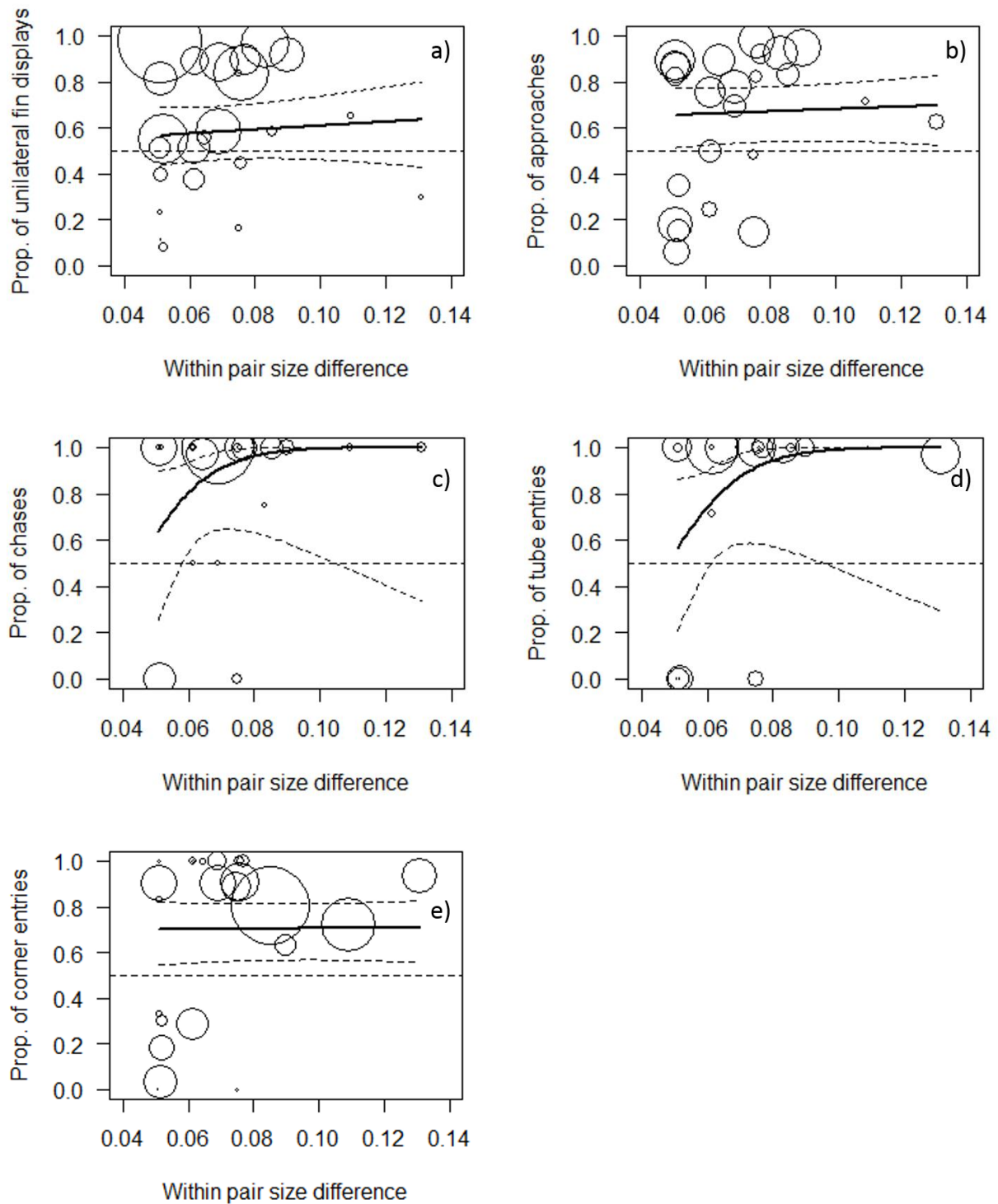
Figure 5. Effect of within pair size difference on the proportion done by the large male out of the total number of a) unilateral fin displays, b) approaches, c) chases, d) tube entries and e) corner entries in a study of size-related intrasexual competition in the *Chrysiptera cyanea*. The within pair size difference was measured as difference between large and small male in TL relative to absolute TL of the smaller male. The one outlier with a very high difference in TL (Table 1) were not included in the figures. The size of points indicates numbers of observations behind the point (sizes not comparable among panels). Solid lines represent relationships estimated by generalised linear models and dashed lines their 95% confidence intervals.
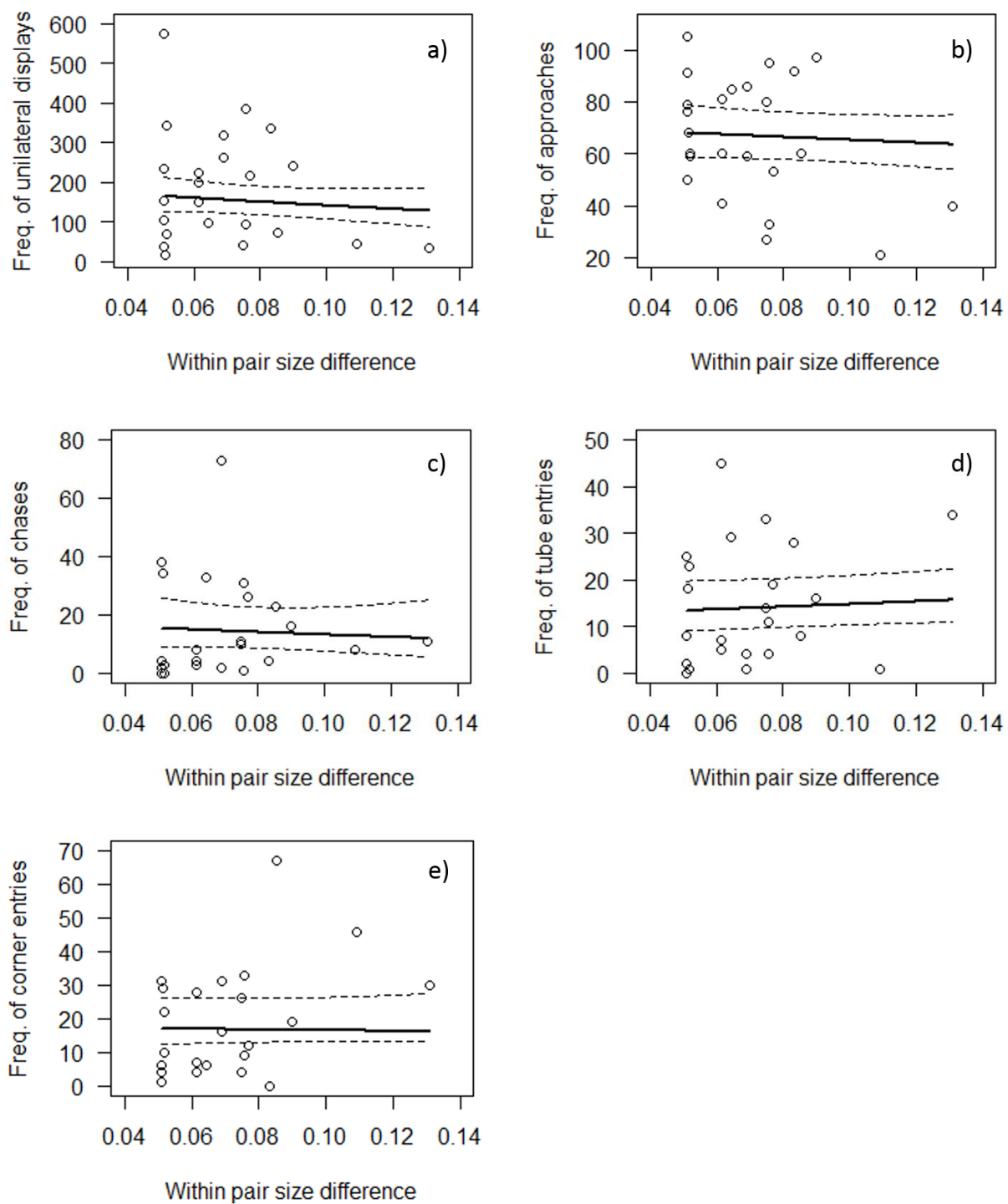
Figure 6. Effect of within pair size difference on frequency of a) unilateral fin displays, b) approaches, c) chases, d) tube entries and e) corner entries done by the large and small male combined in a study of size-related intrasexual competition in the *Chrysiptera cyanea*. The within pair size difference was measured as difference between large and small male in TL relative to absolute TL of the smaller male. The one outlier with a very high difference in TL (Table 1) were not included in the figures. Solid lines represent relationships estimated by generalised linear models and dashed lines their 95% confidence intervals.
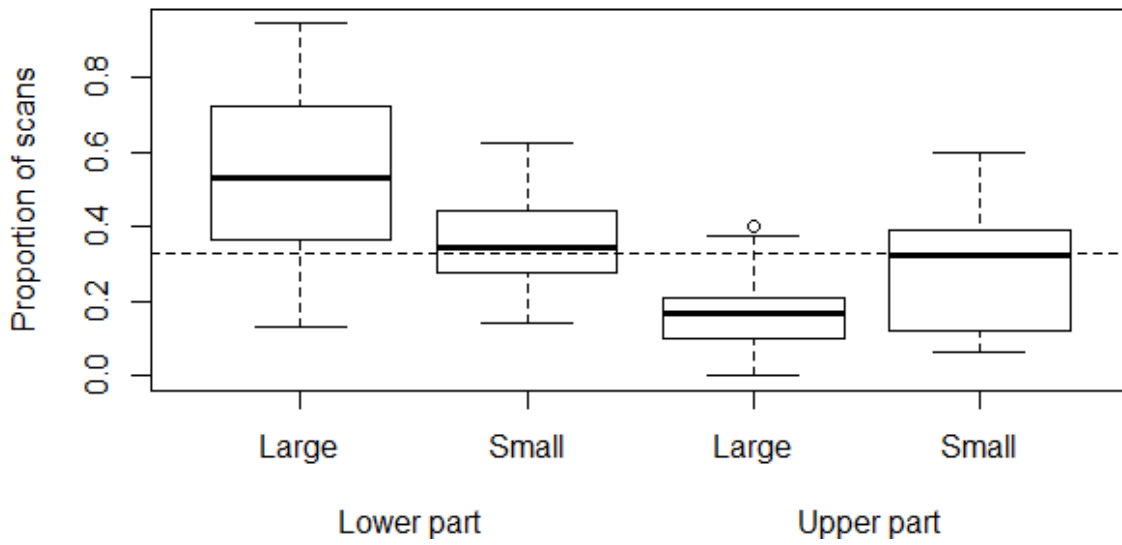
Figure 7. Difference in area use between the large and small male in a study of size-related intrasexual competition in the *Chrysiptera cyanea*. The boxplots represent the proportion of scans for the large and the small male, respectively, in the lower and upper part of the aquarium. The dashed line represents the proportion of scans expected by equal distribution between the three parts (0.33).
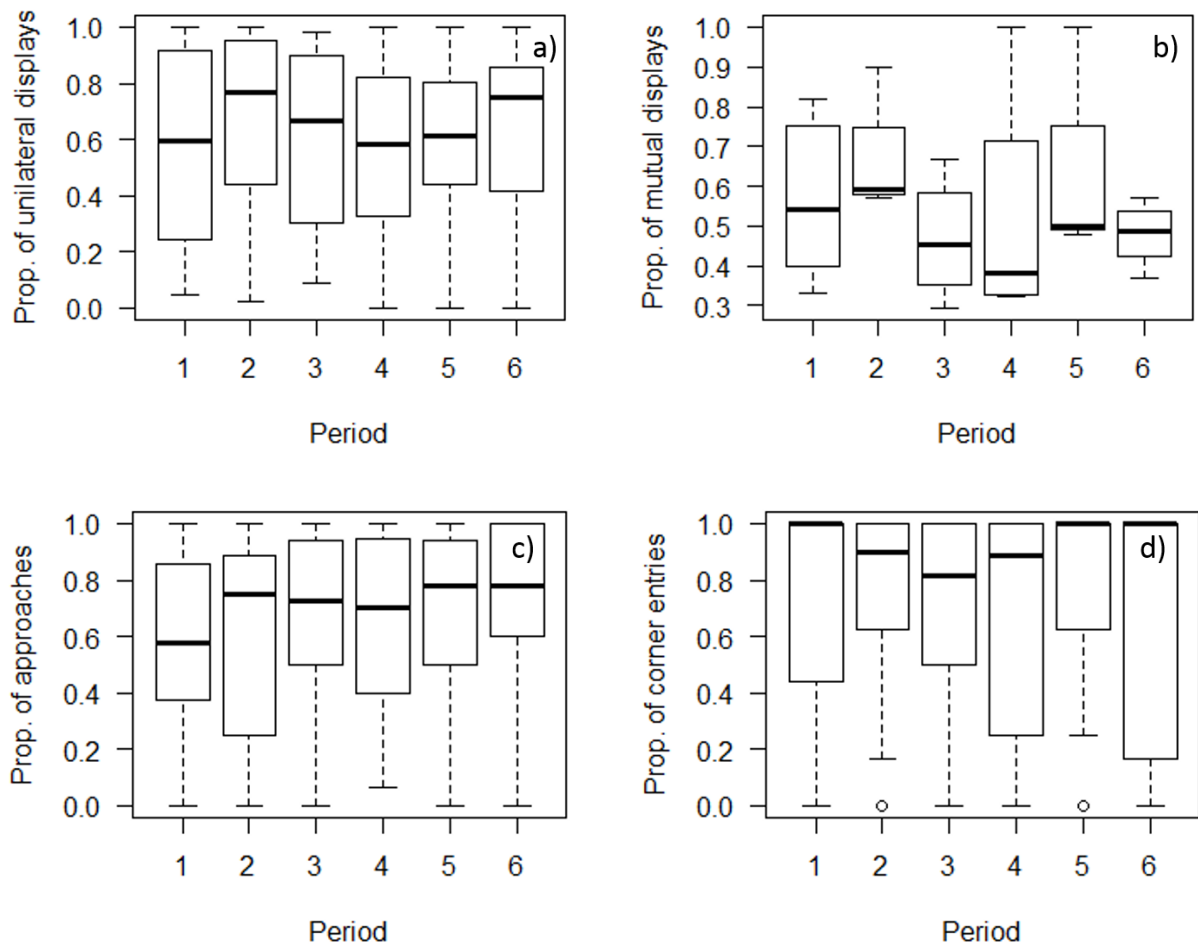
Figure 8. The proportion done by the large male out of the total number of of a) unilateral displays, b) mutual displays, c) approaches and d) corner entries within each of the six time intervals of the trials (periods) in a study of size-related intrasexual competition in *Chrysiptera cyanea.*
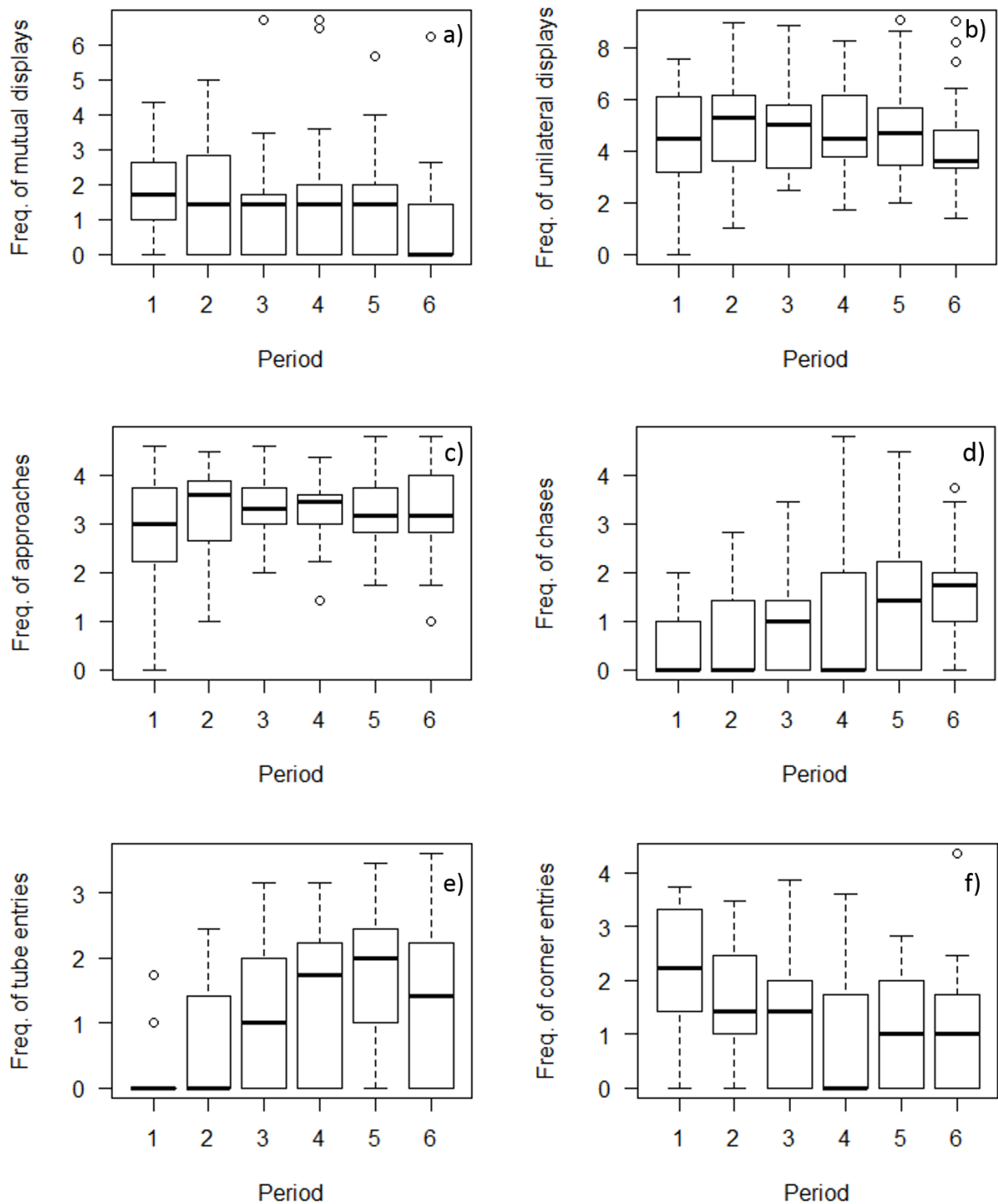
Figure 9. Frequencies of a) mutual displays, b) unilateral displays, c) approaches, d) chases, e) tube entries and f) corner entries done within each of the six time intervals of the trials (periods) by large and small male combined in a study of size-related intrasexual competition in *Chrysiptera cyanea*. The count data used in the boxplots were square root transformed to satisfy the assumption of normality of the residuals when used in ANOVA.