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## **DOCTOR OF PHILOSOPHY**

### **The behavioural ecology of aggression in Lake Malawi haplochromine cichlid fish**

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**The behavioural ecology of aggression in Lake Malawi haplochromine  
cichlid fish**

**PhD Thesis**

Submitted for the degree of

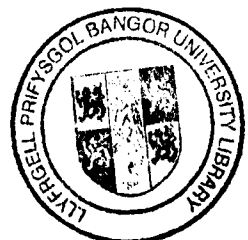
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from the  
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by

**Gavan M. Cooke B.Sc**

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Gavan M. Cooke

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## Abstract

Aggression is ubiquitous in the animal kingdom and a major field of investigation within the area of behavioural ecology. The haplochromine cichlid fish have been used extensively in studies relating not only to the behavioural ecology of aggression but also with respect to how aggression may play a role in the spectacular diversity found within the African Great Lakes.

In the first half of this thesis, I investigate the nature of aggression within the rock dwelling fish of Lake Malawi. Aggression biases within polymorphic populations are initially investigated with field work determining the consequences of rare morph advantage. This is followed by experiments determining which cues males and females may use; lastly a pair of recently diverged allopatric species is studied to determine what may happen should secondary contact occur.

The second half of this thesis focuses on the behavioral ecology of female aggression. Females exhibiting the ancestral condition of post brood care are compared to a species with the more derived condition of no post release care. Non-maternal aggression is investigated with comparisons of the type of behavior used by males and females. When and why females are aggressive is also investigated.

The results of this thesis suggest that overall a common morph bias may exist in some populations and the consequences are manifested in a rare morph advantage. Colour is important in aggression biases but potentially not limited to the dorsal region. Females and males use different kinds of aggressive behaviors and brooding females are able to vary levels of aggression towards different types of threat.

Figure and Tables list.....	3
<b>Chapter 1 Introduction</b> .....	6
Background to the cichlid fish .....	6
Thesis themes.....	20
Aggression and cichlids .....	22
Polymorphism and intraspecific aggression .....	26
Interspecific competition and aggression.....	32
Maternal aggression.....	35
Non-maternal female aggression .....	38
Conclusions.....	40
Thesis outline.....	43
References.....	45
<b>Chapter 2</b> .....	58
<i>Aggression and intraspecific colour polymorphism in haplochromine cichlid fish</i> ....	58
Abstract.....	58
Introduction.....	59
Aims.....	65
Methods.....	65
Data Analysis .....	72
Results.....	72
Discussion .....	78
References.....	84
Appendix.....	87
<b>Chapter 3</b> .....	92
<i>Aggression biases in, and cues used by, geographically isolated cichlid populations</i> 92	
Abstract.....	92
Introduction.....	93
Aims.....	95
Materials and Methods.....	95
Data analysis .....	104
Results.....	104
Discussion .....	111
References.....	116
Appendix.....	118
<b>Chapter 4: Maternal aggression in Malawian cichlids</b> .....	120
Abstract.....	120
Introduction.....	121

Aims.....	124
Materials and methods .....	124
Data analysis.....	129
Results.....	129
Aggression level correlates relating to parental investment theory .....	138
Discussion.....	140
References.....	147
Appendix.....	150
<b>Chapter 5: <i>Female aggression in Malawian mbuna cichlid fish</i></b> .....	151
Abstract.....	151
Introduction.....	152
Aim .....	156
Materials and Methods.....	157
Data analysis .....	162
Results.....	162
Discussion.....	166
References.....	169
Appendix.....	172
<b>Chapter 6</b> .....	174
<i>General discussion, main findings and ideas for future research</i> .....	174
General discussion .....	174
Main findings and ideas for future research .....	180
References.....	185

## Figure and Tables list

<b>Figure 1</b> Photographs of the species, and morphs within species, used in this chapter. Photographs taken by A. Tyers except for the Nkhata Bay O morph male, taken by A. Smith.....	68
<b>Figure 2</b> Mean number of aggressive behaviours (and 95% confidence limits) given to a rare morph intruder or a common morph intruder by the <i>P. zebra</i> focal common morph males. N = 9 focal fish used. ....	72
<b>Figure 3</b> Mean number of aggressive behaviours given to two intruders (one BB morph and one OB morph) by two <i>P. zebra</i> focal types. Error bars = 95% confidence limits. ....	73
<b>Figure 4</b> a) A comparison of the proportion of the two morphs in the general population with the proportion of the two morphs among territorial males found that W morph territorial males are more common than would be expected. b) Mean territory distances for B and W territorial males for each type of neighbouring morph. Error bars show 95% confidence intervals. c) A comparison of grazing behaviour between morphs found that both non-territorial and territorial rare morph (W) fish grazed significantly more than their common morph (B) counterparts. Error bars show 95% confidence intervals. ....	76
<b>Figure 5</b> Median number of aggressive behaviours received from heterospecifics and given towards heterospecifics by territorial males of each morph. Although a trend is seen regarding greater aggression received by B males from heterospecifics, there was no significant difference. Error bars show interquartile range .....	77
<b>Figure 6</b> Aggressive behaviours directed towards territorial intruders of each morph, by territorial <i>P. callainos</i> males. Observations showed a significant own morph aggression bias by the B morph and no significant bias by the W morph. Error bars show interquartile range.....	78
<b>Figure 7</b> Location of study species used in this chapter. A) Male <i>P. emmiltos</i> , B) Female <i>P. emmiltos</i> , C) Male <i>P. zebra</i> , D) Female <i>P. Zebra</i> , E) Male <i>P. thapsinogen</i> and E) Female <i>P. thapsinogen</i> . Photographs by A. Tyers .....	96
<b>Figure 8</b> Photograph of a <i>P. emmiltos</i> male (above left), a <i>P. thapsinogen</i> male (above right. Note the yellow pigmentation in the throat region) and a <i>P. zebra</i> male (bottom). Photographs taken by A. M. Tyers .....	99
<b>Figure 9</b> Photographs of <i>P. thapsinogen</i> female (left) and <i>P. emmiltos</i> female (right). Photographs taken by G. M. Cooke (left) and A. M. Tyers (right). ....	101
<b>Figure 10</b> Photographs of <i>L. fulleborni</i> were used from Konings (pp. 30, 2007) and additional patches of pigmentation were mapped on a simplified fish diagram for comparisons with other species. Most photographs used in this analysis were from Konings (2007) with a small number from the plates included in Ribbink <i>et al.</i> , (1983).....	103
<b>Figure 11</b> Mean number of aggressive behaviours and 95% confidence limits given towards <i>P. zebra</i> and <i>P. emmiltos</i> stimulus fish by focal <i>P. thapsinogen</i> males in the monochromatic and full light experiments. N = 12. ....	105
<b>Figure 12</b> Mean number of aggressive behaviours, with 95% confidence limits, by <i>P. thapsinogen</i> focal female fish towards either <i>P. thapsinogen</i> or <i>P. emmiltos</i> stimulus fish for the three experiments (full light/olfactory cues, full light only and monochromatic light only). N = 8 focal fish tested against three intruder pairs. ....	107
<b>Figure 13</b> Mean number of aggressive behaviours, with 95% confidence limits, by <i>P. emmiltos</i> focal female fish towards either <i>P. thapsinogen</i> or <i>P. emmiltos</i> stimulus fish	



for the three experiments (full light/olfactory cues, full light only and monochromatic light only). N = 8 focals tested against three intruder pairs. ....	108
<b>Figure 14</b> Stylised representation of populations of mbuna at six locations in Lake Malawi, highlighting how different species at different locations may evolve additional patches of pigmentation to reduce heterospecific aggression. <i>L. fulleborni</i> (bottom row) is compared to other populations within its species and its sympatric competitors. Note: At each location rarely, if at all, does two species have similar patches of pigmentation. One example of overlap is at Nkhata Bay where <i>P. zebra</i> and <i>P. callainos</i> both have no additional colouring although one ( <i>P. zebra</i> ) does employ stripes used to highlight dominance. ....	110
<b>Figure 15</b> <i>A. calliptera</i> brooding female and <i>P. thapsinogen</i> brooding female. Photographs taken by A. M. Tyers .....	124
<b>Figure 16</b> Mean aggression displayed to various intruders of the reproductive cycle by <i>P. thapsinogen</i> females with 95% confidence limits. N = 8 focal fish. ....	130
<b>Figure 17</b> Mean aggression by focal <i>P. thapsinogen</i> females displayed to various intruders as organised by time period with 95% confidence limits. N = 8. ....	130
<b>Figure 18</b> Shows the similarity and differences between <i>A. calliptera</i> and <i>P. thapsinogen</i> in response to various intruders over the reproductive cycle. N = 8 for both focal species. Error bars show inter-quartile ranges Note <i>A. calliptera</i> offer parental care in the form for an additional period leading to trilas conducted 25 days after spawning which were not required for <i>P. thapsinogen</i> . <b>A)</b> Comparison of aggression given to conspecifics by <i>A. calliptera</i> and <i>P. thapsinogen</i> . <b>B)</b> Comparison of the amount of aggression given to heterospecific intruders with <i>O. shiranus</i> acting as an intruder for <i>A. calliptera</i> and <i>C. afra</i> for <i>P. thapsinogen</i> . <b>C)</b> Comparison of aggression given to a heterospecific piscivore ( <i>R. sp.</i> 'Chilingali'). ....	133
<b>Figure 19</b> Shows the mean aggression given to different intruders by <i>P. thapsinogen</i> focal females over the entire reproductive cycle. Error bars = 95% confidence limits. ....	135
<b>Figure 20</b> Females varied the amount of aggression given over the reproductive cycle. They also varied the intensity of aggression given to different intruders. Error bars show inter-quartile ranges. ....	137
<b>Figure 21</b> A Pearson product moment correlation was carried out and a significant correlation was found between mean aggression given to intruders and mass of focal for <i>P. thapsinogen</i> in part A ( $r = 0.762$ , d.f. = 7, $p = 0.028$ ). This positive association was only found for Part A of the experiments. (See Appendix IV for scatter chart including all three data sets). 95% Confidence limits shown. N = 8 over 20 trials per female focal fish. ....	139
<b>Figure 22</b> Female colouration (left) compared to male (right) <i>P. thapsinogen</i> . Photographs taken by G. M. Cooke (left) and A. M. Tyers (right). ....	156
<b>Figure 23</b> Experimental setup where the focal was given a choice to attack an intruder next to a refuge or a female not associated with any resource. ....	158
<b>Figure 24</b> Experimental set up when focal females had the choice to attack intruding conspecific females next to a refuge or a territorial male. ....	159
<b>Figure 25</b> Experimental setup when focal was presented with two intruders in a tank containing no obvious resource .....	160
<b>Figure 26</b> Comparison of the types of aggression used by <i>P. zebra</i> males and females in Chapter 1. Male = White, Females = Blue. Focal fish presented with two intruders, one same morph the other a different morph. N = 9 males (63 trials) and N = 16 females (48 trials). Error bars = 95% C.L Box plots = Interquartile ranges. ** = significance to $p < 0.05$ . ....	163

<b>Figure 27</b> Aggression biases by focal fish given in the three experiments relating to different resources by <i>P. thapsinogen</i> females. Error bars show 95% confidence limits. N = 12 for each experiment. ....	164
<b>Figure 28</b> Mean number of aggressive behaviours in each experiment given by <i>P. thapsinogen</i> females. N = 12 for each experiment. Error bars show 95% Confidence limits. ....	165
<b>Figure 29</b> Mean number of aggressive behaviours given to a single intruder at three different times of day by <i>P. thapsinogen</i> females. N = 12 for each trial, error bars show 95% confidence limits. ....	166

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<b>Table 1</b> Summary of major hypotheses relating to mechanisms, functions and benefits of colour polymorphism, with examples. Taken, edited and expanded from Galeotti <i>et al.</i> , (2003). Note: Galeotti <i>et al.</i> , review colour polymorphism in birds yet the hypotheses stated are relevant to the wider animal kingdom. ....	27
<b>Table 2</b> Breakdown of types of behaviour (attacks and displays) including totals for both males and female in laboratory experiments with results of paired t-tests. N = 9 males and n = 8 females. Male focal BB fish were tested against either BB/OB intruders or BB/O intruders over ten minute trials. Female BB focal fish were tested against BB/OB intruders as with OB focal females. Focals tested more than once so results pooled with averages taken. S.E. = standard error. Total aggressive behaviours in <b>bold</b> . ....	74
<b>Table 3</b> All aggressive behaviours given during the two treatments (visual and olfactory cues, monochromatic light) for <i>P. thapsinogen</i> male focal fish, including totals and statistics. Non-contact displays (lateral display, frontal display and quiver) pooled into 'display' category. Overt aggression (lunge and bite) pooled into 'attack' category. S.E = standard error. ....	106
<b>Table 4</b> All aggressive behaviours given during the three treatments (visual and olfactory cues, visual only, monochromatic light) for both focal species ( <i>P. emmiltos</i> and <i>P. thapsinogen</i> ) including totals and statistics. Non-contact displays (lateral display, frontal display and quiver) pooled into 'display' category. Overt aggression (lunge and bite) pooled into 'attack' category. ....	109
<b>Table 5</b> Mann – Whitney U tests for comparison of aggression levels in <i>A. calliptera</i> and <i>P. thapsinogen</i> females for three intruders during the brooding cycle as presented in <b>Figure 18</b> . N = 8 for each focal type. * denotes significance to $p < 0.005$ . The two only significant results reveal that <i>P. thapsinogen</i> focal females were more aggressive to conspecifics than <i>P. calliptera</i> females on day 4 and day 18. ....	133
<b>Table 6</b> Wilcoxon matched pairs test results as multiple tests are made for comparisons of aggression given to intruders between each trial including the non-brooding phase when <i>A. calliptera</i> was focal. <b>Bold</b> indicates which trial showed a significantly greater amount of aggression. Non-significant results not shown see appendix. There were no significant differences between aggression for heterospecifics between any of the trials. ....	138

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# Chapter 1 Introduction

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The aim of this chapter is to provide background for the organisms used, and address the topics and literature associated with the research presented in this thesis. The beginning section gives a background to the fish used throughout this body of research. Following this general background to cichlids is a background to the themes or problems each of the experimental chapters tries to address.

## Background to the cichlid fish

### *Cichlid origins*

Cichlids are almost exclusively fresh water fish and are found in tropical regions such as Central America, South America, Madagascar, Africa and Southern Asia (Barlow, 2002). They belong to the order Perciformes, class Acanthopterygii, and their general body form is typical of Perciformes (Barlow, 2002). They are also placed in the sub-order Labroidei, a group which accounts for about 15% of all the teleost fishes (Stiassny, 1994).

Molecular phylogenies have indicated that cichlids are monophyletic in origin (Streelman and Karl, 1997; Sparks and Smith, 2004) and there is little or no disagreement with this as morphological comparisons strongly lend support to this hypothesis (Kaufman and Liem, 1982; Stiassny, 1987; Stiassny, 1991). The major bio-geographical locations of these animals supports, in tandem with the phylogenetic reconstructions of inter - cichlid relationships, a time of origin before the breakup of the super continent Gondwana some 65 million years ago (Sparks and Smith, 2004). Primitive or early cichlids would have been carried along with drifting landmasses which were previously part of the super continent. This explains why Madagascan

cichlids are more closely related to Indian cichlids even though they are geographically much closer to African cichlids.

The oldest discovered cichlid fossil, *Mahengechromis*, is a mere 46 million years old (Sparks and Smith, 2004) which significantly post-dates the proposed origin of the cichlids. Vences *et al.* (2001) have also published results using 16s and rRNA data which tested the Gondwana break up hypothesis. They found in favour of a Cenozoic origin and stated that their results were more in accordance with the fossil record. This hypothesis would suggest a marine dispersal event instead of a vicariance event caused by the shifting continental plates. Genner *et al.* (2007) sought to solve the puzzle by comparing the fossil data with the Gondwana fragmentation. They also used the rates of evolution for other non-cichlid fish to find an origin of cichlids and the divergence times of the major African cichlid lineages. They found the origin of cichlids to be some 133.2 MYA, which is in closer accordance with the biogeographical evidence as it gives an origin date well before the break up of Gondwana.

### *Cichlidae characteristics*

Morphologically, Cichlids stand out from other groups of fish in several ways (Barlow, 2002). They only possess one nostril on either side of their snout, all other Teleosts (excluding the damselfishes) have two, the significance of this is poorly understood (Barlow, 2002). Even though other fish families contain members with one nostril pair and an interrupted lateral line no other family that has been described contains examples of both (Barlow, 2002). The major aspects of their body plan, such as position of the fins, the nature of their scales, the way important bone structures are arranged, have changed very little despite their extensive radiations (Stiassny, 1991).

*The explosive radiation of East African haplochromine cichlids*

There are a remarkable number of endemic species of cichlids found in African Great Lakes, and Lake Malawi in particular. The lake is believed to contain between 500 and 1000 cichlid species (Turner, 1994; Genner *et al.*, 2004); many of which have yet to be formally described (Genner and Turner, 2005). The Malawi cichlids can be divided into two main groups: the haplochromine and the tilapiine cichlids with the haplochromines being far more speciose. The haplochromine cichlids are probably descended from a fluvial species that invaded the lake early on its history (Fryer & Iles, 1972). *Astatotilapia* as a genus is a prime candidate for a shared ancestor of the haplochromine cichlids as representatives are found in all the Great Lakes but this genus as currently defined is certainly not monophyletic (Snoeks, 2004). Lake Tanganyika is thought to be the source of the haplochromines with subsequent parallel radiations in the other Great Lakes (Salzburger *et al.*, 2005). Recently it has generally been assumed that like Lake Victoria (*e.g.* Meyer *et al.*, 1990) the haplochromine flock of Lake Malawi is monophyletic based on mtDNA analysis (*e.g.* Parker and Kornfield, 1997). However, more recent work using nuclear DNA (Joyce *et al.*, 2011) provided molecular evidence that Lake Malawi “flock” we see today may be the result of several invasions by *Astatotilapia* spp. This stance harks back to a historical position that the Lake Malawi flock was due to multiple invasions (Fryer and Iles, 1972; Mayr, 1963). Furthermore, early hybridisation events may have seeded the most species rich group, the mbuna (Joyce *et al.*, 2011). Aquaria observations suggest that Lake Malawi cichlids hybridise easily and some authors (*e.g.* Smith *et al.*, 2003) believe that hybridisation continues to result in new species in the wild (*i.e.* *P. zebra* x *P. thapsinogen*, resulting in *P.* sp. “Makinjila” a phenotypically and geographically intermediate *P.* sp.).

There is remarkable convergence in forms or guilds found between the lakes, for example methods of feeding and reproduction are shared between lakes but occur in unrelated species (Kocher *et al.*, 1993). The divergence between the major haplochromine groups in Lake Malawi is estimated at between 1 million (Moran *et al.*, 1994) and 700 000 years ago (Won *et al.*, 2006) whilst diversification within these groups is believed to be much more recent (*e.g.* Parker & Kornfield, 1997). The species flock of Lake Malawi is considered one of the most complicated taxonomic puzzles in the world (Barlow, 2002) and as a result species are frequently moved between taxa and genera renamed (*e.g.* Stauffer *et al.*, 1997). The reason for this is due to early classifications being based on morphological and trophic aspects of their biology while subsequent modern molecular work has the potential to clarify taxonomic relationships. There is however much work to be done to give a complete picture of cichlid relationships within the lake.

The reason for such a large number of species is hotly debated, theories include the evolution of the pharyngeal jaw with subsequent modification allowing rapid ecological diversification (Hunter, 1998), to the nature of the environment *i.e.* enclosed boundaries with limited opportunities for dilution of novel adaptations, areas of environmental heterogeneity within the lake, and speciation through sexual selection or hybridisation (Joyce *et al.*, 2011).

#### *Allopatric/sympatric speciation and natural/sexual selection*

The allopatric speciation model states that the most likely cause of speciation is through the splitting of a population by one of two methods, vicariance or dispersal. The arguments for allopatric speciation have been supported by many studies and allopatric speciation is probably the most widely held explanation or most of the

diversity seen; a classic example is the Hawaiian *Drosophilids* which have diverged after isolation on different islands in the archipelago (*e.g.* DeSalle and Giddings, 1986).

Allopatric speciation has had varying levels of popularity as a model for describing the story of the Lake Malawi cichlid radiation and recent evidence suggests that it may be the main contributor to the number of species present. Recent molecular work (Genner *et al.*, 2010) has provided evidence that a fall in lake level, which probably caused the Lake Malawi basin to split into two (Scholz and Rosendahl, 1988), may indeed have contributed to some of the diversity seen today. The echo sounding work of Scholz and Rosendahl (1988) allows one to imagine a situation where a low level lake 25 000 years ago contained a number of shore dwelling cichlids. Along the shore populations were partially separated by stretches of mildly inhospitable features that reduce but do not prevent gene flow between populations. A real world example of this might be the mbuna or rock dwelling cichlids. These are habitat specialists with little or no dispersal phase between populations (van Oppen *et al.*, 1997; Arnegard *et al.*, 1999) and possess a tendency to avoid open water to such an extent that even a barrier of 500m may prevent populations interbreeding (Van Oppen *et al.*, 1997). As the lake level rises, the rock dwelling cichlids continue to hug the rocky shoreline much as they did for millennia before, but now the local populations are getting further and further away from each other as the lake's perimeter expands in tandem with the lake's overall volume (Fryer, 1959). Populations that had some gene flow may now have none at all and are subsequently free to evolve local and reproductive differences without the homogenising effect of gene flow with neighbouring populations. The idea of fluctuating lake levels affecting diversity in the African Great Lakes is appealing, especially when authors like Kaufman (1997) speak of

taxon cycles or Rossiter (1995) of “species pumps” when considering periodic lake level changes. However there may well be other geographic factors that are responsible such as the main lake splitting, or the creation of peripheral satellite lakes which allow populations to diverge over sufficiently long time periods.

### *Sympatric speciation*

In sympatric speciation a population may split into species without any geographical disruption; divergence occurring despite the opportunity for the homogenising effect of gene flow between each insipient species. The theory behind sympatric speciation typically poses two problems: antagonism between selection and recombination and the subsequent coexistence of two ecologically similar (if not identical) species (Coyne and Orr, 2004).

The debates regarding sympatric speciation in African cichlids are numerous (*e.g.* Fryer & Iles, 1972; van Doorn *et al.*, 1998) but there is some support from genetic studies of cichlid populations (*e.g.* Shaw *et al.*, 2000). Some authors, such as Kornfield and Smith (2000), directly state that sympatric speciation has *definitely* been the main mechanism in some cichlid diversification. Some members of the Tilapine cichlids are found in Cameroon lakes and mtDNA analysis of the eleven species found in the Barombi-Mbo crater lake suggest strongly that the species were formed from a monophyletic group without the geographically proximate riverine species that is presumably their ancestor, reinvading repeatedly (Schliewen *et al.*, 1994; 2001). The nature of this diversification is credited to ecological niche filling, as observed in sticklebacks (Gasterosteidae) in the Great Lakes of British Columbia (Canada) which have repeatedly diverged into a limnetic and a benthic form (Rundle, 2000; Schluter *et al.*, 2001). The two types mate assortatively under lab conditions



and, probably due to feeding efficiency, phenotypically intermediate hybrids appear to have a lower fitness than the two parental types (Hatfield and Schluter, 1999; Rundle *et al.*, 2000). Molecular work appears to confirm the repeated parallel evolution of the two lake ecotypes through repeated invasions (Hohenlohe *et al.*, 2010).

### *Sexual selection*

Charles Darwin (1871) believed that sexual selection may play a role in speciation and reviews on haplochromine cichlids (Panhuis *et al.*, 2001) suggest that sexual selection alone can cause sympatric speciation. Darwin (1871) discussed two types of sexual selection, the first relates to female choice. The second type of sexual selection naturally leads on from the first. If it pays females to be choosy about their mates then from male's point of view females are a finite resource that will ultimately be competed for (Darwin, 1871).

Cichlids throughout the world exhibit a wide repertoire of reproductive strategies (for example; monogamy, polygyny and polygynandry (Keenleyside, 1991; Barlow, 2002). Furthermore male and female parental care can vary in relation to the type of reproductive strategy employed, (Keenleyside, 1991; Barlow, 2002). When males compete for females whilst offering little or no parental care, the result can be strong sexual selection on males.

Reproduction in cichlids usually involves one of two methods. The male either fertilises the female's eggs on the substrate or they are fertilised in the female's mouth after which they are brooded for an extended period. In some species care may be provided to free swimming fry after the mouthbrooding period; females retake them into the mouth when under threat.

*The 'mbuna' of Lake Malawi*

The 'mbuna' (small to medium sized Lake Malawi rock dwellers) are a group of haplochromine cichlids that are strikingly coloured (Genner and Turner, 2005).

Within the mbuna there are approximately 300 species fitted into 10 genera (Kornfield and Smith, 2000; Ribbink *et al.*, 1983). Some species are abundant ( $> 10^6$  individuals (Ribbink *et al.*, 1983)) whilst others may have far fewer individuals (Kornfield and Smith, 2000). It must be noted however that the term mbuna has no taxonomic weight and does not designate nor define any evolutionary relationship but its use is common throughout the scientific literature (see Genner and Turner, 2005 for a review of what is known regarding the mbuna).

Just how many species of mbuna, or indeed haplochromine cichlids, are found within the lakes remains unresolved (see Genner and Turner (2005), and in particular Turner (1999) for a review of mbuna and discussion on how species concepts relates to fish in general). This is not because there lacks the will or effort, rather no one species definition (out of at least 11, again see Turner (1999) for species concepts and how they relate to fish) can successfully be applied to all populations in the lake. When working with species isolated by pre-zygotic mate choice it can be argued that the most useful definition is that of the biological species concept (Dobzhansky (1937), mate choice can provide pre-zygotic reproductive isolation; maintaining gene pools as distinct lineages despite the potential for hybridisation. However, in taxa where allopatric populations (reproductively isolated by distance in the wild) differ in traits that are important in reproductive isolation between sympatric species, the allocation of species status to allopatric populations can greatly increase species number.

Laboratory based mate choice trials have found full or partial reproductive isolation between some populations that differ in male courtship colour (*e.g* Knight and Turner,

2004), but to confuse matters further, partial reproductive isolation may also be present between populations that on the surface appear the same (e.g. very similar morphology, feeding habits, courtship signals and secondary sexual characteristics e.g. Blais *et al.*, 2009). Many species remain undescribed and those that have will not necessarily be “good” species in all circumstances.

The other African Rift Lakes (Tanganyika and Victoria) contain parallel examples of a rock dwelling haplochromine cichlid with no recent evolutionary relationship (Kocher *et al.*, 1993). The mbuna have been one of the most extensively studied cichlid groups. Most of the species studied have extremely restricted dispersal rates (McKaye and Marsh, 1983; Kornfield and Parker, 1997) and are *generally* absent from sand, sediment and deep water which acts as a barrier to dispersal. Along rocky outcrops dispersal rates are higher for males than females in some species (Knight *et al.*, 1999). Oral incubation (or mouth brooding) is found in all mbuna (Goodwin *et al.*, 1998) and microsatellite paternity analysis has shown that multiple males (up to six) may sire a single brood (Kellogg *et al.*, 1995; Parker and Kornfield, 1996). Cross fostering experiments carried out by Verzijden and Cate (2007), have shown that assortative mating can be reversed when embryonic fry are artificially placed in the buccal cavity of females from other species (Lake Victoria cichlids; *P. pundamilia* and *P. nyererei*). If assortative mating may be reversed it may be that cues used in mate choice are learned during a critical period during the brooding phase. If these cues are learned by females during this period aggression cues may also be learned by both males and females. Aggression cues are dealt with in two of the chapters in this thesis and discussed further in this introduction chapter.

The frequently studied *Pseudotropheus zebra* species complex (as designated by Ribbink *et al.*, 1983) comprises approximately 27 species and up to six of which can

exist sympatrically at any one location. This group was later assigned to the subgenus *Maylandia* and Stauffer *et al.*, (1997) proposed that some members of the *Pseudotropheus zebra* complex be placed in the genus *Metriaclima*. However many authors consider this to be a junior synonym of *Maylandia*. Others have suggested that no description or diagnostic features were described for *Maylandia* and it can consequently be considered a *nomen nudum* (Konings, 1995). Throughout this text they will be referred to as *Pseudotropheus* as it is the original name.

Males hold territories of approximately 1 metre in diameter (Holzberg, 1978) which they vigorously defend from conspecific males and females that are not reproductively active (Komfield and Smith, 2000). The territories are often densely packed, overlapping with those of other mbuna species (Hert, 1990). Territories are held for up to three years (Hert, 1992). Experimental removal of territorial males has shown rapid re-colonisation by males of the same species that are presumed to be previously non-territorial (Hert, 1992). Similar results have been shown in Lake Tanganyika rock dwelling cichlids (Gashagaza, 1991; Hori, 1991; Kohda *et al.*, 1996). "Sneaky breeders" have been documented in lek-forming cichlids (Rossiter and Yamagishi, 1997) but not within the mbuna. However the potential for such a tactic to evolve may be there; smaller non-territorial males may look very similar to females, often being similar in size, and having the typically drab female appearance. If sneaking does ever occur this may lead to the evolution of alternative reproductive strategies by males which may even have morphological effects where one type of male never attains the size of a typical territorial male. Indeed, sneaky male breeding may result in the polyandry seen in some Malawi sand dwelling haplochromines (Kellogg *et al.*, 1995; McKaye, 1984).

Females are weakly territorial (*e.g.* Genner and Turner, 2005) and are believed to be philopatric in nature (at least in *Pseudotropheus aurora* – Hert, 1992). When they cross into a male's territory and are reproductively active they will be immediately solicited; with males displaying similar behaviours to both attract females and to ward off males. Females recognise conspecific males primarily through visual cues and may use olfactory cues secondarily when making mate choice decisions (Plenderleith *et al.*, 2005). A male will display to the female using lateral displays and quivers. He will then lead the female to its own refuge with a 'lead swim' and the displays have been shown to be highly conserved across related taxa (McElroy and Kornfield, 1993). Mbuna clutch sizes are relatively small (10 – 50 eggs). Being mouth brooders buccal cavity size may limit number of eggs reared compared to substrate brooders (Okuda *et al.*, 1998). However, mouth-brooders may have larger eggs and may simply be investing more parental care in fewer eggs than substrate brooders (G. F. Turner pers. comm.). The fry are released after approximately three weeks into small rocky crevices; the mother will then vacate the area (Trendall, 1988).

### *Cichlids and colour polymorphism*

The same type of chromatic polymorphism is found within the haplochromine cichlids in all three African Great Lakes. Females can be found in three forms, the most common being a drab brown/blue barred type (BB), with at least two rarer morphs *e.g.* orange blotch (OB) and orange (O). Males show the same polymorphism but the rarer morphs are found at much lower frequencies than in females. BB females may be cryptic (*e.g.* Maan *et al.*, 2008), but within territorial males the bars are striking, changing from black bars on a blue/brown background (subordinate/non-territorial) to black bars on a white background (aggressive/territorial/courting). The

O and OB varieties have the bars when dominant but they are often disjointed and much reduced in intensity (if at all visible) and the background colour changes little.

Seehausen *et al.*, (1999) produced a model describing the inheritance of the unusual phenotypes. They proposed the BB (*B*) allele and OB (*b*) allele are co-dominant, with OB individuals being heterozygous (*Bb*) whilst O individuals are homozygous (*bb*). Their subsequent breeding experiments showed evidence of a sex ratio distortion among the offspring of mating's between BB males and OB females; there were far greater numbers of females. This led to suggestions that OB colouration was linked to an emerging female determining chromosome (*W*), which is dominant to the male determining Y chromosome (Seehausen *et al.*, 1999). The female determining gene is thought to be strongly linked to an X chromosome gene creating an overriding dominant gene. Two rarer forms of female were also discovered which led to the inclusion of an autosomal blotch modifier gene into the previous model. This gene is thought to be a 'rescue' gene that counteracts the effect of the overriding female determining gene, reinstating the maleness of some individuals. Seehausen *et al.*, (1999) state this is why we find some individuals that are male and of the rarer morph (either OB or O).

Furthermore, breeding trials (Seehausen *et al.*, 1999) found that BB females preferred BB males, whilst OB females had no preference. With males, the OB males seemed to prefer OB females with similar results between the BB males and BB females. OB males were however more likely to court non-like females than BB males.

Interestingly BB males with OB parents preferred OB females than pure (*BB*) BB females. This led to the suggestion that mate preference may be down to autosomal inheritance (Seehausen *et al.*, 1999; Lande *et al.*, 2001).

Lande *et al.* (2001) proposed that the extra female determining chromosome (W) may be selected for in small, founding populations. If the risk of inbreeding is high when a small subset of an initially small population disperses into a new territory, competition between brothers for mates is intensified (Kocher, 2004). In this situation a skew in the operational sex ratio would be favoured towards females and may allow the original mutation, which created the extra female determining chromosome (W), to be selected for (Lande *et al.*, 2001). In inbred populations (where a few males are responsible for siring most of the offspring), males are susceptible to the sex biasing effects of the W chromosome because descendent males accumulate many deleterious mutations upon their Y chromosome (Lande *et al.*, 2001). The creation of WY females, when mated with XY males, produces YY sons that are almost always unviable due to the accumulation of deleterious mutations, leaving a bias in the sex ratio. The OB colour mutation arises at some point and offers females some undetermined advantage (Lande *et al.*, 2001) with the gene being associated with the new W chromosome. Eventually the W suppressing gene evolves and allows the formation of OB males, at a much lower frequency than is found in females.

More recently, Roberts *et al.*, (2009) suggest that sexual conflict has caused the association of the OB pigmentation gene and the female determining aspect. Sexual conflict occurs when a trait benefits one sex but not the other. OB females may benefit from crypsis which may reduce predation (see below) but in males secondary sexual characteristics are severely disrupted which may reduce fitness (it has also been proposed that males may also suffer increased predation as they employ riskier behaviours to find mates which reduce the anti-predator properties of OB pigmentation (Mann *et al.*, 2008)). This sexual conflict is resolved by female determining genes being tightly linked to the OB pigmentation genes. Roberts *et al.*, (2009) also show that the rare phenotypes have a single origin in Lake Malawi, and may well be the case for Lake Victoria too. The OB -W (female determining gene) can be seen as nascent sex chromosome which is driven by natural selection rather

sexual selection (Roberts *et al.*, 2009) which has previously been used as an explanation for sexual conflict (Arnquist and Rowe, 2005). Versions of this polymorphism are also found in many rock dwelling haplochromine cichlids in Lake Malawi *e.g.* *P. callainos* individuals who are either white (W), as opposed to all blue which is the common form, or white blotch (WB). These individuals carry the OB haplotype (Roberts *et al.*, 2009) at the OB locus meaning OB/O are synonymous with W/WB potentially allowing comparative studies.

It has been proposed that two mechanisms of sexual selection may aid maintenance of colour polymorphism within a haplochromine cichlid population. Firstly, sexual selection through female choice (*e.g.* Knight & Turner, 2004; Seehausen *et al.*, 1999). Females are believed to at least in part base their reproductive decisions on visual cues (*e.g.* Couldridge, 2002) and previous studies suggest that variation in male courtship colours may coincide with partial reproductive isolation between allopatric populations (Knight and Turner, 2004). There is evidence that suggests *Neochromis omnicaeruleus* morphs may mate assortatively (*e.g.* Lande *et al.*, 1999; Seehausen *et al.*, 1999). However another study (Pierotti *et al.*, 2009) suggests that males prefer the rare morph. The second proposed method of the maintenance of colour polymorphism in cichlids relates to intrasexual aggression and this is discussed in detail further on in this introduction.

Some authors (*e.g.* Greenwood, 1956) have proposed that 'blotched' morph individuals may enjoy some additional advantage due to increased camouflage. Yet a recent experimental study by Mann *et al.*, (2008) proposed that the 'blotched' morph individuals (of *N. omnicaeruleus* from Lake Victoria) suffer greater predation through conspicuousness compared to drabber individuals (also proposed by Lande *et al.*, 1999). Blotched individuals are mostly (*P. zebra* 'gold' can be found much deeper, M. Knight pers. comm) found in species that occupy shallow waters in rocky shores (Konings, 2007) and are confined to clear waters (Seehausen and Bouton, 1996). To



the human eye and depending on the exact nature of the rocks that provide their background the OB phenotype can be difficult to detect (for an example of both conspicuous and camouflaged individuals against a rocky substrate see Konings, 2007 page 19).

The cichlid visual system, pigments used and the resulting sensitivity of their colour vision, has been extensively studied (*e.g.* Dalton *et al.*, 2011). They have 7 cone opsin genes (Dalton *et al.*, 2011) with little variation in opsin sequences among the rock-dwelling species of Lake Malawi (Carleton and Kocher, 2001; Spady *et al.*, 2005). They are believed to have colour vision including sensitivity to UV wavelengths (at least in *P. zebra* – Carleton *et al.*, 1999). Habitat type and water depth affect irradiance and radiance of haplochromine cichlid fish at Cape Maclear, Lake Malawi (Sabbah *et al.*, 2011) with probably (depending on variable turbidity) similar effects at other rocky shore locations around the lake. Sabbah *et al.*, also showed that the highest sensitivity for colour could be restricted to shorter wavelengths (at the UV end of the colour spectrum, < 350nm). Although capable of seeing at UV wavelengths, Lake Malawi rock dwelling cichlids appear to have similar colour vision to many primates, including humans.

## Thesis themes

The overriding theme of this thesis is aggression in cichlid fish. Aggression is widespread in the animal kingdom and now considered to be an adaptive strategy that is used to gain or protect resources that can typically increase the inclusive fitness of the individual. This view, of fitness benefits to the individual, is a relatively new one (Archer, 1988). Until the 1960's many studies concluded that, for example, dominance evolved to prevent unlimited fighting in animal communities (*e.g.* Scott

and Fredericson, 1951). Threats and other non-contact behaviours were thought to have evolved to prevent too many individuals of one species being killed (*e.g.* Lorenz, 1966). Contemporary approaches have left behind the group selection-ist stance thanks to the work of Hamilton (1964) and Maynard Smith (1964) who, amongst others, pioneered the formal understanding that selection acts on individuals (and ultimately genes).

Aggression is found in a wide variety of taxa (Archer, 1988) from sessile invertebrates (*e.g.* the sea anemone *Actinia equina*, Brace *et al.*, 1979) to more complex organisms with sophisticated social systems. Aggression can be classified into three functional aspects; protective, parental and competitive (Archer, 1988). It can carry significant costs, not only increasing the likelihood of injury to the individual, but also increasing its energy requirements, risk of predation and time spent away from foraging or reproducing. Simply having elevated levels of testosterone may be metabolically expensive. For example, Yarrow's spiny lizards, *Sceloporus jarrovi*, that have higher levels of testosterone expend more energy than those with lower levels (Marley and Moore, 1991).

Several regions of the brain deal with aggression and regulatory pathways have been shown to be species specific in some cases (Nelson and Chiavegatto, 2001). In a wide variety of vertebrate taxa testosterone has been linked to aggression (*e.g.* Delville *et al.*, 1996, Albert *et al.*, 1986) and aggression is considered a conserved and primitive suite of behaviours (Nelson and Chiavegatto, 2001) yet complex and with multiple causes (Filley *et al.*, 2001). Typically, in humans at least, testosterone and aggression are considered male traits but females as well as males are aggressive (*e.g.* Draud and Lynch, 2002) and female aggression has been relatively understudied (Arnott and Elwood, 2009; Archer, 1988). Females of all vertebrate species studied use

testosterone (*e.g.* Whittinham and Scwabl, 2002) but its exact role remains unclear (Gill *et al.*, 2007, see below for further discussion in “Female aggression” section of this chapter).

Tied into the theme of aggression in this thesis is how natural selection favours individuals of some cichlid species who receive less aggression and what kind of advantage might be manifested due to a reduction in aggression compared to others. Aggression biases within species can have important implications to fitness levels of individuals in polymorphic populations. Who or when to be aggressive may be linked to phylogenetically relatedness *i.e.* some recently diverged species may have evolved sufficient differences that they no longer need be aggressive to one another and selection may favour signals that reduce aggression..

Below are sections that deal with the themes contained in this thesis.

## Aggression and cichlids

### *Signals used by cichlids in aggressive interactions*

As discussed above, aggression may be an important feature in haplochromine behaviour, promoting the coexistence of communities composed of closely related species (Seehausen and Schluter, 2004), signalling either through display or the cues used in aggressive interactions deserves some attention.

Animals use a wide variety of methods for communicating motivational states and understanding what signals individuals are capable of receiving is key. Female haplochromine cichlids have been shown to use olfactory as well as visual cues in mate choice (Plenderleith *et al.*, 2005) but the use of olfactory cues has not been studied in males or in relation to aggression. Visual cues are quite reasonably

expected to be important in territorial fish that patrol territories and are likely to be the first sensory modality used at least by mbuna cichlids when repelling intruders (Genner and Turner, 2005). A large amount of work has shown visual signals to be important in haplochromine cichlids (*e.g.* Pauers *et al.*, 2008; Dijkstra *et al.*, 2005). Female aggression is likely to be less frequent and they do not use the same conspicuous signals employed by males. For example, many mbuna females are drabber; identifying which species females (and subordinate males) belong to is near impossible in the field without catching them (Konings, 2007). Individual fish may need to use further sensory modalities to be sure they are risking costly aggressive encounters appropriately.

Few agonistic interactions actually involve any physical contact. In some cichlids, asymmetries in mass of as little as 2% are often settled without physical contact (Barlow *et al.*, 1986), and even if displays escalate to outright fights, they are often over before one competitor is terminally injured. Animals try to bluff or cheat other animals into backing down before they get injured themselves. For example, many animals try and make themselves appear larger than they really are. Selection favours each additional new method of cheating, as individuals who do not employ these signals lose out and a population ends up with all individuals employing the same signal repertoire. The signals are an honest indicator of an individual's fighting ability because ultimately every individual in a population or species uses them.

Theoretical discussion of honest signals is common (*e.g.* Enquist and Leimar, 1990; Enquist *et al.*, 1990); although different terms have been used, *e.g.* unambiguous signalling, Maynard Smith, 1982; assessment signalling, Maynard Smith and Harper, 1988 and supported by empirical data (*e.g.* Enquist *et al.*, 1990; Leimar *et al.*, 1991; Keely and Grant, 1993).

The signals employed by animals can be quite simple. In many cichlids aggressive signals consist of a few behaviours; the lateral display, which shows off the bulk of the animal's body, giving its challenger an idea of how much it weighs and its corresponding strength. The frontal display is similar, just showing off another dimension of the size of the fish (Baerends and Baerends von Roon, 1950). Some displays, such as the quiver, are more energetically expensive, whilst some are more aggressive without actually resulting in a risky attack (such as the lunge which may lead to nipping or biting) (Baerends and Baerends von Roon, 1950). Finally, and as a last resort if two animals are equally matched, a “jaw – lock” is employed which locks the two fish together, thrashing about until one gives in (Baerends and Baerends von Roon, 1950).

The change from a relatively passive display to a more aggressive and riskier action has been described in the “sequential assessment” model (Enquist and Leimar, 1983) and has been shown to be accurate in a number of taxa (*e.g.* Austad, 1984; Mosler, 1985) including some cichlids (Keeley and Grant, 1993). The non-contact displays are proposed to be for visual assessment and only if the animals are well matched will full contact be made. Keeley and Grant (1993) showed that the convict cichlid's (*Cichlasoma nigrofasciatum*) behaviour supports the model's predictions that when visual cues are diminished non-contact displays are shorter with no effect on displays or actions that require contact. Turner (1993) however, showed that the complete picture might not be that simple; using *Oreochromis mossambicus* in fighting experiments it was found that when residents of territories were smaller than the intruders they began with aggressive contact actions, and not the typical information-giving displays. There appeared to be no simple dichotomy between escalated and non-escalated phases of the contests. These results may be due to a number of

possibilities. Experience is extremely important in survival, and typically within a species smaller fish will be younger and larger fish will be older. Leiser *et al.*, (2004) showed, again using convict cichlids, which smaller and presumably younger fish escalate a lot quicker than larger fish. The question of when to escalate may require a learned component, naïve fish may have to learn the hard way that full contact fighting can be extremely detrimental. The willingness of small fish to escalate despite an asymmetry in size in Turner's (1993) study may be because the smaller fish have no chance of winning unless they employ the riskiest strategy and are willing to bluff their way to success. The same signals often are displayed in different contexts, when they are aimed at females the displays may be identical with intentionally different results (Baerends and Baerends von Roon, 1950).

Many cichlids are described as sexually dimorphic, not just for size but also for secondary sexual characteristics used in signalling, (*e.g.* Konings, 2001) although some female African cichlids *e.g.* *Oreochromis* spp. (Loiselle, 1985; Turner and Falter, 1989; Almada and Oliveria, 1998) have been shown to employ colour changes during mouth brooding but it has rarely been considered in non-brooding female haplochromine cichlids (for an exception see Naish and Ribbink, 1990). Hurd (1997) found that in *Nannacara anomala* there was an association between the visual signal employed and the subsequent aggressive behaviour with medial black lines associated with tail beating and black vertical stripes associated with mouth locking. Whilst not absolute, the relationship between signal and behaviour may indicate cooperative signalling between two individual fish within the same species (Hurd, 1997).

## Polymorphism and intraspecific aggression

### *The problem of polymorphism*

As discussed above, some haplochromine cichlids are polymorphic. Before discussing how colour polymorphism impacts signalling and aggression biases in cichlids, the problem of how polymorphisms are maintained requires some thought.

Polymorphism is described as variation within a single population with the frequency of any single morph not being due to a simple reoccurring mutation (*e.g.* Roulin, 2004). (Although the literature contains one example of what is considered non-genetically based polymorphism – wing colour, determined by sunlight in *Anartia fatima* (Lepidoptera: Nymphalidae), Taylor, 1972). How polymorphisms are maintained in natural populations has been discussed for at least 70 years (*e.g.* Ford, 1945; Huxley, 1955). Fisher (1930) proposed that the existence of stable polymorphism within a species requires a selective balance between the morphs as in theory, without this balance, selection favouring one morph should result in the fixation of that morph. Species may be polymorphic for a number of different types of trait, which may be physiological, behavioural or some other aspect of the phenotype. In the last few decades it has been shown that many genes are polymorphic, as can be chromosomes, and other molecular traits such as enzymes. Chromosomal polymorphism is a condition where one species contains members with varying chromosome counts or shapes. Varying counts may result from chromosomes undergoing fission, splitting into two, *e.g.* *Trichomycterus davisii* (a catfish – Borin and Santos, 2000).

### *Generation and maintenance of polymorphism*

The mechanisms by which polymorphisms are generated are probably better understood than the mechanisms by which polymorphisms are maintained. The starting point for polymorphism must be genetic mutation. There are believed to be more than one mechanism for the maintenance of polymorphisms within populations (Galeotti *et al.*, 2003; for an overview see table 1). Polymorphisms may evolve for the avoidance of predation in heterogeneous environments or to avoid detection as a predator (apostatic selection). Disruptive selection may favour polymorphism via selection for crypsis and sexual selection may promote polymorphism through disassortative mating (benefits of which include heterozygote advantage) or status signalling of costly colour signals.

**Table 1 Summary of major hypotheses relating to mechanisms, functions and benefits of colour polymorphism, with examples. Taken, edited and expanded from Galeotti *et al.*, (2003). Note: Galeotti *et al.*, review colour polymorphism in birds yet the hypotheses stated are relevant to the wider animal kingdom.**

Mechanism	Function	Benefits and cost	Examples	References
Apostatic selection	Disruption of selection image - prey	Higher hunting success of rare morph	Hawks (Falconiformes), Skuas (Charadriiformes)	Galeotti <i>et al.</i> , 2003
	Disruption of search image - predators	Low predation on rare morph	African bush-shrikes (Passeriformes, Laniidae)	Hall <i>et al.</i> , 1966, Owen, 1967
Disruptive selection	Crypsis	Differential hunting success and predation depending on habitat	Mangrove snail, ( <i>Littoraria fuzosa</i> ) Grove snail ( <i>Cepaea nemoralis</i> )	Reid, 1987  Cain and Sheppard, 1950
	Physiological adaptation to environment	Differential thermoregulation  Dispersal or reproduction	Hamster ( <i>Criceus criceus</i> )  Aphids	Huxley, 1942  Braendle <i>et al.</i> , 2006
Non-random mating	Sexual selection (status signalling)	Higher mating success, but higher predation of rare morph	Midas cichlid <i>Theraps (Cichlasoma citrinellum</i> Gecko ( <i>Gonatodes albogularis</i> )	Barlow, 1983  Ellingson, 1994
	Assortative/ Diassortative mating (genotype signalling)	Avoiding inbreeding and/or heterozygote advantage	Scarlet tiger moth ( <i>Callimorpha dominula</i> ) White throated	Sheppard, 1952  Falls and



			sparrow ( <i>Zonotrichia albicollis</i> )	Kopachena, 1993
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Some species exhibit sex limited polymorphism, examples are often seen in female butterflies employing Batesian mimicry (Ford, 1976). Males are monomorphic and invariably exhibit the ancestral form once displayed by both sexes (Kunte, 2008). In Batesian mimics, female only polymorphism is often explained by sexual selection and sex dependant predation pressure (Kunte, 2009). Male only polymorphism also exists, the Pygmy swordtail (*Xiphophorus nigrensis*) has two male colour morphs, blue and yellow. Females generally prefer blue males but yellow male are socially dominant over blue (Kingston *et al.*, 2003)

Due to the perceived likelihood that one morph will out compete other morphs and sweep to fixation within a population, the maintenance of polymorphisms is a classic problem in evolutionary biology (*e.g.* Gray and McKinnon, 2007). A number of mechanisms for the maintenance of polymorphisms within populations have been proposed. These include over dominance, spatial/temporal heterogeneity and negative frequency dependant selection (Futuyma and Anurag, 2009). The latter is believed to be the strongest and most prevalent mechanism (Fisher, 1930; Lewontin, 1958; Fitzpatrick, 2007). Perhaps the best and most common example of negative frequency dependant selection is the balanced sex ratio (Fisher, 1930; Shaw and Mohler, 1953) where individuals of the rare sex are likely to find mating opportunities with the more abundant sex more easily. This suggests that genotypes that produce greater numbers of offspring of the rarer sex will have more grand-offspring and will spread in the population. Eventually the sex ratio of a population will reach a ratio of 1:1 investment in the sexes. Although many prospective cases have been reported

(Takahashi, 2010) and whilst considered common in maintaining balanced polymorphisms within populations, negative frequency dependant selection has until recently rarely been fully demonstrated (Fitzpatrick *et al.*, 2007). However examples do exist; rare morph advantage and negative frequency dependant selection has been demonstrated in the laboratory using *Drosophila melanogaster* (Fitzpatrick *et al.*, 2007). In their study, a behavioural polymorphism of “rover/sitter” (which is controlled by a single gene) was investigated. It was shown conclusively that only whilst rare did one behaviour type have had a higher fitness than the other.

Field studies also support the negative frequency dependant selection model as a mechanism for the maintenance of stable polymorphisms. Olendorf *et al.*, (2006) showed that in guppies (*Poecilia reticulata*) possessing a rarer phenotype increases the fitness of the individual. This has also been shown in damselflies (*Ischnura senegalensis*) although this is sex limited to females (Takahashi *et al.*, 2010). Field work has also shown that morph frequencies may oscillate in predictable cycles, for example, a Lake Tanganyika scale eating cichlid (*Perissodus microlepis*) has been shown to be polymorphic for the behaviour which leads to ‘handedness’ when attacking its prey. In this example the prey’s alertness through experience decreases the fitness of the more common behavioural type (Hori, 1993).

Colour polymorphism is widespread throughout the animal kingdom (Galeotti *et al.*, 2003), for example it is believed that at least 3.5% of bird species are polymorphic for colour (Roulin, 2004). For example, the White throated sparrow (*Zonotrichia albicollis*) which is colour polymorphic for head striping. The colour polymorphism in this example is linked to courtship singing intensity and overall is maintained by negative frequency dependent selection where individuals mate dissasortively (Thomas *et al.*, 2008). However many other taxa possess examples of colour

polymorphism, including both aquatic and terrestrial invertebrates and many other vertebrate groups (Galeotti *et al.*, 2003). For example the Grove snail (*Cepaea nemoralis*) is colour polymorphic which have antipredatory benefits in heterogenous environments (Cain and Sheppard, 1950). Mammals are also known to be polymorphic: Albert's squirrel (*Sciurus aberti*) is polymorphic for coat colour (Ramey and Nash, 1976).

### *The maintenance of colour polymorphisms in cichlids through aggression*

Intrasexual aggression may be responsible for maintenance of colour polymorphisms within haplochromine cichlids (*e.g.* Mikami *et al.*, 2004; Seehausen & Schluter, 2004; Dijkstra *et al.*, 2007; 2009). Within a polymorphic population negative frequency dependant selection may favour the least abundant morph (*e.g.* Dijkstra *et al.*, 2009). For example, in a population where males are aggressive and female mate choice is based on the quality of a male's territory, a mutation that occurs in genes that code for male nuptial colour could give an advantage to the male for a couple of reasons, both based on a negative frequency dependent advantage for the new colour type. Either the "new/rare" colour may intimidate males of the "normal" colour, resulting in acquisition of a higher quality territory for the rare male (where rare-rare disputes are settled on size and fighting ability and rare-normal disputes are settled on colour). Or, a new rare colour male may not be recognised as conspecific and therefore left to gain a good quality territory, and increased reproductive success, due to lack of competition. This type of scenario has been postulated for some cichlid fish species in the African Great Lakes that are colour polymorphic (*e.g.* Dijkstra *et al.*, 2005). This thesis investigates how aggression is biased to different morphs that are variable in frequency.

Whilst rare males may enjoy a greater advantage through negative frequency dependant selection, females can also benefit. Some female haplochromine cichlids are believed to be at least weakly territorial (Dijkstra *et al.*, 2007; Seehausen *et al.*, 1999) and are frequently seen to be aggressive in aquaria. Furthermore, females may defend fry or places where fry are released (Fryer & Iles, 1972; Seehausen, 1996) which may be another situation in which rare morph individuals gain an advantage. (Female aggression both in a maternal and non-maternal context is discussed in detail further on in this chapter).

Rare morphs can only gain an advantage through being rare if they are not recognised as competitors by the more abundant morph/s. In male haplochromine cichlids it appears that aggression choices are correlated with an individual's own colour in some species (*e.g.* Dijkstra *et al.*, 2006). However own morph aggression bias is not absolute. In the Lake Victoria cichlid *Pundamilla* sp. a bias towards only one morph was found (Dijkstra *et al.*, 2005). Common morph only aggression biases have also been proposed (but not shown) in a Lake Malawi polymorphic species (*e.g.* *P. zebra* – Pierotti *et al.*, 2007). Aggression choices or biases appear not be under the control of imprinting in haplochromine cichlids (Verzijden *et al.*, 2008). Experiments using Lake Victoria haplochromine cichlids did not reveal maternal phenotype biases after cross fostering. Males do not appear to be subjected to imprinting but that does not mean aggression biases cannot be learnt. Dijkstra *et al.*, (2008) showed that in a Lake Victoria haplochromine cichlid (*Pundimilla* sp.) that are recently diverged, blue males that had no prior experience of red males did not show any aggression bias when presented with a blue and red intruder. However, when blue males had some prior experience of red males they showed aggression biases towards other blue males.

## Interspecific competition and aggression

Whilst intrasexual selection clearly has a large impact on individuals, species are not isolated entities in space and time – competition between species can be fierce and aggression is often used. Interspecific exploitative competition can be a potent mechanism of selection on traits relating to a species adaptation to its ecological niche (Grether *et al.*, 2009; Schluter, 2000) and has been frequently studied. Numerous studies have focused on one species interacting another, such as parasitism and mutualism. There has been much less focus on interspecific interference competition relating to non-ecological niche factors (Grether *et al.*, 2009). Interspecific interference competition is likely to occur between sister taxa that still share many ecological and/or physiological traits. Many species that evolve in allopatry find themselves in secondary contact with ancestral/sibling species and here interference competition may be fierce. On secondary contact, two recently diverged species are initially unlikely to respond adaptively to one another as they have evolved independently (Grether *et al.*, 2009). If in competition, sympatric taxa may be selected to diverge on traits that affect encounter rates, competitor recognition cues or fighting ability. Even if trophic resources are not finite, traits may still diverge to reduce accidental and unnecessary interspecific aggression (Grether *et al.*, 2009; Cody, 1969; 1973), especially if the two species are recently diverged, in which case behavioural signals are likely to be conserved and a high potential for hybridisation may result in interspecific male – male competition for females. There have been few attempts to address this hypothesis though notable exceptions come from the studies on East African haplochromine cichlids (Seehuusen and Schluter, 2004; Young *et al.*, 2009). These studies are particularly relevant as haplochromine cichlids have been the focus of many sexual selection studies, as have traits usually used in conspecific competitor

recognition (Grether *et al.*, 2007). Sexual selection research has often focused on mate choice, yet the signals investigated in these studies are often used in intrasexual competition too (Berglund *et al.*, 1996; Heinsohn *et al.*, 2005). Where intrasexual competitor signals are similar between recently diverged taxa, selection may favour character displacement; an overlooked area of evolutionary biology (Grether *et al.*, 2007). Reproductive character displacement has been discussed in the context of reinforcement and may cause secondary sexual characteristics to diverge if hybrid fitness is low (Coyne and Orr, 2004). Secondary sexual characteristics may often be conspicuous cues such as courtship call, behaviour or colour signals.

#### *Agonistic character displacement*

Colour diversity in coral reef fish was initially proposed to be a result of heterospecific aggression. If the fish use visual signals to repel competitors, then colour divergence between highly specialised species (those not in ecological or trophic competition) may reduce unnecessary and costly interspecific competition (Lorenz, 1966).

A commonly used indicator of agonistic character displacement is a phenotypic shift in one population with respect to another allopatric population of the same species (Grether *et al.*, 2009). There is strong evidence of agonistic character displacement in pied flycatchers (*Ficedula hypoleuca*). At some locations males exhibit black and white plumage. Where they are sympatric with the congeneric *F. albicollis* (which are competitively dominant) the black feathers are brown, making them more similar in appearance to females of both species. Browner males receive less aggression and are more successful at establishing territories (Atlatlo *et al.*, 1994).

Differences in male signal traits are associated with reduced interspecific aggression in cichlid fish (Pauers *et al.*, 2007; Dijkstra *et al.*, 2006), damselflies (Anderson *et al.*, 2010; Tynkkynen *et al.*, 2004) and other taxa (Grether *et al.*, 2009). Aggression biases in some cases are stronger in sympatric populations, leading to suggestions that agonistic character displacement may be a significant process in evolutionary ecology (Grether *et al.*, 2009), facilitating co-existence of recently diverged species (Seehausen and Schluter, 2004). Field studies have suggested that there is reduced aggression between differently coloured but closely related cichlid fishes in Lakes Victoria (Seehausen and Schluter, 2004) and Malawi (Young *et al.*, 2009).

Reduced interspecific aggression need not only apply to males. Female haplochromine cichlids can be aggressive (*e.g.* Dijkstra *et al.*, 2008) and at least weakly territorial (Genner and Turner, 2005). Various traits that are thought to be important in interspecific aggression choices between males are also found in females (dorsal fin colour and other patches of pigmentation), and if a reduction in interspecific aggression for females results in a fitness benefit then this may amplify divergence of traits normally associated with male signalling.

The haplochromine cichlid fish of the African Great Lakes are ideal candidates for investigating the potency of agonistic character displacement and also the result of similar species meeting on secondary contact. This is because many species have geographic races that differ slightly in traits associated with secondary sexual characteristics such as dorsal fin colour, throat pigmentation or a combination (for an example see *Labeotropheus fuelleborni*, Konings 2007, page 30). Courtship behaviour is also highly conserved, even between disparate haplochromine cichlids (Myers, 1960; Ribbink, 1990) which can potentially lead to heterospecific male- male competition. Many sympatric taxa differ strikingly in colour while differing little in

ecology, suggesting divergence in colour may be due to some non-trophic interference competition if not sexual selection. Lastly, and perhaps most interestingly, secondary contact between recently diverged species/allopatric races is plausible. Water levels, in Lake Malawi for example, have been shown to rise and fall over geological time (e.g. Scholz *et al.*, 2007) potentially forcing allopatric species together. Young *et al.*, (2009) indirectly addressed this issue by focusing on cichlids at Thumbi West Island (Lake Malawi). This island provides the setting for an accidental experiment in what may happen when recently diverged taxa meet on secondary contact. During the 1970's many species from Nkhata Bay and other areas in the north of the lake were collected for the hobbyist aquarium industry and deposited at Thumbi West Island which happened to be near the base of a major aquarium fish exporter. After more than 30 years, Young *et al.*, (2009) found that the translocated fish neither outcompeted the native residents, nor did they disappear themselves, they concluded that this was due to the ability of dissimilar coloured species to coexist and speculated that species with dissimilar colours interacted less aggressively. Seehausen and Schluter (2004) proposed that whole cichlid communities in Lake Victoria may be influenced by aggressive interactions based on colour cues in secondary sexual characteristics.

### **Maternal aggression**

The second half of the research in this thesis focuses on female aggression in haplochromine cichlids and begins with maternal aggression. Maternal aggression is a part of parental care and additional investment in offspring as costs can be far from trivial (Archer, 1988). To understand maternal aggression, parental care and parental investment may need to be understood.



### *Parental care and parental investment*

'Williams principle' was one of the first theoretical treatments of parental investment theory and stated that parental care had not only a benefit but a cost to lifetime reproductive output (Williams, 1966a, b) and the aggressive defence of young may have significant costs for future reproductive success. Trivers (1972) presented two hypotheses which may allow predictions for when females alone should bear the costs of parental care and aggression in the face of predatory threats. The first relates to gametic investment, the second to uncertainty of paternity and potentially explains many examples of maternal aggression seen in a broad range of animal taxa.

### *Defence of young*

Parental aggression describes the act of protecting offspring from a predatory threat using aggressive behaviours at a cost to the parent (Archer, 1988). Predation of offspring is more likely to occur than predation on parents or adults, which are better equipped to defend themselves (Archer, 1988). Predation of gametes or juveniles is found throughout the animal kingdom (Ridely, 1978; Hrdy, 1979; Labov *et al.*, 1985) and parental aggression for the defence of eggs is found in many invertebrates *e.g.* polychaetes (Ridley, 1979), arachnids (Saito, 1986a, b) and crustaceans (Montgomery and Caldwell, 1982). Vertebrates also exhibit egg guarding behaviour *e.g.* teleost fish (Eisenberg, 1981), lungfish (Archer, 1988), amphibians (Ridely, 1978), and reptiles (Archer, 1988). Parental aggression is also seen in later stages of offspring development and is found in some invertebrates (*e.g.* tree hoppers of the family Membracidae, Tallamy, 1984) and in a wide variety vertebrates (Archer, 1988). It is likely that active defence of offspring by females is a more successful strategy against opportunistic conspecifics than heterospecific predators; which are

likely to be larger with greater weaponry (Maestripieri, 1992). Due to the significant predatory threat to offspring, it could be argued that active defence of young through maternal aggression is likely to evolve (Archer, 1988).

### *Cichlids and maternal aggression*

The vast majority of studies relating to maternal aggression focus on mammals, particularly rodents (Archer, 1988) yet teleost fish (including cichlids) have long been used in behavioural ecology and also show maternal defence of young through mouthbrooding and, in some cases, fry guarding. The cichlid fish are an extremely diverse group of teleost fish demonstrating great variability in reproductive strategies (Keenleyside, 1991; Barlow, 2003) and with many frequently used species much of their ecology and evolution is already known. Carlisle (1985) found that during field experiments with the South American cichlid *Aequidens coeruleopunctatus* females were more likely to abandon (artificially manipulated smaller broods) in the presence of a potential predator. Conversely it was shown that artificially larger broods changed female behaviour to give more care and the author argued that females opted for future prospects of offspring as opposed to past investment in terms of maximising inclusive fitness. Wisenden *et al.*, (2008) has discussed differences in male and female investment, with male investment associated with brood size whilst female investment relates to age of fry in a biparental species. Taborsky and Foerster (2003) have shown that the African cichlid *Ctenochromis horei* will hold onto broods longer than usual in the presence of a predator suggesting some flexibility or those maternal strategies may be context specific. Two species have been shown to increase aggression as broods develop (Oliveira and Almada, 1995: *Oreochromis mozambicus*, and Alonzo *et al.*, 2001: *Archocentrus nigrofasciatus*). Turner and Falter (1989) described how, in a dichromatic maternal mouth brooding cichlid species

(*Oreochromis niloticus*: Baerends and Baerends van Roon, 1950), females may display male breeding colours during the mouthbrooding phase. Visual displays have costs, changing colour can be metabolically expensive and may bring the attention of aggressive conspecifics or predatory threats and therefore must have some adaptive advantage in maternal care.

### Non-maternal female aggression

Female aggression in the animal kingdom is common though relatively understudied (Archer, 1988; Arnott and Elwood, 2009). We are just beginning to untangle the molecular basis of female aggression and whether phenotypic convergence between males and females is common or whether females and males use the same genes and hormonal pathways (*e.g.* Davis and Marler, 2003; Rubenstein and Wikelski, 2005; West-Eberhard, 2003; Aubin-Horth *et al.*, 2007). In males, testosterone appears to promote aggression during reproduction (Hau, 2007) but in females the link between aggression and testosterone is not as obvious. The handful of studies so far has led to contradictory results. In some cases there is clear evidence of testosterone during female aggression (*e.g.* Hegner and Wingfield, 1986; Woodley and Moore, 1999) but in others none was detected (*e.g.* Davis and Marler, 2003; Hay and Parhurst, 2005), leading to the tentative conclusion that testosterone may mediate female aggression in some contexts but not others (Gill *et al.*, 2007).

Most studies of female aggression relate to parental care (*i.e.* maternal aggression) which has been shown in a wide variety of taxa (see Archer, 1988). Female territoriality has been proposed to have originally evolved as a method for reducing conspecific predation of young; females may occupy territories for the sole purpose of settling contests with conspecifics before offspring are raised (Sherman *et al.*, 1981;

Wolff, 1985; 1998). There are far fewer studies focusing on female aggression where parental care is not invoked as a functional explanation.

It is possible that similar aggressive behaviours arise between males and females through different developmental pathways. This is believed to be the case in the Galápagos marine iguana, *Amblyrhynchus cristatus* (Rubenstein & Wikelski, 2005). Davis and Marler (2003) propose differential male and female biochemistry in *Peromyscus californicus* (the Californian mouse) regarding aggression and territoriality. This is opposed to when females employ hormones *etc.* normally selected for in males, as may be the case in the cichlid *Neolamprologus pulcher*, (Aubin-Horth *et al.*, 2007), although they were unable to disentangle reproductive status with dominance status (McGlothlin *et al.*, 2007).

#### *Female – female competition for mates*

There are a few examples which suggest that in some specific cases aggression may actually be used by females to gain access to mates. The conditions may be relatively rare as it has been proposed that similar parental roles, a female biased operational sex ratio (OSR) and variation in male quality are required (Berglund *et al.*, 1993). Yet despite these relatively rare conditions female competition for males has been shown. For example, in the red deer (*Cervus elaphus*) females are more aggressive in harems where mating order is at stake (Bebie and McElligott, 2006). Mating order may matter, it has been suggested that in Topi (*Damaliscus lunatus*), dominant males suffer sperm depletion (Bro-Jorgensen, 2002) and this may be the case in other ungulates too (*e.g.* Preston *et al.*, 2001). Female birds have also been shown to use aggression in competition for males (*e.g.* Lefelaar & Robertson, 1985). Female ornaments have also been shown experimentally to be used by males in mate choice

decisions and sexual selection through male choice may be driving the evolution of female signals (Amundsen and Forsgren, 2001). Although rare, sexually selected female aggression is conceivable given the right conditions.

Females may also be competing for refuge from predators or for food. However, aggression to obtain such resources may not specifically be a female trait; rather a trait found in the species as a whole. Where males and females are both aggressive over resources there is a propensity to focus on one sex or the other (Arnott and Elwood, 2009). Where there is strong dimorphism between sexes (for example, males have weaponry and females do not) and/or differences in the frequency of aggressive encounters (where males compete for females *and* resources such as food), natural selection may shape male and female behaviours separately (Archer, 1988). There are no studies that deal directly with the why's and how's of cichlid aggression that is non-maternal in nature. There are studies that deal with aggression biases, or dominance in Lake Victoria haplochromine cichlids (e.g. Dijkstra *et al.*, 2006; 2008). It has been proposed in these studies and others that frequency dependant selection may promote stable chromatic polymorphism. Understanding why and/or when females might be aggressive might reveal the significance of aggression across species as a whole and not just in territorial males and this is investigated towards the end of this thesis.

## Conclusions

### *Colour polymorphism maintained by aggression*

When all varieties of polymorphisms are considered, it is clear that nearly all species in the animal kingdom are polymorphic in probably many different ways. When the focus is narrowed to traits that have been commonly studied, for example colour, still

many species can be considered polymorphic. Negative frequency dependant selection appears to be responsible for much of the physiological and in particular colour, polymorphisms we see (Fisher, 1930; Lewontin, 1958; Ayala and Campbell, 1974). There is an increasing body of evidence suggesting that negative frequency dependant selection is a potent force in maintaining diversity within species through aggression (*e.g.* Dijkstra *et al.*, 2006; 2008). Haplochromine cichlids present an interesting example of polymorphisms. Colour polymorphisms and even sex linked colour polymorphism are seen in the animal kingdom but the complicated way in which the underlying genetics (see Seehausen *et al.*, 1999; Lande *et al.*, 2001; Kocher, 2004) determine which colour phenotype an individual develops may result in unusual consequences or a reduced likelihood of a stable equilibrium. There has been much discussion on whether much or some of the spectacular diversity of the East African cichlids is due to sympatric speciation by sexual selection (*e.g.* Turner and Burrows, 1995; Seehausen *et al.*, 1999; Barluenga *et al.*, 2006) but it seems this generation of polymorphism may provide too few males for divergence to occur in sympatry (due to the inability to mate assortatively because of very low rare male numbers). However, the maintenance of stable polymorphisms is still fascinating. Laboratory aggression trials using fish from Lake Victoria have revealed that overall a common morph biases may exist and that rare morphs may possess a negative frequency dependant advantage (*e.g.* Dijkstra *et al.*, 2005; 2006; 2008). What remains to be shown is just how this advantage is manifested in natural settings and whether same may be true in the other Great African Lakes, *e.g.*, Lake Malawi.

### *Interspecific aggression*

Aggression between individuals of the same species has been frequently studied. There are numerous reasons why animals use aggression to defend resources from

conspecifics. There are far fewer studies investigating interspecific aggression (Grether *et al.*, 2009). Fewer still have studied aggression between allopatric and recently diverged sister taxa. This is probably for good reason as normally interactions between allopatric populations are a null point as they are unlikely to meet. However, the haplochromine cichlids of Lake Malawi are unusual, not only in their spectacular diversity that may have evolved relatively quickly, but also because some recently diverged allopatric taxa could face secondary contact in the (geologically) near future .

The mbuna of Lake Malawi are highly territorial and use aggression frequently to compete for access to mates. Investigating how allopatric sister taxa, with very similar secondary sexual characteristics, see one another may reveal the nature of coexistence on secondary contact. One study (Pauers *et al.*, 2008) has investigated how some heterospecific mbuna react to one another and hint at what cue (dorsal fin colour) may be involved. However, there are no studies investigating allopatric taxa or investigations specifically focused on other potential colour cues used in aggression (*e.g.* throat pigmentation) or even other sensory modalities (*e.g.* olfaction).

### *Maternal aggression*

Females of many species clearly invest resources into offspring and promote their lifetime inclusive fitness with the ultimate goal of increasing reproductive output. Whether to invest in the present or the future is determined by a number of factors affecting individual females and varies in different species (*e.g.* Trivers, 1972; Williams, 1966a, b; Dawkins and Carlisle, 1975; Maynard Smith, 1977; Gross, 2005).

Cichlids have been used extensively in research relating parental care and investment (*e.g.* Carlisle, 1985) due to the ease at which they are manipulated in controlled

settings and the number of different parental/reproductive strategies they employ. Most work has focused on how cichlid parents interact with fry in varying conditions (*e.g.* Baerends and Baerends van Roon, 1950; Fryer and Iles, 1972) with fewer studies on how cichlid females react to predators of fry (Oliveira and Almada, 1995). Furthermore, the fascinating group of haplochromine cichlids have not yet been looked into.

### *Non-maternal female aggression*

Nearly all aggression studies focus on males, and whilst males are generally more aggressive, females of many species are increasingly being seen as aggressive (Archer, 1988; Arnott and Elwood, 2009). Much research remains on the hormonal nature of a female aggression whether it is common that females are aggressive via different genes/hormones (*e.g.* Davis and Marler, 2003; Rubenstein and Wikelski, 2005; West-Eberhard, 2003; Aubin-Horth *et al.*, 2007) and whether testosterone is required in female aggression across all contexts (Gill *et al.*, 2009).

### **Thesis outline**

The focus of the thesis is aggression between and within species of haplochromine cichlids and how natural selection may favour individuals who receive less aggression or give aggression to the “right” individuals at the appropriate time. As stated above the nature of aggression biases has been investigated in Lake Victoria haplochromine cichlids but not in their Lake Malawi counterparts. **Chapter 2** investigates the nature of aggression biases within polymorphic mbuna cichlid species. Do the rarer morphs receive less aggression than common morphs? This chapter also investigates the frequency of territory holding male rare morphs compared to common morph males, do they do better than expected and does this tie in with receiving less aggression?



**Chapter 3** Investigates the use of colour in dorsal fins as an aggression cue using two allopatric Malawian mbuna cichlids. Is dorsal colour alone sufficient to result in assortative aggression by two otherwise very similar mbuna species? This chapter also investigates the cues used by females in aggression. The two mbuna species investigated differ only in throat colour, sharing similar dorsal fin colour: are these subtle differences enough to promote assortative aggression in two closely related species?

Aggression may be a costly behaviour and risky behaviour but aquaria observations suggest brooding cichlid females use aggression during the brooding phase. **Chapter 4** compares two haplochromine cichlid species in a maternal aggression context. One species is considered the ancestral form in that it provides post release brood care to its fry whereas the second species does not. The chapter looks at levels of aggression towards different types of intruder during the brooding period.

Female aggression is a relatively understudied field. Females may be aggressive or a number of reasons and the reasons that haplochromine cichlids are aggressive and may add to our understanding of the importance of aggression in the diversity we see in the African Great Lakes. **Chapter 5** investigates non-maternal female aggression in two Malawian haplochromine cichlids. The type of aggressive behaviours they use is compared to males, and the hypothesis that females may be using aggression to compete for males or refuges is also investigated. Furthermore, hypotheses based on when during the day females may be aggressive are also investigated.

Lastly, **Chapter 6** describes and discusses the main findings of this thesis and suggests future avenues of research based on the findings herein.

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## Chapter 2

# Aggression and intraspecific colour polymorphism in haplochromine cichlid fish

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### Abstract

Over the last decade theoretical and experimental work has developed the idea of sexual selection being responsible for the maintenance of colour polymorphisms

within a number of East African Cichlid fish species. An extension of this idea focuses on male territoriality/aggression and how a negative frequency dependant fitness advantage to rare morphs may promote stable polymorphic populations. The aim of this study, using Malawian rock dwelling cichlids, was to investigate how different colour morphs behave towards each other and whether or not the rare morph individuals may gain an advantage by being rare. Two way laboratory aggression trials were performed using males and females of *Psuedotropheus zebra*, with field work focusing on two *P. callainos* morphs. Field work determined ratios of morphs in the general population compared to ratios of territorial males, distance and morph of nearest territorial neighbour, feeding rates and aggression given and received. Results suggest that in Lake Malawi rock dwelling cichlids rarer morphs may receive less aggression than the common morph. Furthermore the least abundant *P. callainos* morph may gain an advantage that is manifested in greater numbers of rare territorial males than expected, increased grazing rates and reduced aggression received.

## **Introduction**

Polymorphism is described as variation within a single population with the frequency of any single morph not being due to a simple recurring mutation (e.g. Roulin, 2004; Gray and McKinnon, 2007) and some haplochromine cichlid populations are polymorphic at seemingly stable frequencies (Seehuusen *et al.*, 1999). How colour polymorphisms are maintained in natural populations has been discussed for at least 50 years (e.g. Huxley, 1955; Dijkstra *et al.*, 2010). Recently work has focused on



whether sexual selection drives polymorphism towards divergence in sympatric populations in haplochromine cichlids (Dijkstra *et al.*, 2005, 2006, 2008).

### *Generation of polymorphisms*

Of the great number of haplochromine cichlid species many are polymorphic for colour and there has been some focus on the well known 'blotched' polymorphism which is seen in the haplochromine tribe within African Great Lakes (Lande *et al.*, 2001; Kocher, 2004). In Lake Victoria there are a number of species (*e.g. Neochromis omnicaeruleus*) that have 'white blotch' and 'orange blotch' individuals alongside a more common 'plain' morph (Dijkstra *et al.*, 2007). A similar situation is found in Lake Malawi where 'orange blotch' and 'orange' individuals are found in many 'mbuna' (rock dwelling) species again alongside a more common 'plain' morph (Genner and Turner, 2005). However Lake Tanganyika has far fewer, if any examples yet shows a similar radiation of haplochromine cichlids. *Tropheus moori* populations can contain a colour morph that looks similar to the orange blotch phenotype found in both Lake Victoria and Lake Malawi although these individuals are rare and one of many types of colour variants found within this species (Konings, 2007.). The genetic basis for this polymorphism has been debated (*e.g.* Kocher, 2004; Streelman *et al.*, 2003; Lande *et al.*, 2001; Allender *et al.*, 2003; Roberts *et al.*, 2009) with a complex genetic process having been proposed which requires the 'blotched' pigment phenotype being linked to a emerging female sex determining gene (*e.g.* Kocher, 2004; Lande *et al.*, 2001; Seehuusen *et al.*, 1999; Roberts *et al.*, 2009). What is clear is that the vast majority rare morph individuals are female (Lande *et al.*, 2001). Recent work (Roberts *et al.*, 2009) has shown that there is likely to be a single genetic origin for OB morphs within Lake Malawi making comparisons of recognition mechanisms of morphs across genera appropriate. This supersedes earlier work

(Allender *et al.*, 2003) which stated that the OB morph was caused by a novel mutation in each lineage (see Chapter 1 for further discussion on this topic).

### *Maintenance of polymorphisms*

Polymorphism may evolve under natural or sexual selection (Roulin, 2004).

Heterogeneous environments may promote the coexistence of different colour morphs within the same population. The Grove snail (*Cepaea nemoralis*) has a number of different morphs which are more or less successful depending on the specific location this widespread species is found in and which visual based predators are operating (Cain and Sheppard, 1950). In cichlids, common morph females are often less conspicuous (see Chapter 1 for discussion on cichlid vision) than their brightly coloured male counterparts and it has been suggested that blotching may provide an alternative form of crypsis by increasing background matching with the algae covered rock substrate (Roberts *et al.*, 2009). There is, however, some dispute about the camouflage properties of blotch phenotypes and whether they increases (*e.g.* Seehausen *et al.*, 1999; Maan *et al.*, 2008) or decreases (*e.g.* Greenwood, 1956; Snoeks, 1994; Roberts *et al.*, 2009) detectability and predation risk (see also Konings, 2007 p19 for photographic examples of cryptic and conspicuous OB individuals).

Disruptive selection may promote stable colour polymorphism within one population if the environment is heterogeneous or individuals move between differing light conditions (Gray and McKinnon, 2007). Rock dwelling haplochromine cichlids operate in visually diverse rocky shores that have extremely variable rock sizes. It also true that light or the wavelengths of light available is strongly determined by depth (see Seehausen *et al.*, 2009 for how depth and light availability may promote sensory drive in haplochromine cichlids) from the surface and species such as *P. zebra* (which are polymorphic for colour) have ranges between zero and 40 metres at some locations

(Ribbink *et al.*, 1983) and it has been suggested that the OB morph may be cryptic to avian predators in the shallows whilst conspicuous at deeper depths (Streelman *et al.*, 2003).

### *Colour polymorphism maintenance through sexual selection*

Colour polymorphism is cited as a key component when discussing how sexual selection may cause divergence (*e.g.* Turner and Burrows, 1995; Salzburger *et al.*, 2006; Knight & Turner, 2004; Seehausen *et al.*, 1999). In recent years there has been a focus on sexual selection and its role in the diversification of haplochromine cichlids (*e.g.* Seehausen & van Alphen, 1998; Knight & Turner, 2004). It has been proposed that two mechanisms of sexual selection may aid maintenance of colour polymorphism within a population within the haplochromine cichlids. Firstly, sexual selection through female choice (*e.g.* Knight & Turner, 2004; Seehausen *et al.*, 1999). Females are believed to at least in part base their reproductive decisions on visual cues (*e.g.* Couldridge, 2002). There is evidence that suggests that the Lake Victorian cichlid *N. omnicaeruleus* morphs may mate assortatively (*e.g.* Lande *et al.*, 1999; Seehausen *et al.*, 1999), however another study (Pierotti *et al.*, 2009) suggests that in *P. zebra* (Lake Malawi) males prefer the rarest morph.

Secondly, intrasexual aggression may also be responsible for maintenance of colour polymorphisms between individuals of the same population (*e.g.* Mikami *et al.*, 2004; Seehausen & Schluter, 2004; Dijkstra *et al.*, 2007; Dijkstra *et al.*, 2009).

Haplochromine cichlids sometimes bias aggression to their own morph (Dijkstra *et al.*, 2007). Common morph only aggression biases have also been proposed (but not shown) in the Lake Malawi *P. zebra* (Pierotti *et al.*, 2007)

Learning or imprinting with respect to morph association may play a role in mate choice (Verzijden & ten Cate, 2007) and it may be common in birds (ten Cate & Vos, 1999) and is also documented in mammals (Kendrick *et al.*, 1998). Cross fostering experiments using Lake Victoria haplochromine cichlids (Verzijden & ten Cate 2007) strongly suggest that females choose males that exhibited the biological mother's phenotype. However, other studies (using Central American cichlids) do not fully support this (Siepen & Crapon de Caprona, 1986). The former experiment used species that 'mouth guard'; mothers will take fry back into the buccal cavity when they are threatened. This is an ideal opportunity for visual imprinting - if females exhibit the relevant phenotypic diversity (Verzijden *et al.*, 2008). Aggression choices or biases however appear not be under the control of imprinting in at least some haplochromine cichlids (Verzijden *et al.*, 2008) where experiments, again using a Lake Victoria haplochromine cichlid, did not reveal maternal phenotype biases after cross fostering. Males do not appear to be subjected to imprinting but that does not mean aggression biases cannot be learnt. Dijkstra *et al.* (2008) showed that in a Lake Victoria haplochromine cichlid (*Pundimilla* sp.) blue males that had no prior experience of red males did not show any aggression bias when presented with a blue and red intruder. However, when blue males had some prior experience of red males they showed aggression biases towards other blue males.

#### *Rare morph advantage – frequency dependant selection*

Within a polymorphic population negative frequency dependent selection may favour the least abundant morph (*e.g.* Dijkstra *et al.*, 2009). This occurs because of own morph aggression biases favour rarer morph individuals that are likely to engage in fewer aggressive encounters. If the common morph represents the ancestral condition there may be a genetically determined bias towards attacking common morphs, which

would allow a frequency dependant advantage to rare morphs (Seehausen *et al.*, 1996). Any advantage may allow greater ease of forming a territory - a pre-requisite for reproduction in males (*e.g.* Maan *et al.*, 2004). A rare morph male that has fewer conflicts (due to a lack of recognition of the rare phenotype being conspecific) whilst rare may have more feeding opportunities, less risk of injury and a reduced chance of predation through ease of refuge acquisition (Dijkstra *et al.*, 2007).

Whilst rare morph males may enjoy a greater advantage through negative frequency dependant selection, females can also benefit. Female haplochromines are believed to be at least weakly territorial (Dijkstra *et al.*, 2007; Seehausen *et al.*, 1999) and are seen to be frequently aggressive in aquaria. Furthermore, females may defend fry or places where fry are released (Seehausen, 1996) which may be another situation in which rare morph individuals gain an advantage. Indeed, female competition has been proposed as an important mechanism for maintenance of polymorphism in some haplochromine cichlids (Dijkstra *et al.*, 2007).

Some authors (*e.g.* Snoeks, 1994) have proposed that 'blotched' morph individuals may enjoy some additional advantage due to increased camouflage. Yet a recent experimental study by Mann *et al.*, (2008) proposed that the 'blotched' morph individuals (*N. omnicaeruleus* – Lake Victoria) suffer greater predation through conspicuousness compared to drabber individuals (also proposed by Lande *et al.*, 1999). Whilst a potentially sensible explanation as to why so few male 'blotched' morph fish exist it fails to explain why in *P. callainos* (Lake Malawi) white morph males maybe very abundant at some locations (see Fryer, 1959). 'White' morph fish appear to be much more conspicuous, compared to 'blotched' morph fish in other species, and are found in shallow waters (Ribbink *et al.*, 1983) where similar avian

predators (e.g. *Haliaeetus vocifer*, *Phalacrocorax lucidus*, *P. carbo*, *Ceryle rudis*, *Ardea* spp.) often feed with similar predation regimes as those found in Lake Victoria.

## **Aims**

The first aim of this study was to investigate whether there is a bias in aggression towards morphs from within *Pseudotropheus zebra*.

- Do morphs show assortative aggression in laboratory conditions?

Should own type biases occur in aggression choices then the least abundant morph may have a frequency dependant advantage. Therefore the second aim of this investigation is to look for evidence of any advantage a rare morph may have by observing fish in the wild.

- Do rare males receive less aggression in the wild?
- Are there more territorial rare morph males than expected?
- Do rare morphs gain in other ways?

## **Methods**

For a full description of the study species used in this investigation see the appendix at the end of this chapter.

### *Laboratory trials – males and females*

Territorial resident BB *P. zebra* males were tested for aggression biases when simultaneously presented with a male intruder of one of the rare morphs and BB morph from the same population (Nkhata Bay). Due to numbers remaining after the first set of experiments only BB males were used as residents.

Nine BB males were isolated from isolated stock tanks and used as territorial residents (mean standard length = 98.77mm). Four pairs of BB and OB males and three pairs of BB and O males were separated from stock tanks. All stimulus pairs were sized matched as closely as possible for standard length ( $\pm 5$ mm, mean length of all intruders = 97.71mm). The isolated males were held in small glass tanks but adjacent to other Mbuna species. Males were 'coloured' up, indicating that they considered themselves to be dominant individuals which also provided evidence that the focal fish were healthy and in good condition (G.F. Turner pers. comm.)

Each focal was tested with all seven pairs of stimulus fish resulting in 63 trials. Focal fish were not used as stimulus fish. Each pair was unique. Trials lasted for ten minutes and involved placing stimulus fish into plastic jars equidistant from a central brick refuge which the focal invariably used as a refuge/territory. Tanks were 1.5 meters long, with air drive filters placed centrally alongside a 300 watt aquarium heater. All cues were made available to the intruders by piercing the plastic jars, allowing chemical communication. All types of aggressive behaviours were recorded. Stimulus fish were added randomly to either side of the central brick refuge. Focal fish were given 24 hours to acclimatise and given at least 24 hours between being tested with the different intruders. All males were lab bred offspring from wild caught *P. zebra* stocks (Nkhata Bay).

*P. zebra* females were wild caught from Chiofu Bay, Lake Malawi in 2008. Ideally females from the same population as the males (Nkhata Bay) would have been used but no females from this source were available. Mate choice experiments (Blais *et al.*, 2009) found that the populations showed a significant degree of assortative mating, suggesting the two populations may well be insipient species. As the aim of this investigation focuses on what *Pseudotropheus* spp. are doing with respect to

aggression biases and the manifestations of negative frequency dependent advantage comparing overall trends may be appropriate with due caution. Roberts *et al.*, (2009) report that there is likely to be a single origin of OB/O polymorphism within Lake Malawi, so although the populations (which may well be insipient species) differ, responses to common or rare morphs may be identical.

16 (eight BB morph and eight OB morph) females were available and paired (one of each morph) by size matching (standard length to within  $\pm 4$ mm). Each focal female (8 of each morph) was tested with 3 randomly chosen pairs of the size matched fish.. 48 trials (24 for each focal type) were conducted in total. Trials lasted for five minutes; this was shorter than the male trials and allowed a greater number of trials to be performed in a shorter time period. Five minutes has been used by other researchers using female haplochromine cichlids (*e.g.* Dijkstra *et al.*, 2008) which found that females were sufficiently aggressive within this time frame to provide enough data for meaningful statistical analysis. Only healthy females were used as indicated by their behaviour (posture *i.e.* fins clamped, feeding vigorously, good body shape). When being used as focal fish females would invariably use some colour changes typically seen in male signalling. Although not as conspicuous as males they were clearly being territorial, another indication of good condition and health.

Both males and females were kept in temperatures between 24 and 26 degrees Celsius, kept on 12L12D light cycles and fed daily on tropical fish flake.

The results for each focal type (male's females, BB or OB morph) were pooled and a mean number of aggressive behaviours given to each type of intruder were calculated. This gave an impression on what a focal might do on a consistent basis rather than a



single trial. This might be important given the relatively low ( $n = 9$  for males and  $n = 8$  for either female morph) number of focal fish for each species tested.

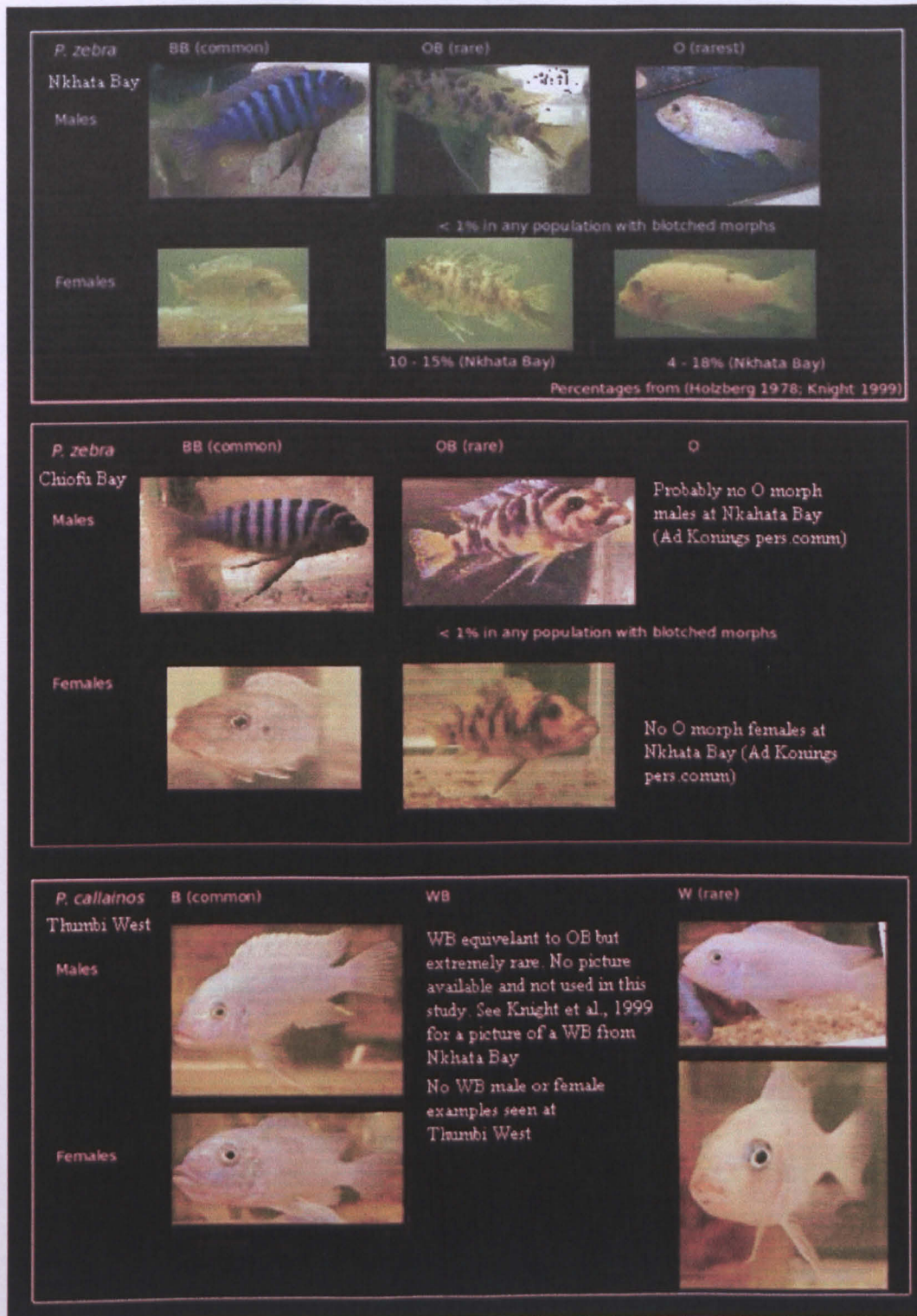


Figure 1 Photographs of the species, and morphs within species, used in this chapter. Photographs taken by A. Tyers except for the Nkhata Bay O morph male, taken by A. Smith

### *Field work*

Field work was carried out over nine days during July of 2010 at Thumbi West Island, Lake Malawi (S 034° 43.539 E 014° 01.123). The field work for this study focused entirely on *P. callainos* and was chosen due to the abundance of the 'rarer' morph (W) within this species compared to *P. zebra* OB or the O morph at this location. Only two rare morph females and no rare morph males were found for *P. zebra* were found. Ideally comparing laboratory tested species with their wild equivalents would have been ideal but this was not possible as explained above. As the two species are within the same genus, with practically identical life histories/behaviour/morphology and share the same genetic mechanism responsible for the colour polymorphisms (Roberts *et al.*, 2009), it is arguable that the species may be comparable.

### *Morph Frequencies*

Initially the proportion of each morph in the general population was required to determine whether the W morph had some advantage that manifested itself via disproportionately high number of territorial W males. This was achieved by counting each fish of the focal species along three 30 metre transects at a depth (2-5m) where they are most abundant (see Ribbink *et al.*, 1983 for abundances at this location) the author of this chapter carried out all three counts. Fishing twine was cut to a 30m length and weighted with stones and laid at a depth of approximately four metres. Snorkelling along this transect, fish were counted and recorded with regard to their colour. Fish approximately 0.5m either side of transect were included and comprised of territorial and non- territorial individuals. This gave an estimate of the frequency of

each morph at this particular location. Without catching individuals it was impossible to determine the ratio of each morph for each sex. A conservative assumption would be that each sex contained an equal proportion of W and B morphs given that at other locations and in other species the 'rare' morph frequency found in males may be as little as less than 1%.

### *Territory mapping*

The frequency of territorial males of each morph was counted from maps drawn from 5x5m quadrats and the method is as follows. Using the same fishing twine 5 x 5 metre lengths were cut and made into squares by tying 4 ends together. A swimmer (G. M. Cooke) took these to a suitable location within the same bay that the general population frequencies were surveyed. Once the quadrat was in place an undergraduate volunteer (A. Scott) recorded all the rocks and boulders found within this quadrat. Accuracy was achieved by drawing a scaled quadrat outline on a plastic dive slate in pencil. Once all rocks and boulders were transposed territorial fish of the focus species were observed until it was apparent where the central point of its territory was. The central point of the territory was determined by observing where the territorial fish swam to when it courted a female in an attempt to spawn with it. It was found that the territorial males would often swim, to what was always a crevice or rocky cave, frequently irrespective of a female's presence. These central points were used in calculating the number of territorial individuals for each morph and mean distances to nearest territorial neighbour of either morph. After a dive slate containing the quadrat was completed it was traced onto a transparent piece of graph paper (see figure A2.4 in the appendix). This allowed measurements of territory distances to be calculated with a reasonable degree of accuracy. In total nine quadrats were analysed this way. Not all territorial males within the quadrat were measured

with respect to nearest neighbour. This was because males on the edge of the quadrat may have had their nearest neighbour outside of it. To compensate for this an inner scaled 3 x 3m square was drawn over the traced quadrats. Males within this inner square had their nearest neighbours measured. Males outside of this inner square were counted as neighbours to males within the inner square but not as focal males for measurement to nearest neighbour.

The territory distance data was analysed and presented with respect to the distances each territorial male (B or W) kept the different types of other territorial neighbours: The distance to the nearest B neighbour was compared for B and W focal morphs, for example. This is as opposed to comparing the distances each territorial morph kept each type of territorial neighbour which might be influenced by the fact that there were more territorial B males than W males. The latter method was also employed and the results can be found in the appendix.

#### *Focal watches*

The method was similar for both morphs and both types of individual (territorial and non-territorial). Non-territorial individuals were watched first. An individual was chosen and followed for five minutes. For the non-territorial fish initially both grazing and gulping (of particulate food matter) were recorded but gulping became difficult to distinguish on days with high turbidity and was subsequently abandoned. The conspecific morph that directed aggression towards non-territorial focal was also not recorded. This was because it would often happen whilst the focal fish was grazing and the attacker often caused the focal fish to flee. This however was not the case for territorial individuals where it much easier to see who the aggression was being directed at.

## Data Analysis

Data sets were analysed with respect to distribution and equality of variances. If assumptions for parametric testing were not met transformation attempts were made.

See Appendix for analysis of data sets.

## Results

### Laboratory trials – *P. zebra*

Focal *P. zebra* BB males showed significantly more aggressive behaviours towards BB morph males than to O morph (Paired t-test,  $t = 6.779$ , d.f. = 8,  $p < 0.001$ ) or OB morph (paired t-test,  $t = 6.235$ , d.f. = 8,  $p = 0.001$ ).

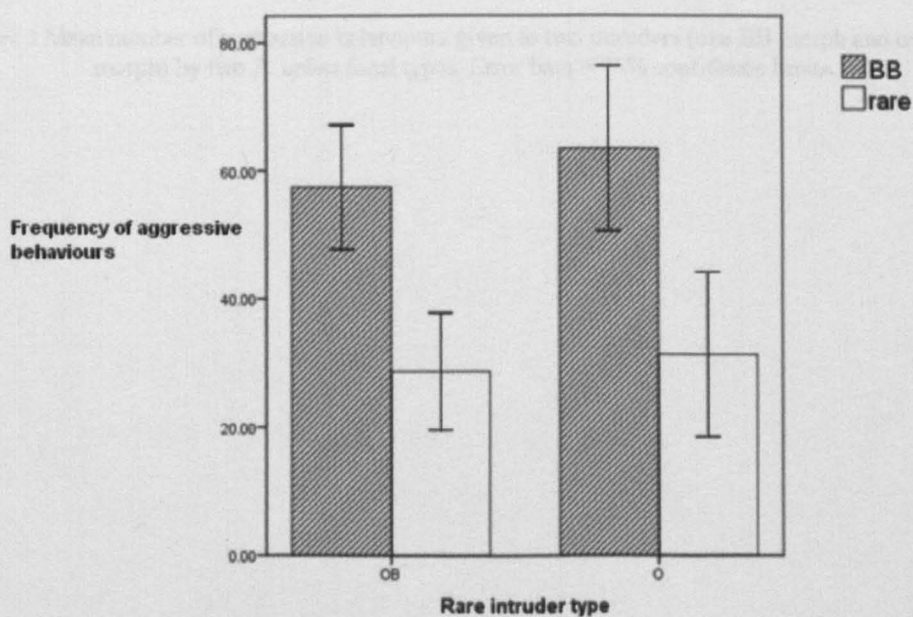


Figure 2 Mean number of aggressive behaviours (and 95% confidence limits) given to a rare morph intruder or a common morph intruder by the *P. zebra* focal common morph males. N = 9 focal fish used.

BB *P. zebra* focal females showed a significant bias towards attacking BB females (Paired t-test,  $t = 2.100$ , d.f. = 7,  $p = 0.03$ ). OB females showed no significant bias towards either intruder morph (Paired t-test,  $t = 0.222$ , d.f. = 7,  $p = 0.4139$ ).

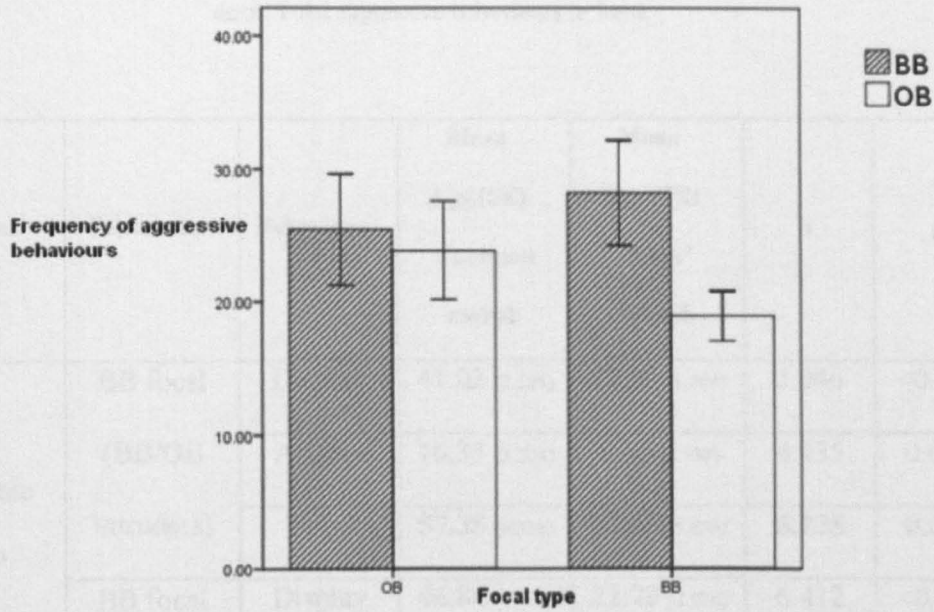


Figure 3 Mean number of aggressive behaviours given to two intruders (one BB morph and one OB morph) by two *P. zebra* focal types. Error bars = 95% confidence limits.

Table 2 Breakdown of types of behaviour (attacks and displays) including totals for both males and female in laboratory experiments with results of paired t-tests. N = 9 males and n = 8 females. Male focal BB fish were tested against either BB/OB intruders or BB/O intruders over ten minute trials. Female BB focal fish were tested against BB/OB intruders as with OB focal females. Focals tested more than once so results pooled with averages taken. S.E. = standard error. Total aggressive behaviours in **bold**.

Sex	Experiment	Behaviour	Mean Agg.(SE) Common morph	Mean Agg.(SE) 'Rare' Morph	t	p
Male <i>P.</i> <i>zebra</i>	BB focal (BB/OB intruders)	Display	41.02 (2.684)	22.00 (1.884)	5.046	<0.001
		Attacks	16.33 (3.204)	6.65 (2.730)	4.135	0.001
		<b>Total</b>	<b>57.35 (4.212)</b>	<b>28.65 (3.525)</b>	<b>6.235</b>	<b>0.001</b>
	BB focal (BB/O intruders)	Display	46.84 (3.400)	22.28 (2.934)	6.412	<0.001
		Attacks	16.50 (3.339)	8.94 (3.086)	3.207	0.006
		<b>Total</b>	<b>63.34 (5.628)</b>	<b>31.22 (5.559)</b>	<b>6.779</b>	<b>&lt;0.001</b>
Female <i>P.</i> <i>zebra</i>	BB focal (BB/OB intruders)	Display	12.91 (2.526)	7.33 (1.264)	2.687	0.015
		Attacks	15.29 (3.188)	11.62 (2.219)	1.360	0.10
		<b>Total</b>	<b>28.20 (5.710)</b>	<b>18.95 (3.480)</b>	<b>2.100</b>	<b>0.03</b>
	OB focal (BB/OB intruders)	Display	14.36 (3.099)	11.86 (2.819)	0.610	0.28
		Attacks	11.11 (2.744)	12.03 (2.310)	0.270	0.39
		<b>Total</b>	<b>25.47 (5.844)</b>	<b>23.89 (5.129)</b>	<b>0.222</b>	<b>0.41</b>

*Field work – ratios of each P. callainos morph*

51 *P. callainos* B non-territorial individuals were counted compared to 23 W non-territorial individuals, whereas there were 83 territorial B males compared to 59 W

territorial males (see appendix I for table of actual counts) with the difference in observed and expected ratios of territorial males of each morph being significantly different ( $G = 6.91$ , d.f. = 1,  $p < 0.009$ ), with there being significantly more W territorial morphs than expected.

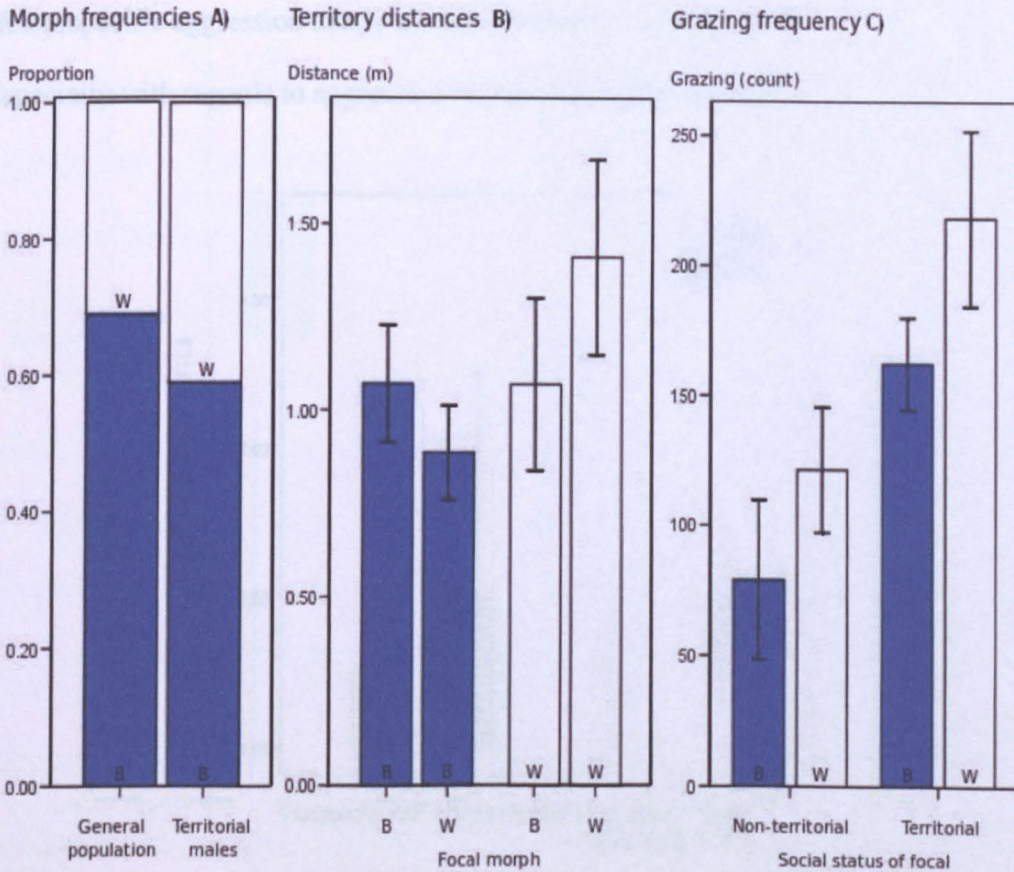
*Field work – territory distances between P. callainos morphs*

When data for both morphs is combined there is a significant difference (ANOVA:  $F_{1, 103} = 9.53$ ,  $p = 0.003$ ) in the distance between focal fish and nearest same morph (mean 1.24m) and nearest different morph (mean 0.98m). Distances to nearest neighbours were compared for each morph. When nearest B neighbour from focal fish of each morph was compared it was found that B territorial males kept other B territorial fish further away than W territorial males did although this was not quite significant (ANOVA,  $F_{1, 52} = 3.451$ ,  $p = 0.069$ ). W focal males kept other W territorial males significantly further away than B territorial males did (ANOVA,  $F_{1, 52} = 3.489$ ,  $p = 0.049$ ), as would be expected given that B are more common

*Field work – grazing by P. callainos individuals*

Over 10 minute focal watches territorial W males ( $n = 9$ ) grazed significantly more often (ANOVA,  $F_{1, 16} = 10.374$ ,  $p = 0.005$ ) than territorial B males ( $n = 9$ ), and non-territorial W individuals ( $n = 9$ ) grazed significantly more often (ANOVA,  $F_{1, 16} = 6.292$ ,  $p = 0.023$ ) than non-territorial B individuals ( $n = 9$ ).





**Figure 4** a) A comparison of the proportion of the two morphs in the general population with the proportion of the two morphs among territorial males found that W morph territorial males are more common than would be expected. **b)** Mean territory distances for B and W territorial males for each type of neighbouring morph. Error bars show 95% confidence intervals. **c)** A comparison of grazing behaviour between morphs found that both non-territorial and territorial rare morph (W) fish grazed significantly more than their common morph (B) counterparts. Error bars show 95% confidence intervals.

### Field work – aggression towards and received by *P. callainos* morphs

W and B territorial males did not differ in the number of attacks on heterospecifics (Mann-Whitney U:  $Z = -1.325$ ,  $n = 9$ ,  $p = 0.185$ ). W and B territorial males did not differ significantly in the number of attacks received from heterospecifics (Mann-Whitney U:  $Z = -1.701$ ,  $n = 9$ ,  $p = 0.083$ ), however there was a trend towards blue morph males receiving more aggression.

Heterospecific aggression nearly always involved *P. elongatus* 'slab'.

Especially with regards to aggression *received* from heterospecifics

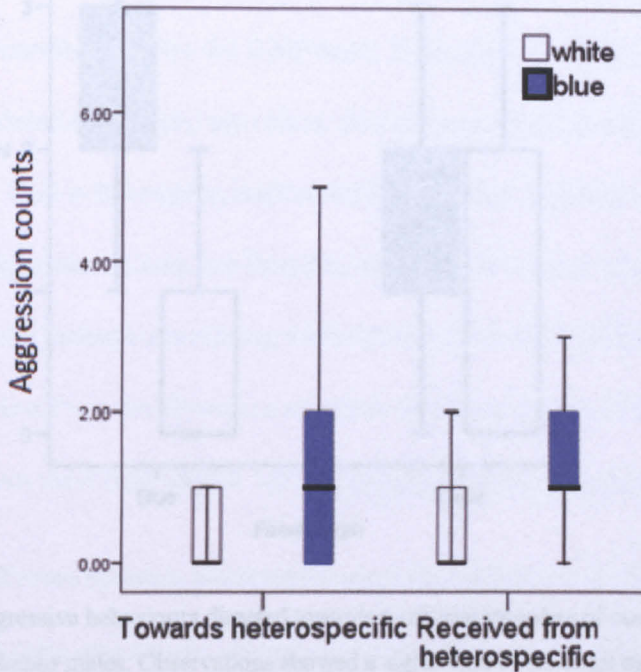
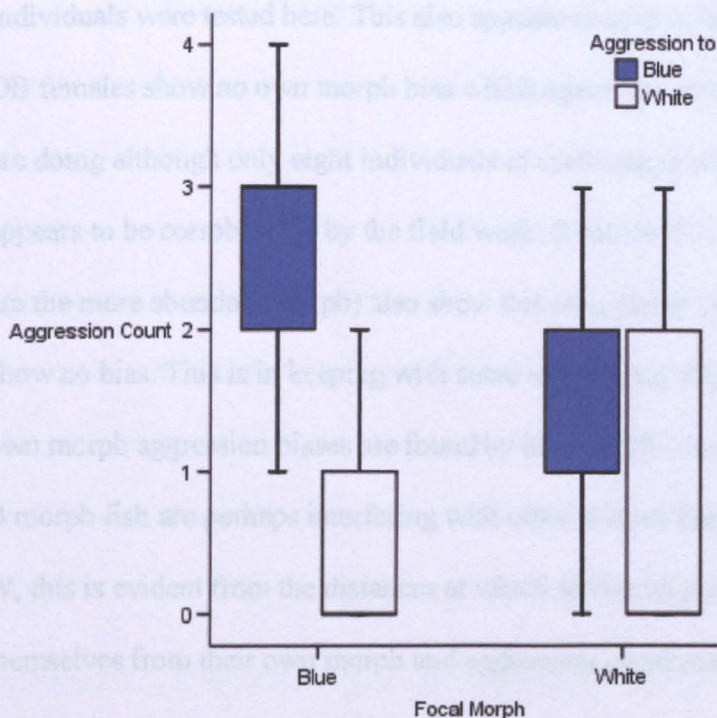


Figure 5 Median number of aggressive behaviours received from heterospecifics and given towards heterospecifics by territorial males of each morph. Although a trend is seen regarding greater aggression received by B males from heterospecifics, there was no significant difference. Error bars show interquartile range

## Discussion

*P. callainos* B territorial males directed significantly more aggression at B individuals (Wilcoxon matched pairs:  $Z = -2.328$ ,  $d.f = 17$ ,  $p = 0.002$ ). However, *P. callainos* W territorial males showed no significant difference in the number of aggression behaviours directed at either morph (Wilcoxon signed ranks for matched pairs:  $Z = -0.604$ ,  $d.f = 17$ ,  $p = 0.546$ ) over a ten minute period.



**Figure 6** Aggressive behaviours directed towards territorial intruders of each morph, by territorial *P. callainos* males. Observations showed a significant own morph aggression bias by the B morph and no significant bias by the W morph. Error bars show interquartile range.

## Discussion

The results of this study suggest that, in Lake Malawi rock dwelling cichlids, rare morph individuals may receive less aggression than common morph individuals and that this advantage can possibly be translated into a fitness increase in natural settings. This is further suggested by the observation that there are significantly more territorial W males than expected and that W individuals are receiving less aggression and grazing more often B individuals.

In laboratory trials the BB males significantly show an own morph bias when presented with their own morph and a rare morph (either OB or O) and this may be indicative of what populations of these fish may do on average although only nine

individuals were tested here. This also appears to be true for female BB females yet OB females show no own morph bias which again may represent what wild females are doing although only eight individuals of each morph were tested. This bias appears to be corroborated by the field work. B morph *P. callainos* individuals (which are the more abundant morph) also show this own morph bias whilst W individuals show no bias. This is in keeping with some studies (e.g. Dijkstra *et al.*, 2006) where own morph aggression biases are found by one morph but not another. Furthermore, B morph fish are perhaps interfering with other B more than W are interfering with W, this is evident from the distances at which territorial males of each morph find themselves from their own morph and aggression given to non-territorial individuals.

Comparing different species, particular during the laboratory work and the field work may be problematic and should always be treated cautiously. Allender *et al.*, (2003) showed that the OB morph had repeatedly appeared in mbuna populations which may reduce the appropriateness of comparing *P. zebra* laboratory aggression bias trials and *P. callainos* field results provided here. However, more recent work (Roberts *et al.*, 2009) contradicts Allender *et al.*, stating that there may well be a single origin of OB (and therefore O/W too). This would make comparing aggression biases and the manifestations of reduced aggression more appropriate.

The results from this study suggest, along with other studies (e.g. Dijkstra *et al.*, 2006), that rare morphs have an advantage and that this advantage is potentially negatively frequency dependant. The field work aspect appears to show that the rare morph (W) may have an advantage. W individuals graze significantly more often than B individuals which may have daytime anti-predator benefits. Epilithic algae' grazing has been cited as an important method of nutritional gain in mbuna (Reinthal, 1990) although planktonic diatoms also make up a considerable part of the diet of some

species (e.g. *Cynotilapia afra*). By grazing, the fish are close to an immediate refuge should a predator strike. Avian predators, such as fish Eagles, are likely to be more successful predating individuals in open water than predating individuals that are close to a rocky refuge (although this has never been tested). Presumably the non-territorial B individuals have to make up the lost grazing opportunities by foraging in the water column where avian predators are likely to be more successful especially at the shallow depths preferred by *P. callainos* (see Ribbink *et al.*, 1983) whereas individuals who are grazing are naturally much closer to an escape into a rocky shelter.

Mann *et al.*, (2008) showed that some OB morph haplochromine cichlids are potentially more vulnerable to predation, using field experiments. White blotch (WB) *P. callainos* individuals are extremely rare, much rarer than the W morph at Thumbi West Island; it may be that predation has a significant affect on *P. callainos* WB individuals as they unlikely ever to be camouflaged against rocks as OB individuals can be in other polymorphic haplochromine cichlids (see Konings, 2007, page 19). However, W morphs are arguably even more conspicuous (see Chapter 1 for discussion on cichlid visions) than the equivalent orange (O) individuals in other haplochromine cichlid species (e.g. *P. zebra*) and perhaps conspecific WB individuals too. In many other non- cichlid species predation is likely to be an important mechanism in the generation of colour polymorphism and maintained by anti-predator benefits in heterogeneous environments (e.g. Hall *et al.*, 1966; Owen, 1967; Reid, 1987; Cain and Sheppard, 1950) whereas in this system polymorphism may be maintained despite the potential (Mann *et al.*, 2008) for increased predation of one morph. This may be due to status signalling where conspicuous signals are favoured by females as an indicator of high fitness (e.g. Barlow, 1983; Ellingson, 1994).

However, there is scant evidence that female haplochromine cichlids preferentially mate with rare morph males, although some evidence suggests that males may choose the rare morph females (in *P. zebra* at least, Pierotti *et al.*, 2009). It is perhaps also unlikely that rare morph individuals will disrupt the search image of predators (see Hall *et al.*, 1966; Owen, 1967) that operate on rocky shores as there is no evidence to suggest that predators choose specific fish species or morphs given that many species coexist and are similarly available to predators. The rocky shore is made up of numerous species that vary strongly in colour so the “oddity effect” (*e.g.* Snekser *et al.*, 2010) may not have as strong an effect as seen when large shoals contain single species with members all looking alike.

As stated above, this study has shown that territorial blue males graze less which seems to coincide with an increased amount of conspecific and heterospecific aggression received. If one morph received less heterospecific aggression then it may have an advantage and disruptive selection on traits that reduce heterospecific aggression may play a significant role in shaping cichlid communities (Seehausen and Schluter, 2004). If conspecific aggression biases can be learnt then heterospecific aggression can be too. As *W P. callainos* individuals increase in frequency then heterospecifics may target them more.

The clearest and potentially most important indicator that rare morph advantage exists is found in the frequency of W morph territory holders which is significantly greater than expected from counts of each morph in the general population. Although the comparison should be between non territorial males and territorial males of each morph this was impossible from visual identification alone, due to the difficulty of telling non-territorial males from females. The assumption that males are found at 50:50 W/B frequencies potentially leads to a conservative result as in reality, based on

other species and locations, W morph individuals (especially males) are probably at much lower frequencies in the general population (e.g. Holzberg, 1978, Ribbink *et al.*, 1983), meaning that, as territorial males, they should be expected to be present at even lower frequencies than calculated here. Frequency dependant selection due to reduced conspecific aggression may be allowing W males to form territories more easily than B morph males. Frequency dependant selection has been demonstrated in other cases (e.g. Fitzpatrick *et al.*, 2007; Olendorf *et al.*, 2006; Takahashi *et al.*, 2010; Hori, 1993) and might be a feature in the maintenance of polymorphism in some haplochromine cichlid species (Dijkstra *et al.*, 2006). However, due to the genetic constraints on the number of W males available and evidence suggesting rare males are indeed very rare elsewhere it is not clear whether they will achieve a frequency whereby any advantage becomes reduced. However, to further complicate matters there are W only populations (G. F. Turner pers. comm.) in the northern region of the lake which either suggests fixation of this morph in these populations or a different genetic origin for these white cichlids, meaning they could be a different species converged on the white colouration of the *P. callainos* rare morph.

Heterospecific aggression is possibly the consequence of competition for food or space for reproduction or offspring release and relatively straightforward visual cues appear to be enough to make adaptive aggression choices (e.g. Pauers *et al.*, 2008). However, sexual competition or other causes of intraspecific aggression appears to be more complex. There is an increasing amount of evidence (e.g. Dijkstra *et al.*, 2006; Dijkstra *et al.*, 2008) that suggests that intraspecific aggression biases may not be based on own type visual cues, rather a common morph bias may exist. How this is comes about is uncertain, it may be simply that all individuals inherit a predisposition towards attacking the common morph, increasingly likely if the common morph is the

ancestral form. However, there potentially involves a degree of learning. Female aggression may come from imprinting on maternal phenotypes as has been shown via cross fostering experiments in *Mbipia mbipi*/*Mbipia lutea* (Verzijden *et al.*, 2008) and *P. pundamilia*/*P. nyererei* for mate choice (Verzijden and ten Cate, 2007). Although these species employ 'mouth guarding' care after release, whereas *P. zebra* and *P. callainos* do not have this extended fry care so it is unclear when or how they would imprint. What is important with respect to this current investigation into haplochromine cichlid aggression is that the potential for rare morph advantage exists.

Ideally and estimate of fitness levels for each morph type would be calculated over the lifetime. This would reveal a) if rare morphs have some real advantage by being rare and b) if this advantage does indeed reduce when numbers increase. It may be that rare phenotypes have some other advantages that means becoming common does not reduce the advantage over previously common phenotypes. Conversely, rare phenotypes may suffer some unknown cost that means they never attain the same numbers as the more common morphs. A lifetime fitness analysis may reveal the nature of advantages to rare morphs and whether or not they actually are negatively frequency dependant and should be pursued as an approach.



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## Appendix

### *Study species*

*P. zebra* is found at many rocky shore locations around the lake and often is most abundant around 5-10m in depth (Ribbink *et al.*, 1983). Females can exhibit three different sympatric colour morphs: Barred (BB), the most common, is dark brown with dark vertical bars. In other species and studies these are also known as the 'plain' morph as they lack bars (*e.g.* Dijkstra *et al.*, 2007). Orange blotched (OB) females have orange background colour with numerous dark blotches, the orange (O) morph is overall orange, but a few dark blotches may be present (Genner & Turner, 2005). The proportions of each morph vary in wild populations. Knight & Turner (1999), for example, found O to make up 4-18% of a population and OB 10-15%. Blotched or orange males can be found in natural populations but are rarer still (less than 0.5% of the population at Nkhata Bay for example, Knight & Turner, 1999). In many haplochromine cichlids (including *P. zebra*) this colour polymorphism appears to be almost entirely female-limited (Lande *et al.*, 2001). Males nearly always have a bright blue background colour with dark vertical bars and are BB (Genner & Turner, 2005).

*P. callainos* is also found at many locations around the lake, always in the rocky shore with greatest abundance at shallow (< 5m) depths (Ribbink *et al.*, 1983). At least two

morphs are frequently seen; a 'white' (W) and 'blue' morph (B). Occasionally partially blotched white morph individuals are seen though these appear to be very rare. The two *P. callainos* morphs were once considered to be *P. zebra* morphs; this was from observations at Nkhata Bay (Fryer, 1959) where they occupy a similar depth range, and possess very similar feeding adaptations and very similar morphology and behaviour. Interestingly, in the Nkhata Bay region frequency of B and W morph fish varies from location to location (Holzberg, 1978, G. F. Turner pers.com). At some places there appear to be only W or B morphs and where they are found together the B morph tends to be more abundant. A number of translocations occurred during the 1970's and they are now found at Thumbi West Island where an endemic *P. zebra* population is also found (Ribbink *et al.*, 1983, Konings, 2001). The ratio of B to W morph fish that were translocated to Thumbi West Island is unknown.

## **Data Analysis**

### *Laboratory trials*

As each focal was tested more than once (both males and females), an average was calculated for the aggression given to both intruder types by each focal. The average for each focal fish was then pooled giving an overall impression for aggression biases. Testing each focal more than once allowed greater confidence in what an individual might do on average and does not rely on one off interactions. Averaging each focal fishes response removes the potential for pseudo-replication when analysing the data. However, averaging a focal fishes behaviour smoothes out individual behaviour, the aim of this study was not to focus on individual variability in response to two intruders but to elucidate what a population might do an average towards to intruders.

Therefore averaging the behaviour for each focal fish was appropriate for the aim of this study.

All types of behaviour were recorded and pooled into two types: Attack behaviours (lunge and bite) or display behaviours (lateral display, frontal display and quiver). Both types of behaviour were analysed separately and also totalled for comparisons of all aggressive behaviour given.

Data for focal BB males was divided into rare intruder type (O/BB or OB/BB). The data for the O/BB intruders did not differ significantly from a normal distribution (Kolmogorov-Smirnov test,  $Z = 0.690$ ,  $p = 0.727$ ) nor have significantly unequal variances (Levenes  $p = 0.465$ ). After arcsine transformation the OB/BB dataset did not differ from a normal distribution (Kolmogorov-Smirnov test,  $Z = 0.736$ ,  $p = 0.651$ ) nor show significantly unequal variances (Levenes  $p = 0.283$ ). Therefore, for both intruder types a parametric test (Paired T-test) was used to compare means of aggressive behaviour given to each intruder type.

The female averages were split into focal fish type (BB or OB) and each tested for a normal distribution. The BB data set did not differ significantly from a normal distribution (Kolmogorov-Smirnov test,  $Z = 0.803$ ,  $p = 0.539$ ) and variances did not differ significantly from equal (Levenes  $p = 0.558$ ). The OB data set did not differ significantly from a normal distribution (Kolmogorov-Smirnov test,  $Z = 0.901$ ,  $p = 0.391$ ) and variances did not differ significantly from equal (Levenes  $p = 0.524$ ). Therefore, for both focal fish types a parametric test (Paired t-test) was used to compare means of aggressive behaviour given to each intruder type.

**Table A2.1** 3 x 30 m transects were carried out to determine the ratio of each morph (white and blue) in the general population for *P. callainos* at Thumbi West Island, Lake Malawi.

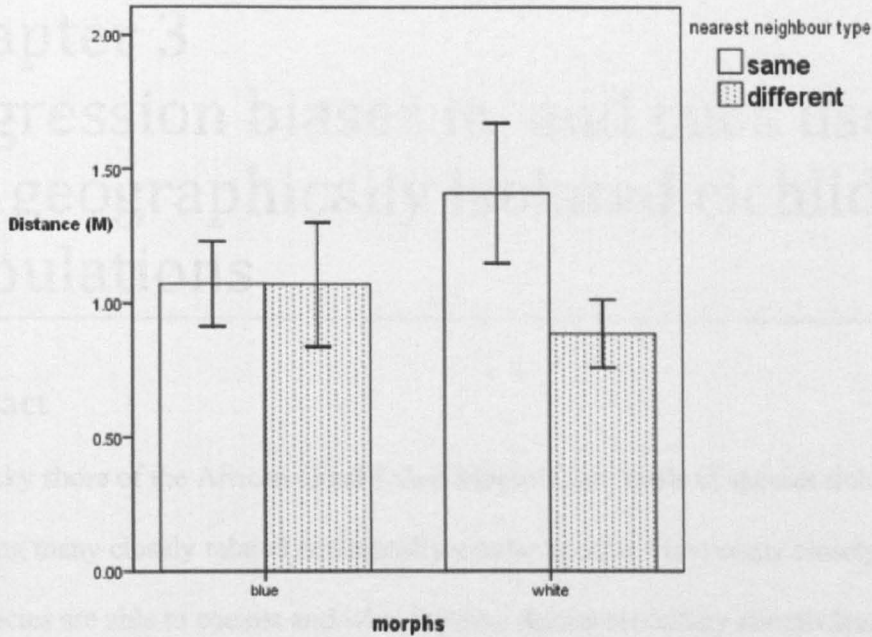
Transect	N of Blue morph	N of white morph
A	12	6
B	19	8
C	20	9
<b>Total</b>	<b>51</b>	<b>23</b>

**Table A2.2** Territorial males for each morph (blue and white) for *P. callainos* at Thumbi West Island, Lake Malawi, were counted after the quadrats were transcribed onto graph paper.

Quadrat	N of Blue morph	N of white morph
A	7	10
B	10	5
C	6	6
D	11	9
E	13	4
F	11	10
G	8	6
H	7	6
<b>Total</b>	<b>83</b>	<b>59</b>

It was found that *P. callainos* blue territorial males kept either morph (blue or white) at non-significantly different distances from themselves (ANOVA,  $p = 0.994$ ).

However, *P. callainos* white males had white males at a significantly further distance than blue males (ANOVA,  $p < 0.001$ ).



**Figure A2.1.** White male territorial focus point is significantly (ANOVA:  $F_{1,48} = 13.89$ ,  $p < 0.001$ ) closer to blue males (mean 0.89m) than it is to white males (mean 1.41m). There is no difference (ANOVA:  $F_{1,48} = 0$ ,  $p = 0.994$ ) in the distance between blue focal males and their closest blue and white males (mean 1.07m). With both morphs combined there is a significant difference (ANOVA:  $F_{1,98} = 6.23$ , d.f. = 1,  $p = 0.014$ ) in the distance between focal fish and nearest same (mean 1.24m) and different (mean 0.98m) morph.  $N = 25$  territorial males for each morph comparing its two nearest neighbours of each morph. Error bars = 95% Confidence limits.



# Chapter 3

## Aggression biases in, and cues used by, geographically isolated cichlid populations

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### Abstract

The rocky shore of the African Great Lakes support high levels of species richness, including many closely related ecologically similar species. How many closely related fish species are able to coexist and what happens during secondary contact has been the focus of recent work. Males of rocky shore haplochromine cichlids are strongly territorial for mate acquisitions and females may defend refugia. Many allopatric populations differ in colour, possibly due to divergence under sexual selection. Could such colour differences among closely related allopatric populations help permit them to coexist in sympatry? These questions were investigated by laboratory dichotomous choice aggression trials. In the first experiment, male fish were presented with 2 intruders from allopatric populations, one with a similar dorsal fin colour (orange) and the other with a different one (blue). With only visual cues available, resident males showed significantly more aggression to intruders from the population with similar dorsal fins colours, but this bias disappeared when colour differences were masked. A similar result was found with females of one of a pair of populations that differed in throat colour (*P. thapsinogen*), but females of the other population (*P. emmiltos*) showed no significant bias in aggression even under broad spectrum illumination. This indicates that divergence in colour might facilitate species co-existence but that not all populations differentiate all colour differences.

## Introduction

Closely related sympatric mbuna species may occupy very similar trophic niches, differ little in morphology (Genner and Turner, 2005), show extremely conserved behaviour (Myers, 1960) and only differ in colour traits associated with secondary sexual characteristics (Konings, 2007). Many sympatric species and allopatric populations of haplochromine cichlid fish differ strikingly in male colour (Allender *et al.*, 2003) and this is particularly true for the 'mbuna' or rock dwelling cichlids of Lake Malawi (Konings, 2007). Furthermore, geographical variants of fish considered the same species may differ slightly in traits such as dorsal fin or throat colour (*e.g.* *Labeotropheus fuelleborni*, Konings 2007, page 30). Divergence in signals associated with sexual selection is thought to play a key role in speciation in cichlid fishes (Seehuasen, 1996; Maan *et al.*, 2004), as well as in other taxa, for example Hawaiian *Drosophila* spp. (Boake *et al.*, 2000; Coyne *et al.*, 2002).

Male mbuna cichlids compete aggressively for territories and those males that are unable to secure a territory are unlikely to breed successfully (Genner and Turner, 2005). Territorial males are generally more aggressive to conspecifics intruders, with which they compete for mates as well as other resources such as food and refuge (Genner *et al.*, 1999).

Differences in male signal traits are associated with reduced interspecific aggression in sympatric cichlid fish (Pauers *et al.*, 2007; Dijkstra *et al.*, 2006), birds (Alatalo *et al.*, 1994), damselflies (Anderson *et al.*, 2010; Tynkkynen *et al.*, 2004) and other taxa (Grether *et al.*, 2009). Aggression biases in some cases are stronger in sympatric populations, leading to suggestions that agonistic character displacement may be a significant process in evolutionary ecology (Grether *et al.*, 2009), facilitating co-existence of recently diverged species (Seehausen and Schluter, 2004). Field studies

have suggested that there is reduced competition between differently coloured cichlid fishes in Lakes Victoria (Seehausen and Schluter, 2004) and Malawi (Young *et al.*, 2009).

Reduced interspecific aggression need not only apply to males. Females can be aggressive during the mouthbrooding stage (see chapter 4) and territorial (Dijkstra *et al.*, 2008; Genner and Turner, 2005). Divergent traits that are thought to be important in interspecific aggression choices between males are also found in females (dorsal fin colour and other patches of pigmentation), and if a reduction in interspecific aggression for females results in a fitness benefit then this may amplify divergence of traits normally associated with male signalling.

It has been suggested that dorsal fin colour is important (Pauers *et al.*, 2008) in choosing who to be aggressive towards but many haplochromine cichlids also have patches of bright pigmentation elsewhere (see chapter 1 for discussion on cichlid visual capabilities) and recent work (Pauers, 2011) suggests that environment and food availability may have a small role in colour patterning. Of the four populations of so called “red top zebras” from Lake Malawi, in only one population (*P. thapsinogen* found at Eccles Reef) do individuals possess yellow throats in addition to the yellow dorsal fin, the others being grey/black. Aquarium observations suggest that these additional patches may serve some purpose as they are expanded during displays and appear to be used in an intimidatory fashion. Water levels in Lake Malawi have been shown to rise and fall over geological time (*e.g.* Scholz *et al.*, 2007) which is likely to force allopatric populations to re-join, coexistence of near identical fish species may be promoted by subtle colour differences alone (Seehausen and Schluter, 2004).

## Aims

To determine the cues used in aggression by two *Pseudotropheus* spp.

- Is dorsal colour important in choosing who to be aggressive towards when faced with two heterospecifics?
- Is olfactory signalling important in aggression?

To assess the importance of additional patches of pigmentation that some mbuna posse.

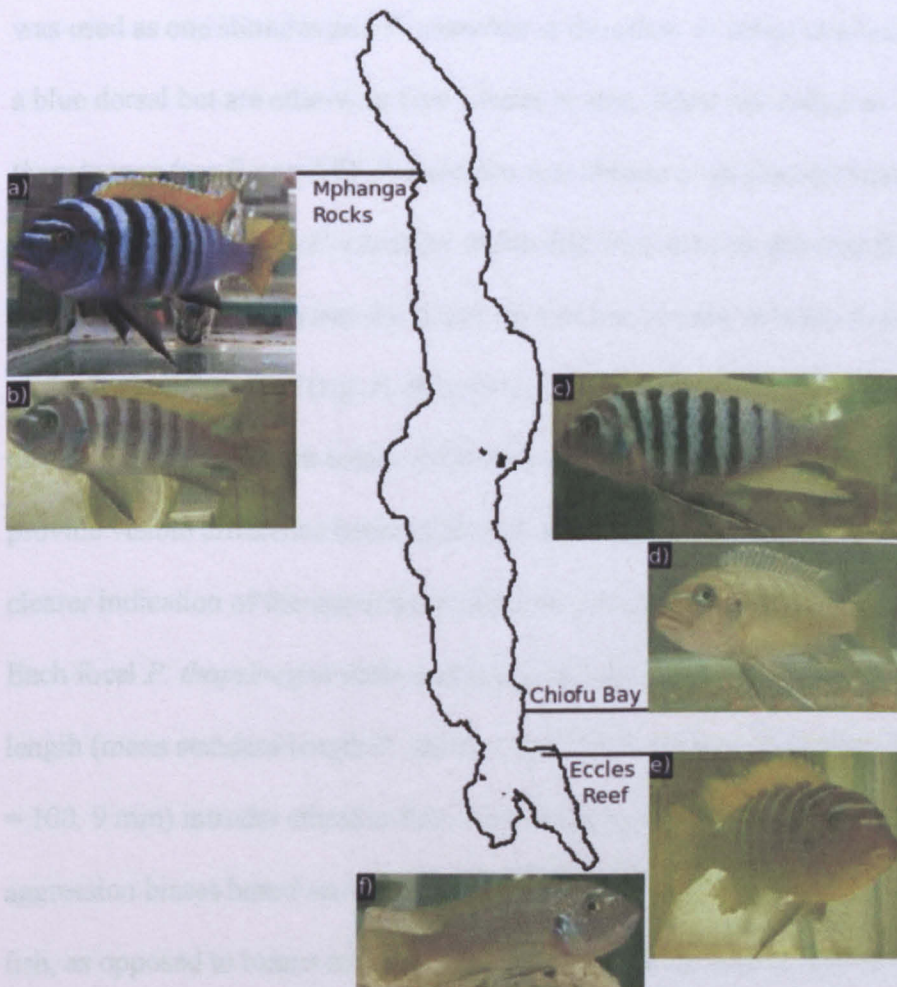
- Do two species that differ significantly only in a single patch of pigmentation, show assortative aggression biases?
- Is there evidence from the literature that sympatric species evolve additional patches of pigmentation which may reduce unnecessary aggression?

## Materials and Methods

### *Experimental animals*

The study populations used were members of the *Pseudotropheus zebra* species complex, part of the subgenus *Maylandia* (also known as the genus *Metriaclima* (Stauffer *et al.*, 1997), endemic to Lake Malawi. All were formerly in the species *P. zebra*, but several populations have been given formal species names (*e.g.* *P. emmiltos*) on the basis of differences in colour pattern and subtle differences in morphology (Smith and Kornfield, 2002). *P. thapsinogen* males and females were wild caught from Eccles Reef in 2008 and used as focal fish in Part A and B. *P. emmiltos* males and females were wild caught from Mphanga Rocks in 2008 and used as focal fish and stimulus fish in Parts A and B. *P. zebra* stimulus males used in Part A only were wild caught from Chiofu Bay in 2008. Allender *et al.*, (2003) created a molecular phylogeny of this group of Malawian cichlids which suggests that the

proximate dissimilar colour forms (*i.e.* *P. zebra* (Chiofu Bay) and *P. thapsinogen*) are more closely related than geographically separated similar colour forms (*i.e.* *P. thapsinogen* and *P. emmiltos*). Blais *et al.*, (2009) investigated the strength of reproductive isolation in these three species (as well as *P. zebra* from Nkhata Bay) and found some assortative mating between *P. emmiltos* and *P. thapsinogen* and amongst their conclusions state that cues other than colour dorsal fin colour (*e.g.* throat pigmentation, olfactory cues, UV reflectance) may play a role in mate choice.



**Figure 7** Location of study species used in this chapter. A) Male *P. emmiltos*, B) Female *P. emmiltos*, C) Male *P. zebra*, D) Female *P. Zebra*, E) Male *P. thapsinogen* and E) Female *P. thapsinogen*.

Photographs by A. Tyers

*Part A: Male trials*

Twelve *P. thapsinogen* males were used as focal fish for this experiment and isolated from stock tanks before experiments began. One of the males was a dominant male, occupying the one territorial refuge in the tank. The remaining 11 males were subordinate. Giving the males time to acclimatise in the experimental setup allowed them to exhibit colour changes associated with dominance and provided an indicator that they would be aggressive towards intruders. All males used were considered healthy and in good physical condition *i.e.* not underweight nor clearly ill. *P. zebra* was used as one stimulus and *P. emmiltos* as the other. *P. zebra* was used as they have a blue dorsal but are otherwise very similar in size, shape and colour to *P. thapsinogen* (see figure 3.2). *P. emmiltos* was chosen as an intruder because they also possess a yellow dorsal. *P. emmiltos* males that were used as stimulus fish lacked the black barring extending into the dorsal fin that can be seen in individuals from some of the “red top” species (*e.g.* *P. thapsinogen*). This was important in the second half of this experiment where colour differences were masked the black bars would still be provide visible difference between the two intruder stimulus fish. This allowed a clearer indication of the importance of dorsal colour used in aggression choices. Each focal *P. thapsinogen* male was assigned a pair of size matched for standard length (mean standard length *P. zebra* = 101.6 mm, mean standard length *P. emmiltos* = 100.9 mm) intruder stimulus fish. Size matching intruders attempted to control for aggression biases based on asymmetries in the condition of the intruding stimulus fish, as opposed to biases relating to the species (and therefore cues) of the intruder. The first experiment comprised of each focal being presented with its own intruder pair in a 1.5 meter tank containing a central refuge. Two transparent jars equidistant from the central refuge area held intruder males. Transparent jars were un-perforated

preventing chemical communication between focal and stimulus fish. A focal male fish was added to the set up and allowed to acclimatise for 48 hours. This gave the resident male time to become territorial over the refuge provided which was essential for optimising male response towards intruders. One male intruder was randomly assigned a left or right side transparent jar. Trials lasted 10 minutes as in previous experiments using males (Chapter 2, Dijkstra *et al.*, 2006). All 12 focal *P.*

*thapsinogen* males were tested this way.

After each male had been tested in the first experiment, the second experiment began.

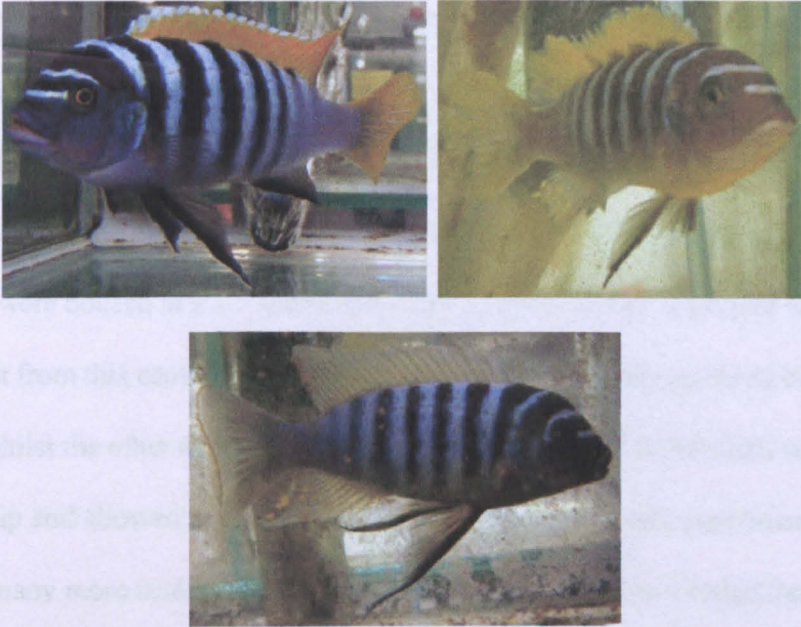
Each focal male had at least 24 days between the first and second experiment with focal males being used in the same order to allow similar rest periods for each male.

The second experiment repeated the previous design except this time the transparent jars were masked by wrapping them in orange/red optical filter acetate (Lee filters 026) and the jars were not perforated. The filter was chosen based on previous usage by researchers working on monochromatic light experiments in mbuna cichlid fish (Plenderleith, 2005) and only transmits light at the wavelengths between 500–700 nm, effectively eliminating the difference between the orange and blue dorsal fin colour.

A similar filter (Lee filters 106) was used successfully in a recent study designed to test assortative mating in *P. zebra*, *P. emmiltos* and *P. thapsinogen* in full and monochromatic light conditions (Blairs *et al.*, 2009). This filter has a very similar range of light transmission but was not available at the time of the experiments performed here. Each focal was again tested with its own pair which was again randomly assigned to either the left or right jar.

All fish were kept on a 12L12D light cycles and fed aquarium flake. Tanks were maintained at 23 – 26 degrees C. Focal males were ‘coloured’ up, indicating that they

considered themselves to be dominant individuals which also provided evidence that the focal fish were healthy and in good condition (G.F. Turner pers.comm)



**Figure 8** Photograph of a *P. emmilotos* male (above left), a *P. thapsinogen* male (above right. Note the yellow pigmentation in the throat region) and a *P. zebra* male (bottom). Photographs taken by A. M. Tyers

### *Part B: Female Trials*

In part B females were used exclusively as males were unavailable, two species (*P. thapsinogen* and *P. emmilotos*) were used in experiments designed to see if subtle differences in pigmentation may lead to assortative aggression biases in these two very similar allopatric members of the *P. zebra* complex.

Females came from mixed sex stock tanks containing only their own species. Eight females of each species were used as focal fish. The sixteen focal fish were sized matched in pairs (one of each species) and three stimulus pairs were randomly assigned to each focal. Each focal *P. thapsinogen* or *P. emmilotos* female pairing were



size matched for standard length (mean *P. thapsinogen* = 82.3 mm, mean *P. emmiltos* = 86.25 mm). Size matching the pairs of intruders attempted to control for aggression biases based on asymmetries of the intruding stimulus fish, as apposed biases relating to the species (and therefore cues) of the intruder. All females used as stimulus or focal fish were healthy and considered in good condition *i.e.* not underweight nor showing any signs of illness.

Each focal was presented with three pairs of intruders in all three treatments. In all trials for each of the three treatments (see details for each of three treatments below) focal fish were housed in a 1.5 metre tank with a central refuge. Two jars were placed equidistant from this central refuge. One intruder was randomly assigned one end of the tank whilst the other occupied the remaining end. A focal female fish was added to the set up and allowed to acclimatise for 24 hours. The female experiments involved many more trials so time was constrained which led to a reduction in acclimatisation period. During initial trials of the setup it was found that 24 hours was sufficient for females to become aggressive towards intruders. The time of each trial was also reduced to 5 minutes which has been used in previous studies using haplochromine female aggression studies (Dijkstra *et al.*, 2008).

In the first treatment visual and olfactory cues were available by placing the intruders in translucent and perforated transparent plastic jars. The trial began when the two intruders were added simultaneously to the jars. All sixteen focal fish (eight of both species) were tested this way before moving onto the second treatment.

The second treatment differed from the first in that this time jars without holes were used for the intruder stimulus fish, preventing potential olfactory communication. The same three pairs of intruders, used in the first treatment, were used again for the same focal fish but they were randomised with respect to the order they were presented and

side of intrusion with respect to the first treatment. Again, all sixteen focal fish were tested this way before the third and final treatment.

The third treatment replicated those above but in this case the transparent jars were wrapped in red/orange acetate (same filter as part A – again without perforation) in an attempt to remove colour differences between the two intruder fish species (*i.e.* throat colour). Again the focal fish were presented with the same triplicate of intruder pairs, which were again randomised with respect to order and side of central refuge.

The results for each focal in any given treatment were pooled and a mean number of aggressive behaviours given to each type of intruder was calculated. This gave an impression on what a focal might do on a consistent basis rather than a single, one off trial. This might be important give the relatively low ( $n = 8$ ) number of focal fish for each species tested.

Data was then analysed with the focus on how each species biased aggression at the two intruders per treatment. Experiments were carried out over a six week period in 2009. All fish were kept on a 12L12D light cycles and fed aquarium flake, tanks were maintained between 23 and 26 degrees C.



**Figure 9** Photographs of *P. thapsinogen* female (left) and *P. emmitos* female (right). Photographs taken by G. M. Cooke (left) and A. M. Tyers (right).

The experiments for this chapter were performed between April 2008 and September 2009. Part A was carried out to completion before the beginning of part B. In both Parts A and B stimulus fish behaviour was not quantified due to the difficulty of

recording both focal and stimulus behaviour simultaneously. However, it was noted that stimulus fish always initially explored the jar in which they were enclosed and would react aggressively to aggressive behaviours given by the focal fish.

*Part C: Evidence from the literature for disruptive selection on pigmentation patches*

From two major sources of mbuna information (Ribbink *et al.*, 1983; Konings, 2003) one species of mbuna was observed to vary subtly in pigmentation on its body in different populations around the lake (*Labeotropheus fulleborni*). Each of the six populations chosen shows similar colour traits except for occasional additions of patches of yellow/orange/black pigmentation which are found in the dorsal, edge of fins, throat or entire body. Ribbink *et al.*, (1983) provided information on which species were most abundant at similar depth ranges as *L. fulleborni*. Species were included if they shared some life history aspect such as feeding strategy (all grazers as opposed to grazers and scale biters for *e.g.*) or rock crevice spawning – traits that might cause them to share space or resources leading to heterospecific aggression necessary or otherwise.

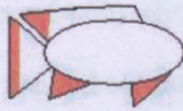
Konings (2007) is an authority on the cichlids of Lake Malawi with many photographs of species and their variation in different populations around the lake. Photographs of fish from other species, which also possess additional patches of pigmentation and coexisted with *L. fulleborni*, were studied. The term “additional” here denotes patches that are likely to be secondary sexual characteristics used in courtship/aggression, are conspicuous and may be behaviourally mediated (either through colour changes or body language that amplifies the patch, increasing conspicuousness). In other words, as blue or brown is likely to be the least conspicuous colour, based on the environment (open water column or rocky shore) any patch of colour in addition to blue/brown was treated as additional. Lateral or horizontal stripes were also not included as nearly all mbuna species poses one or the other. This method also does not include rare morphs of some species (*e.g. L.*

*fulleborni*, *P. zebra*, *P. callainos*) which are in much lower frequency than the morphs used here and may be not be the result of disruptive selection to reduce unnecessary heterospecific aggression (although see Chapter 2 for the advantages being novel may have for reduced conspecific aggression). Whilst possibly crude, this analysis of the already available information may reveal trends within communities that can leader to future work. The method does not take into account body shape differences. However, behaviours in disparate haplochromine cichlids are highly conserved and small differences in morphology may not be sufficient to remove unnecessary heterospecific aggression in species that are behaviourally indistinguishable.

#### Thumbi West



*Labeotropheus fulleborni*



#### Chinyamwezi Island



*Labeotropheus fulleborni*



#### Chinyankwazi Island



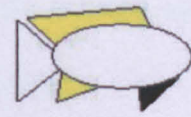
*Labeotropheus fulleborni*



#### Makanjilla point



*Labeotropheus fulleborni*



#### Nkhata Bay



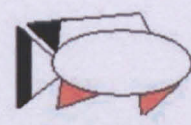
*Labeotropheus fulleborni*



#### Boadzulu Island



*Labeotropheus fulleborni*



**Figure 10** Photographs of *L. fulleborni* were used from Konings (pp. 30, 2007) and additional patches of pigmentation were mapped on a simplified fish diagram for comparisons with other species. Most photographs used in this analysis were from Konings (2007) with a small number from the plates included in Ribbink *et al.*, (1983).

## Data analysis

Data sets were analysed with respect to distribution and equality of variances. If assumptions for parametric testing were not met transformation attempts were made.

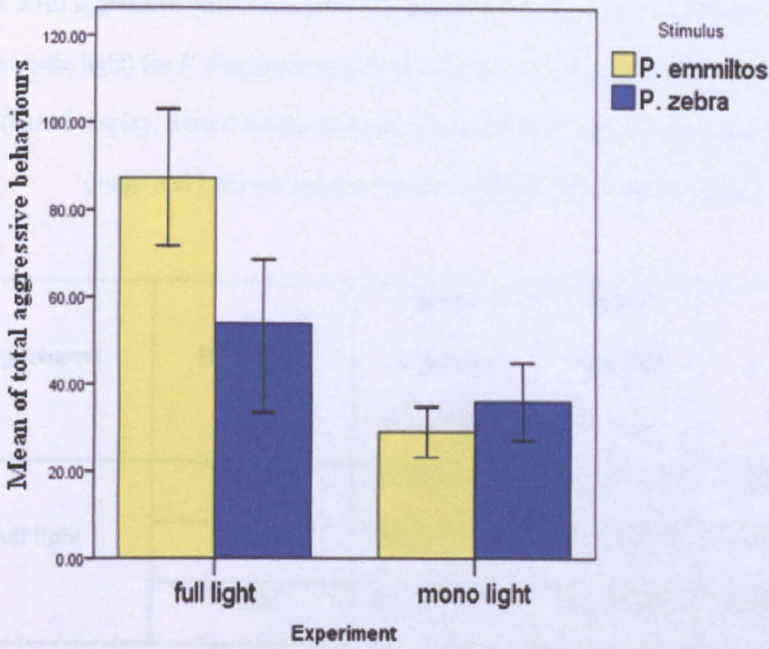
See Appendix for analysis of data sets.

## Results

### *Part A: Males*

Focal *P. thapsinogen* male fish showed a significant (Paired t-test,  $t = 2.250$ , d.f. = 11,  $p = 0.02$ ) aggression bias towards *P. emmiltos* intruders (mean = 87.33) compared to *P. zebra* intruders (mean = 54.00) when full visual cues were provided. *P.*

*thapsinogen* male focal fish did not show a significant tendency to be aggressive to *P. emmiltos* male intruders (mean = 28.58) compared to *P. zebra* intruders (mean = 36.41) in the mono-chromatic light treatment (Paired t-test,  $t = -1.396$ , d.f. = 11,  $p = 0.09$ )



**Figure 11** Mean number of aggressive behaviours and 95% confidence limits given towards *P. zebra* and *P. emmitlos* stimulus fish by focal *P. thapsinogen* males in the monochromatic and full light experiments. N = 12.

Types of aggressive behaviours used (*i.e.* attack or display) showed similar significant differences during the first experiment which were again lost in the second experiment (see table 3.2).

Table 3 All aggressive behaviours given during the two treatments (visual and olfactory cues, monochromatic light) for *P. thapsinogen* male focal fish, including totals and statistics. Non-contact displays (lateral display, frontal display and quiver) pooled into 'display' category. Overt aggression (lunge and bite) pooled into 'attack' category. S.E = standard error

Experiment	Behaviour	Mean	Mean	t	p
		Agg.(SE) <i>P. emmiltos</i>	Agg.(SE) <i>P. zebra</i>		
Full light	Display	64.08(6.357)	38.50(7.235)	2.053	0.03
	Attacks	23.25(2.834)	15.50(2.227)	1.974	0.04
	<b>Total</b>	<b>87.33(7.098)</b>	<b>54.00(9.301)</b>	<b>2.250</b>	<b>0.02</b>
Monochromatic light	Display	20.25 (2.111)	26.08(2.861)	-1.510	0.08
	Attacks	8.33(1.127)	10.33(1.298)	-0.830	0.21
	<b>Total</b>	<b>28.58(2.261)</b>	<b>36.41(4.048)</b>	<b>-1.396</b>	<b>0.09</b>

#### Part B: Female trials

When given the choice of a conspecific and a similarly coloured allopatric heterospecific, *P. thapsinogen* female focal fish showed a significant conspecific aggression bias under visual and olfactory (*P. thapsinogen* mean = 49.48, *P. emmiltos* mean = 25.52, paired t-test,  $t = 5.495$ , d.f. = 7,  $p < 0.001$ ) and visual only conditions (*P. thapsinogen* mean = 40.70, *P. emmiltos* mean = 19.03, paired t-test,  $t = 8.234$ , d.f. = 7,  $p < 0.001$ ). However, females did not show an aggression bias in monochromatic light (*P. thapsinogen* mean = 33.20, *P. emmiltos* mean = 31.06, paired t-test,  $t =$

08.34, d.f = 7, p = 0.21).

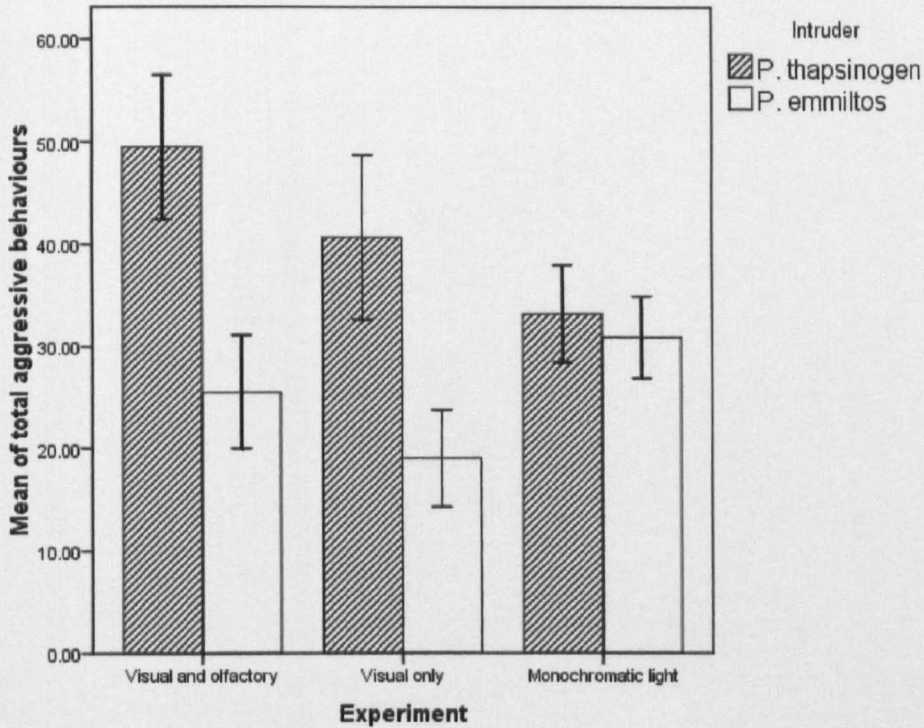


Figure 12 Mean number of aggressive behaviours, with 95% confidence limits, by *P. thapsinogen* focal female fish towards either *P. thapsinogen* or *P. emmiltos* stimulus fish for the three experiments (full light/olfactory cues, full light only and monochromatic light only). N = 8 focal fish tested against three intruder pairs.

*P. emmiltos* female focal fish showed no significant bias towards either intruder in the visual and olfactory treatment (mean aggression to *P. emmiltos* = 16.80 and to *P. thapsinogen* = 18.24, Paired t-test,  $t = -0.557$ , d.f = 7,  $p = 0.29$ ), the visual only treatment (mean aggression to *P. emmiltos* = 17.90 and to *P. thapsinogen* = 19.60, paired t-test,  $t = -1.497$ , d.f = 7,  $p = 0.089$ ) and monochromatic treatment (mean aggression to *P. emmiltos* = 18.99 and to *P. thapsinogen* = 21.28, Paired t-test,  $t = -0.695$ , d.f = 7,  $p = 0.183$ ).



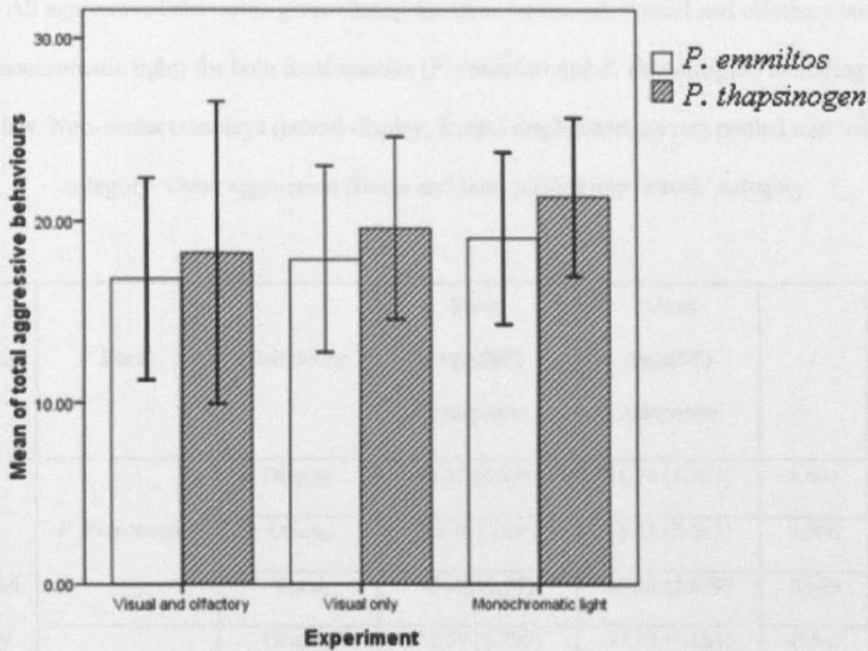


Figure 13 Mean number of aggressive behaviours, with 95% confidence limits, by *P. emmiltos* focal female fish towards either *P. thapsinogen* or *P. emmiltos* stimulus fish for the three experiments (full light/olfactory cues, full light only and monochromatic light only). N = 8 focals tested against three intruder pairs.

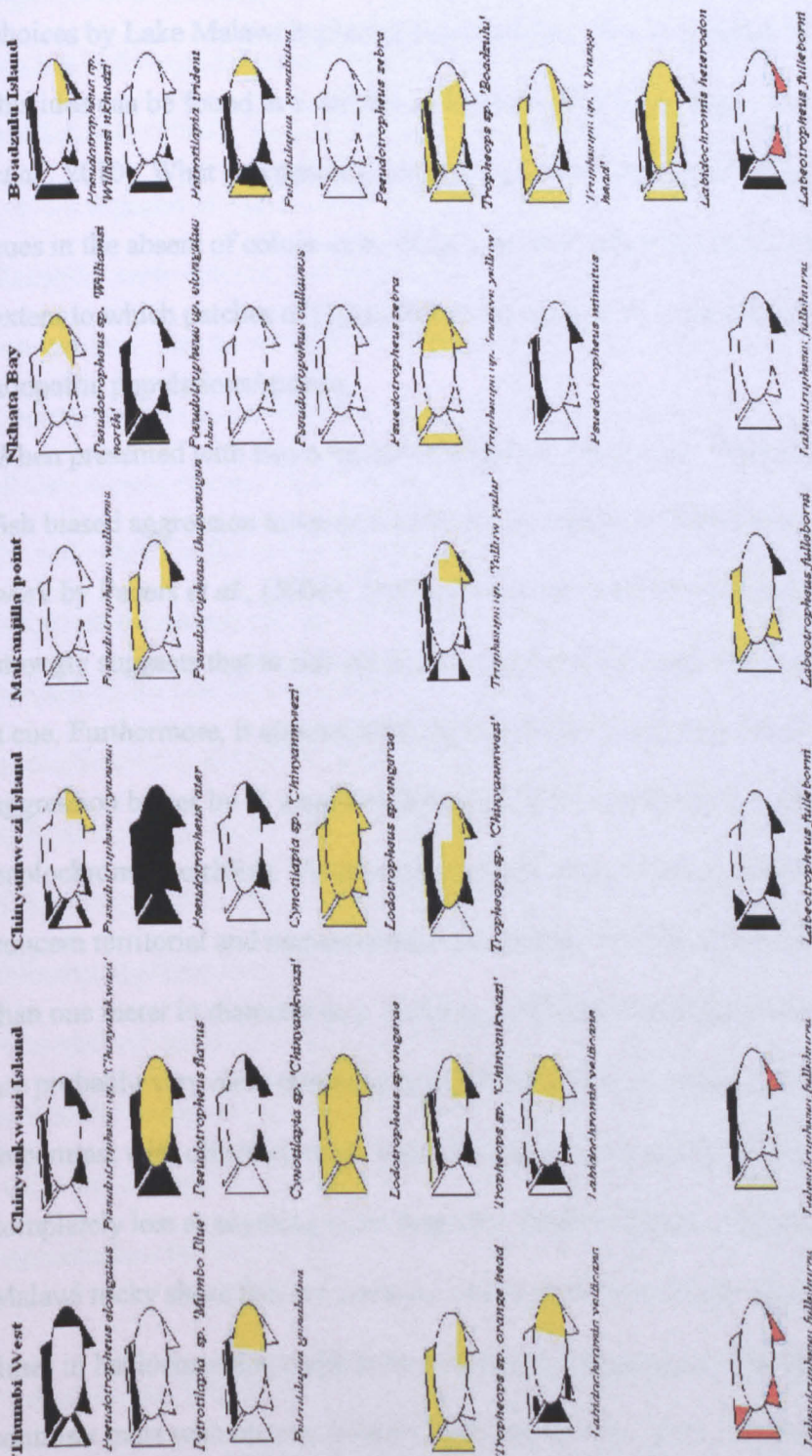
Types of aggressive behaviours used (*i.e.* attack or display) by *P. thapsinogen* focal fish showed similar significant differences during the visual and olfactory and visual only experiments which were again lost in the monochromatic light experiment. *P. emmiltos* focal fish showed no significant bias when display or attack behaviours were analysed separately (see Table 3.3).

**Table 4** All aggressive behaviours given during the three treatments (visual and olfactory cues, visual only, monochromatic light) for both focal species (*P. emmilto*s and *P. thapsinogen*) including totals and statistics. Non-contact displays (lateral display, frontal display and quiver) pooled into 'display' category. Overt aggression (lunge and bite) pooled into 'attack' category.

Experiment	Focal	Behaviour	Mean	Mean	t	p
			Agg.(SE) Sympatric	Agg.(SE) Allopatric		
Visual and olfactory	<i>P. thapsinogen</i>	Display	24.32 (2.274)	11.78 (1.503)	4.604	0.001
		Attacks	25.16 (2.24)	13.74 (2.165)	4.506	0.001
		<b>Total</b>	<b>49.48 (2.97)</b>	<b>25.52 (2.359)</b>	<b>5.949</b>	<b>&lt;0.001</b>
	<i>P. emmilto</i> s	Display	11.10 (1.799)	11.95 (1.684)	-0.547	0.30
		Attacks	5.70 (1.131)	6.29 (1.997)	-0.404	0.34
		<b>Total</b>	<b>16.80 (2.342)</b>	<b>18.24 (3.511)</b>	<b>-0.577</b>	<b>0.29</b>
Visual only	<i>P. thapsinogen</i>	Display	24.92 (1.500)	11.03 (1.598)	8.667	< 0.001
		Attacks	15.78 (2.632)	8.00 (1.756)	5.610	< 0.001
		<b>Total</b>	<b>40.70 (3.394)</b>	<b>19.03 (2.01)</b>	<b>8.234</b>	<b>&lt;0.001</b>
	<i>P. emmilto</i> s	Display	14.1 (1.719)	14.83 (1.215)	-0.680	0.25
		Attacks	3.8 (0.807)	4.77 (1.201)	-1.268	0.12
		<b>Total</b>	<b>17.9 (2.17)</b>	<b>19.60 (2.11)</b>	<b>-1.497</b>	<b>0.089</b>
Monochro matic	<i>P. thapsinogen</i>	Display	14.91 (1.556)	14.24 (0.999)	0.486	0.32
		Attacks	18.29 (0.933)	16.82 (1.601)	0.87	0.20
		<b>Total</b>	<b>33.20 (2.009)</b>	<b>31.06(1.692)</b>	<b>0.834</b>	<b>0.21</b>
	<i>P. emmilto</i> s	Display	10.70 (0.827)	11.29 (1.104)	-0.378	0.357
		Attacks	8.29 (0.933)	9.99 (1.601)	-1.613	0.07
		<b>Total</b>	<b>18.99 (2.002)</b>	<b>21.28 (1.838)</b>	<b>-0.965</b>	<b>0.183</b>

When aggression to both stimulus fish was combined *P. thapsinogen* female focal fish were significantly more aggressive than *P. emmilto*s female focal fish during the visual and olfactory trials (t-test,  $t = -6.155$ , d.f. = 7,  $p < 0.001$ ).

## Part C: Evidence from the literature for disruptive selection on pigmentation patches



**Figure 14** Stylised representation of populations of mbuna at six locations in Lake Malawi, highlighting how different species at different locations may evolve additional patches of pigmentation to reduce heterospecific aggression. *L. fulleborni* (bottom row) is compared to other populations within its species and its sympatric competitors. Note: At each location rarely, if at all, does two species have similar patches of pigmentation. One example of overlap is at Nkhata Bay where *P. zebra* and *P. callainos* both have no additional colouring although one (*P. zebra*) does employ stripes used to highlight dominance

## Discussion

The results of this study strongly suggest that colour cues are important in aggression choices by Lake Malawi haplochromine cichlids. This is unsurprising as support for this idea can be found in a number of recent studies (*e.g.* Pauers *et al.*, 2008; Dijkstra *et al.*, 2010). What is potentially interesting is that females do not appear use olfactory cues in the absent of colour cues. Also, it appears there that may be differences in the extent to which patches of pigmentation are used as cues between recently diverged allopatric populations/species.

When presented with two congeneric allopatric intruders *P. thapsinogen* male focal fish biased aggression towards the fish most similar to itself which supports earlier work by Pauers *et al.*, (2008). This bias was lost under the monochromatic light which strongly suggests that in this set up the male focal fish were using dorsal fin colour as a cue. Furthermore, it appears unlikely that smell or olfactory signals have any role in aggression biases by *P. emmiltos* females which may also be true for many haplochromine cichlids. Visual cues are much more effective over the distances that concern territorial and non-territorial mbuna fish. Territories are typically no smaller than one meter in diameter (*e.g.* Holzberg, 1978) at which distance colour differences are probably very clear (see chapter 1 for discussion of cichlid colour vision). This is in contrast with olfactory cues, which by their nature, are likely to be diluted or completely lost at anything other than very small distances. Yellow pigments in Lake Malawi rocky shore fish are common and are believed to have evolved numerous times in haplochromine cichlids found generally because in clear lake waters yellow contrasts most with blue in cichlid vision (Boughmen, 2001; Deutsch, 1997) so additional patches of yellow pigmentation are likely to be conspicuous. Figure 3.8 highlights how this may be affecting whole communities. Within *L. fulleborni* there is

variation that relates to location. Where each *L. fulleborni* populations is found it appears that other species do not share the location of yellow, orange or black pigmentation. This may mean that disruptive selection may drive novel pigmentation patches reducing unnecessary heterospecific aggression, as proposed by Seehausen and Schuler (2004). Young *et al.*, (2009) observed that species that had similar body colours engaged in significantly more aggressive interactions than those with dissimilar body colours, a simple novel patch of another colour, shown off to be very conspicuous, may be enough to reduce aggression between heterospecifics. The figure created here has a number of drawbacks: Not all information was available regarding all fish at each site and Konings (2007) photographs may not be representative of all the individuals within a species; many species show some individual variation in colour (not counting true polymorphism with distinct common and rare morphs). It is perhaps likely that if mate choice trials were carried out between the allopatric populations of *L. fulleborni* you find some degree of reproductive isolation as is seen in *P. zebra* (Blais *et al.*, 2009). If populations are evolving pigmentation differences to avoid aggression this may have consequences for speciation if indeed females use colour cues predominantly.

The method used here is arguably subjective and future work should determine a way to quantify the nature of pigment patches (*e.g.* dorsal fin colour, throat colour, chest colour etc.) in species in sympatry to see if, for example, patches of yellow pigmentation do not overlap between species, this could then be repeated lake wide.

*P. thapsinogen* female focal fish show a significant bias in directing aggression at conspecifics when presented with two very similar intruders. As they differ in throat pigmentation it appears that this additional pigmentation might be used as a species

specific cue at least in aggression. This is supported by observations of aggressive behaviour; these fish will expand their throat membrane during non-contact aggressive displays. It has been proposed that disruptive selection on nuptial colours may reduce interspecific aggression in sympatric cichlids and field studies have provided support for this idea (Seehausen and Schluter, 2004). Some studies have suggested that sympatric co-occurrence of competing species has led to selection for divergent signals to reduce competition (Alatalo *et al.*, 1994; Anderson and Grether, 2010; Tynkkynen *et al.*, 2005). However, a study of North American salamanders showed markedly higher levels of aggression to heterospecifics when they co-occurred in sympatry, suggesting selection for enhanced aggression in the presence of resource competitors (Deitloff *et al.*, 2009). Both responses indicate evolutionary change in sympatry (character displacement) in response to heterospecific competition. If disruptive selection is acting to reduce interspecific aggression then the evolution of additional yellow pigments would probably be most effective.

It is interesting that *P. emmiltos* female focal fish do not appear to bias aggression to their own species. It may be down to chance that different populations just happen to base species recognition on different signals. Different species in different allopatric communities may evolve particular species recognition signals depending on other signals present in potentially competing heterospecifics *i.e.* *P. thapsinogen* may find themselves coexisting with a closely related or similarly adapted heterospecific.

Selection might favour reduced or unnecessary aggression leading to *P. thapsinogen* using additional cues (throat or other pigmentation) when biasing aggression.

However there is a lack of information on the source location, and fish therein, to back this up. Lastly, the yellow throat of *P. thapsinogen* may be a derived state and

may have evolved for intraspecific signalling in some way and therefore would not be surprising if that *P. thapsinogen* responds to this signal and *P. emmiltos* do not.

The idea that small changes in phenotype may allow coexistence of closely related species in one genus may be plausible but it may not be true for all haplochromine genera. *Pseudotropheus* spp. are broadly considered trophic generalists in their environment (Genner *et al.*, 1999) and a small number of generalists in Lake Malawi are believed to tolerate other heterospecific generalists (Genner *et al.*, 1999). This is not considered true for 'specialists' such as *Tropheops* spp. which will not tolerate heterospecifics with similar specialised feeding behaviour. So, whilst disruptive selection may be driving small changes in phenotype of species at a given location, possibly promoting short term local coexistence and coexistence during phases of secondary contact over longer time periods when/if allopatric races meet again, it may not be the case for all mbuna or haplochromine cichlids in general.

Recent laboratory trials (Turner *et al.*, 2011 - submitted) have shown that allopatric populations of the same species (*P. zebra*) are at least partially assortatively aggressive to members of their own population, suggesting a possibility of coexistence should some or all populations meet again. Furthermore this assortative aggression correlated with partial reproductive isolation in another laboratory experiment using the same populations (Knight and Turner, 2004). Divergence in male signals due to disruptive selection which reduces heterospecific aggression may lead to reproductive isolation of populations, should visual cues be important at any stage of mate choice, which they appear to be. If divergent allopatric populations are partially reproductively isolated before secondary contact occurs (*e.g.* Knight & Turner, 2004) the result, through interbreeding between races, may be stable polymorphism of male nuptial colouration under sympatry: which has been proposed

as a pre-requisite in models describing how Lake Victoria haplochromine cichlids may have diverged in sympatry (Dijkstra *et al.*, 2005).

*P. thapsinogen* females were significantly more aggressive as focal fish when all cues were provided. This may be true for when they are stimulus fish too but was not recorded here. This could have the effect of pulling the focus of focal *P. emmiltos* females which may explain the non-significant results found when they were focal fish. At the same time it may explain the significant results of *P. thapsinogen* females as the conspecific stimulus fish may provide a positive feedback loop, creating a bias towards conspecifics based on behaviour and not by the cues available. This behavioural difference may be due to different selective pressures at the location *P. thapsinogen* are found compared to *P. emmiltos* *i.e.* competition for shared resources may be for some reason stronger. This may be due to seasonal peaks of increased productivity which relates to food abundance as reported by Marsh *et al.*, (1986) at other locations as resources may more limited outside of the peak seasons. These potential differences in life history traits need to be quantified before any further conclusions can be drawn.

The use of females in Part B was due to a lack of male fish remaining, future work would ideally use males as they are typically more aggressive than females.

In conclusion this study has shown that for *P. thapsinogen* at least, dorsal colour is probably important when biasing aggression against one similar and one dissimilar allopatric sister taxon. Furthermore it appears that olfactory cues are of little importance in aggression biases. Additional or novel pigmentation *may* be enough for some populations to be assortatively aggressive which has consequences for secondary contact.



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## Appendix

### *Data analysis*

#### *Part A: Male trials*

There was no significant differences in variances (Levenes' test,  $p = 0.754$ ) and the data were normally distributed (Kolmogorov – Smirnov test,  $Z = 0.481$ ,  $p = 0.975$ ) for the full light treatment, therefore a paired t-test was used to look for differences in aggression directed at the two allopatric intruding males. However, whilst the data for the mono-chromatic light treatment showed a normal distribution ( $Z = 0.948$ ,  $p = 0.329$ ) variances were not equal ( $p = 0.023$ ). Subsequent log transformation removed the significant difference in variances ( $p = 0.176$ ) and a paired t-test was used to look

for statistically significant differences in aggression directed at the two allopatric intruders.

*Part B: Aggression Female trials*

All six data sets showed a no significant deviation from a normal distribution and with variances not deviating significantly from equal parametric tests were performed.

**Table A3.1** Kolmogorov – Smirnov tests for data collected for each species in all three treatments. *P. thapsinogen* and *P. emmiltos* were used as focals, n = 8 for both. Each focal used in all three treatments and tested three times with three pairs of intruders (one *P. emmiltos* and one *P. thapsinogen*).

Species/Treatment	Kolmogorov – Smirnov		Levene's test	
	Z	p	Levene statistic	p
<i>P. thapsinogen</i> visual and olfac	0.608	0.584	0.445	0.516
Visual only	0.776	0.854	1.086	0.315
Mono-chromatic light	0.694	0.722	0.139	0.715
<i>P. emmiltos</i> visual and olfac	0.778	0.581	0.615	0.446
Visual only	0.483	0.974	0.115	0.739
Mono-chromatic light	0.767	0.598	0.212	0.652

## Chapter 4: Maternal aggression in Malawian cichlids

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### Abstract

Aggression directed towards predatory threats of fry during the entire reproductive cycle is investigated in two haplochromine cichlid fish species. *P. thapsinogen* mouthbrooding females were used as a focal species to ascertain levels of aggression against two congeneric intruders during the mouthbrooding period. Although there was no significant differences in the levels of aggression directed at the different intruders a significant drop in aggression during the middle of the brooding phase was found. *P. thapsinogen* females were again tested, this time with three disparate intruders. *A. calliptera* females were also tested and compared as they exhibit some post release care which is believed to be the ancestral condition in haplochromine cichlids. Again a significant drop during the middle phase of mouthbrooding was shown for both species investigated, furthermore a significant tendency to attack conspecifics over distantly related heterospecifics and a heterospecific piscivore. However, a relative increase in aggression towards the piscivore during the time when fry are likely to be released was seen which may suggest a plastic response in parental care based on the type of threat and the timing of the threat. Levels of aggression were in some cases correlated with other factors such as size of intruder, all of the results are discussed in terms of parental investment theory.

## Introduction

Parental aggression describes the act of protecting offspring from a predatory threat using aggressive behaviours at a cost to the parent (Archer, 1988). Due to the significant predatory threat to offspring, it could be argued that active defence of young through maternal aggression is likely to evolve (Archer, 1988). As aggression can carry significant costs natural selection may limit the use aggression by mothers to only when absolutely necessary.

### *Past investment vs. future potential*

Parental brood care may carry large costs including injury, greater predation risk and energy loss (Clutton-Brock and Vincent 1991, Smith and Wootton, 1995) and theory suggests parents should invest in their offspring according their reproductive value (Trivers 1972, Dawkins and Carlisle, 1976). Trivers (1972) identifies the point at which a parent should desert offspring in order to maximise their lifetime inclusive fitness and suggests that it would be wasteful to stop care if investment has already been made regardless of the potential impact on lifetime reproductive output.

However, others suggest a flaw in reasoning (the 'Concord fallacy' - Dawkins and Carlisle, 1976) and suggest that rather than continuing investment to justify the energy already used and the risk already taken, females should consider how much further investment is required not just how much has been put in when considering at what point to stop care and concentrate on future reproductive opportunities (Dawkins and Carlisle, 1976; see also Boucher, 1977). This contrasts with Trivers who's reasoning suggests that females ought never to abandon offspring despite the possibility that it may increase life time reproductive output.

Carlisle (1985) experimentally manipulated cichlid broods and showed that in *Aequidens coeruleopunctatus*, females are more likely to increase risk for larger

broods than smaller ones indicating that the strategy employed reflected future prospects for reproductive success (*i.e.* Dawkins and Carlisle, 1976) rather than the cumulative costs of past investment (*i.e.* Trivers, 1972). Maynard Smith (1977) proposed that cumulative past investment may be a reliable indicator of potential future success (see also Sargent and Gross, 1986) and where this is true a species may not be able to respond adaptively to future prospects. Indeed, other studies have shown that a number of species do commit the 'Concord fallacy' and continue care even when detrimental to lifetime fitness potential (*e.g.* savannah sparrows (*Passerculus sandwichensis* – Weatherhead, 1979) and digger wasps (*Sphex ichneumoneus* – Dawkins and Brockmann, 1980).

Parental investment theory should be applicable to maternal aggression as the costs of aggression can be high and may have serious consequences for future reproductive output. However, some threats may be more or less frequent and the aggressive response of brooding females towards potential predators may vary in relation to past investment or future potential of offspring production (*e.g.* Alonzo *et al.*, 2001; Taborsky and Foerster, 2004)

#### *Cichlids and maternal aggression*

Cichlids are an extremely diverse group of teleost fish demonstrating great variability in reproductive strategies (Keenleyside, 1991) and can be useful for studying parental behaviour (Goodwin *et al.*, 1988). For example, biparental, maternal or paternal care may be exhibited with substrate spawning or mouth brooding. Cichlid fry are very small compared to many of the fish that are found in the same environment and will be under great predation threat, from both specialised predators to opportunistic con or heterospecifics (see Alonzo *et al.*, 2001, for the response of a bi-parental cichlid

*Archocentrus nigrofasciatus* to 8 different predators where conspecifics posed the biggest threat).

Much of the ecology and evolution of cichlid species which are frequently used in scientific studies is already known with many having been used in aggression studies. Wisenden *et al.*, (2008) investigated differences in male and female investment in a bi-parental species; finding male investment to be associated with brood size whilst female investment relates to offspring age. Taborsky and Foerster (2004) have shown that females of the African cichlid *Ctenochromis horei* hold onto broods longer than usual in the presence of a predator suggesting that some flexibility is possible or that maternal strategies may be context specific. Thünken *et al.*, (2010) investigated brood quality and parental response in *Pelvicachromis taeniatus* and found that parents reduce care sooner in broods containing smaller fry. Suggesting that, in this species parents are able to adjust levels of care/effort in response to perceived offspring quality (future potential). An increase in levels of parental care aggression as a brood develops has been shown in two species of cichlid (*Oreochromis mossambicus*, Oliveira and Almada, 1998; *Archocentrus nigrofasciatus*, Alonzo *et al.*, 2001). Turner and Falter (1989) described how, in a dichromatic maternal mouth brooding cichlid species (*Oreochromis niloticus*: Baerends and Baerends van Roon, 1950), females may display male breeding colours during the mouthbrooding phase. Visual displays have costs; they can bring the attention of aggressive conspecifics or predatory threats and therefore must have some adaptive advantage in maternal care.



## Aims

Using cichlids two issues are addressed:

- Does maternal aggression (*i.e.* maternal effort) remain constant throughout the brooding phase in two haplochromine cichlids?
- Do two haplochromine species that exhibit different post fry release care react similarly to predatory threats?

## Materials and methods



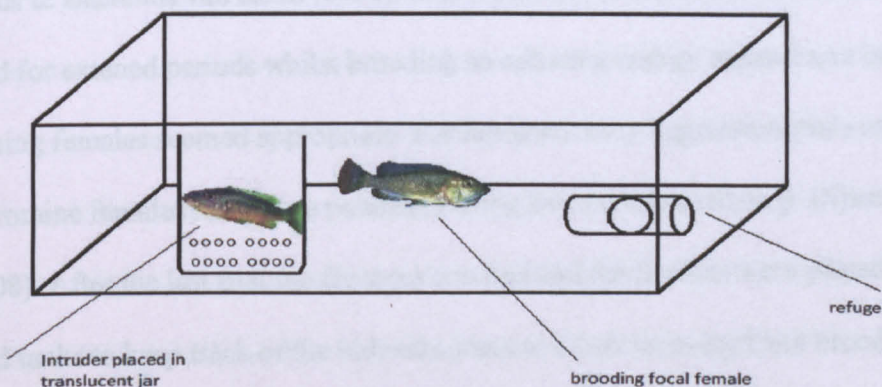
**Figure 15** *A. calliptera* brooding female and *P. thapsinogen* brooding female. Photographs taken by A. M. Tyers

### *Part A: Variation over the reproductive cycle*

In part A, *P. thapsinogen* females were used exclusively. Eight female focal fish were tested every 5 days against 5 unique stimulus fish until they had release all of their fry. The five unique stimulus fish were conspecific males, conspecific female, heterospecifics male (from the same genus), heterospecific females (from the same genus) and a brooding conspecific female. One month after the last trial the female focal fish were tested again to compare the levels of aggression when brooding to background levels of aggression. This ‘control’ may not be sufficient as ideally the

females would be tested in the non-brooding phase for as long as the brooding phase, however time and space requirements limited the study.

Females were wild caught from Eccles reef, near Makanjilla Point, on the eastern shore of Lake Malawi (S13° 46.239 E 034° 57.759.) and shipped back to the UK in 2008. On arrival they were kept in large (200 litre) single species stock tanks. Two metre tanks were partitioned and made ready for the maternal aggression experiments. Two metre tanks were divided into four compartments, partitioned with opaque plastic. Each experimental compartment consisted of 1 heater, one air filter and one piece of PVC tubing to act as a refuge for the focal female. Experimental tanks were constantly heated between 24° and 26° Celsius.



**Figure 4.2:** Experimental set up for congener maternal aggression experiments. In this instance *A. calliptera* as focal with *R. sp.* 'Chilingali' as an intruder.

*P. thapsinogen* females were allowed to breed freely with dominant males in their stock tanks and each day females were caught and checked for broods. Brooding females were placed in an experimental test chamber and given 72 hours to acclimatise before the first trial began. For each brooding female weight and standard length were measured (mean mass = 17.1g  $\pm$  0.1g, mean SL = 79 mm  $\pm$  1mm, n = 8) before introduction to the experiment. Fry numbers for each focal were counted and

recorded (mean number of fry = 23.5 S.E. = 3.86, n = 8). Where possible these were females not used as focal females and only 1 brooding females was used as a focal and stimulus fish. Heterospecific stimulus fish were caught from Chiofu Bay (Lake Malawi) during the same shipping period in 2008. For each trial one stimulus fish was placed in a perforated transparent plastic jar in the tank with the focal female. Two trials were conducted initially followed by three the next day. At least 1.5 hours was given between each trial. After 5 days the females were tested again. The order of stimulus fish remained the same: conspecific female, conspecific male, heterospecific female, heterospecific male, brooding conspecific female. The potential issues of order effect (Martin and Bateson, 2002) are addressed in the discussion. Each trial lasted five minutes and all aggressive behaviours were recorded as described by Baerends & Baerends van Roon (1950). Five minutes was chosen as the females do not feed for extended periods whilst brooding so reducing energy expenditure of the weakening females seemed appropriate. Furthermore many aggression trials using haplochromine females have been published using five minute trials (*e.g.* Dijkstra *et al.*, 2008). After the last trial the fry were counted and the females were placed in isolated tanks to keep track of the individual and to allow recovery from brooding. One month after the last trial each female was tested again after 48 hours acclimatisation, in the same tank in which they were first tested, with the same set up and with the same intruders that they were presented with during the mouth brooding phase of the experiment.

*Part B: Variation in levels of aggression and response to different intruders by two cichlids*

Two species of haplochromine cichlid were tested with three different intruders over the entire reproductive cycle including a non-brooding phase. Eight focal fish were

used for each species which differ in post release care; one offers no post release care whilst the other is known to guard fry for a short period after initial release.

*A. calliptera* wild caught females (caught in July 2009) were allowed to spawn with dominant males in stock tanks during September 2009. On a daily basis females were checked for broods and brooding females placed in an experimental tank consisting of a piece of PVC piping and a perforated plastic jar used to hold intruding stimulus fish. The *A. calliptera* experimental tanks were part of a large recirculation system and heated/filtered externally to between 24 and 26 degrees Celsius. Three species of intruders were used to assess the nature of maternal aggression in this species. The first species used were conspecifics (females only). Secondly a sympatric heterospecific piscivore (wild caught *Rhamphochromis* sp. 'Chilingali' – fig. 4.3) was used to test aggression levels against a sympatrically occurring fry predator. Lastly, a cichlid species from a relatively distant genus (wild caught *Cynotilapia afra* from Nkhata Bay) was used to assess how focal females reacted to a species it may normally be in no competition with or feel no predatory threat from. Each focal female was weighed and measured after the experiment (mean mass =  $5.92\text{g} \pm 0.1\text{g}$ , mean SL =  $60.6\text{ mm} \pm 1\text{mm}$ ,  $n = 8$ ) and number of fry were recorded (mean number of fry = 25.7, S.E. = 1.08,  $n = 8$ ). After a period of acclimatisation (3 days) the stimulus fish were in turn added to the perforated jar in the experimental tank. All aggressive behaviours to each intruder were counted over a 5 minute period. There was a gap of at least 1 hour between each experiment. 7 days after the first set of experiments the females were tested again and every 7 days until all the fry were released. When some or all fry were released the female was tested for the last time. After this last trial the female was removed, separated from her fry and allowed to recover in a tank of her own. One month after the last trial the female focal fish was

then tested again with the same intruders. Exception was made for two female focal fish that had spawned in isolation and were found to be brooding unfertilised eggs, these were allowed to lose their eggs (probably through ingestion) and recover before being tested for the last time. This 'control' (as well as in part A) may not be sufficient as ideally the females would be tested in the non-brooding phase for as long as the brooding phase, however time and space requirements limited the study.

In the second half of part B, *P. thapsinogen* females were allowed to spawn freely with a dominant male in their stock tanks. Females were from the same stocks as part A and it is likely some will have participated in both experiments. Each day females were checked and brooding females were removed and placed in their experimental tanks. The brooding *P. thapsinogen* female focal fish were also tested with three intruders, one conspecific female, one piscivore (again *R. sp.* 'Chilingali') and one other cichlid, *Oreochromis shiramus*, which served as a similar intruder to *C. afra* i.e. distantly related non-threatening to adults, but still a potential threat to fry. As with the *A. calliptera* trials each female was given three days to acclimatise and tested with each intruder every 7 days until all fry were released (mean number of fry = 33.8 S.E. = 5.22, n = 8). All focal females were weighed and measured after the last experiment (mean mass = 20.4g ± 0.1g, mean SL = 84.9 mm ± 1mm, n = 8). Experiments began in December 2009 and after all trials each female was removed and placed in isolation to allow tracking and recovery. After one month of recovery each female was then tested again the same experimental tank with the same intruder stimulus fish. As above, this 'control' may not be sufficient as ideally the females would be tested in the non-brooding phase for as long as the brooding phase, however time and space

requirements limited the study. In both parts of this study the light cycle was 12L: 12D. All fish were fed aquarium flake.

In all experiments and in both parts of this study all types of aggressive behaviour were pooled, a number of types of behaviours (*e.g.* quiver) were only performed by a small number of individuals. As the female focal fish were mouth brooding the use of physical aggression (*e.g.* lunge) appeared to be rare and bites non-existent. Therefore splitting behaviour into visual displays or physical attacks seemed redundant.

### Data analysis

All data were tested for normality (Kolmogorov – Smirnov test) and homogeneity of variances (Levene's test) before carrying out statistical analyses. Where the assumptions were not met data transformation were considered before non-parametric tests were used. See Appendix for analysis of data sets. Tukey HSD post hoc tests were used when applicable due to the reduced likelihood of Type II errors compared to other post hoc tests (*e.g.* Scheffe's test and LSD test).

## Results

### *Part A.*

*P. thapsinogen* females did not show significant differences in the levels of aggression given to various intruders over the reproductive cycle when tested with 5 intruders (ANOVA  $F_{4, 4} = 1.267$ ,  $p = 0.281$ ). However, *P. thapsinogen* focal fish did show a significant difference in the levels of aggression between days ( $F_{4, 4} = 7.052$ ,  $p < 0.001$ ), with Tukey post hoc test revealing significant differences between day 5 and day 10 ( $p = 0.008$ ) and between days 5 and 15 ( $p = 0.018$ ), day 9 and the non-brooding trial ( $p < 0.001$ ) and day 15 and the non-brooding trial ( $p = 0.001$ ).

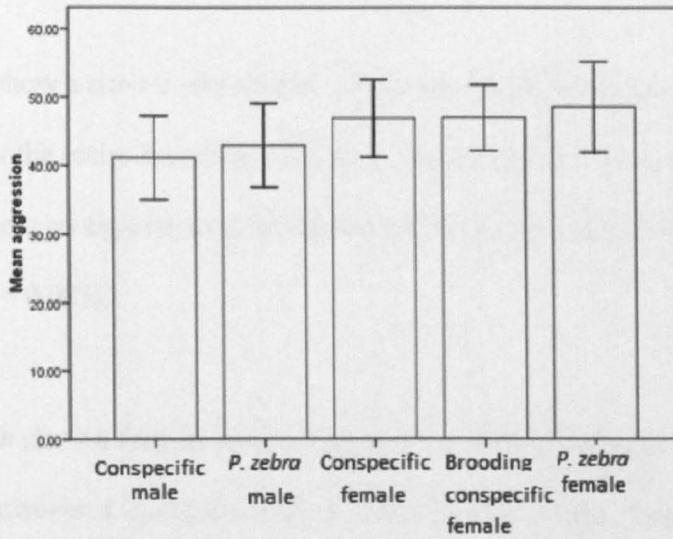


Figure 16 Mean aggression displayed to various intruders of the reproductive cycle by *P. thapsinogen* females with 95% confidence limits. N = 8 focal fish.

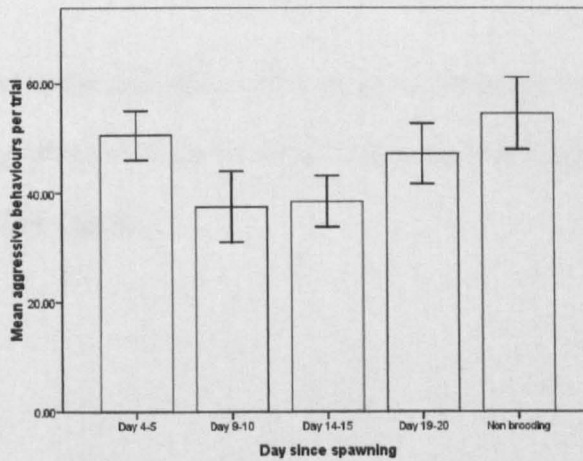


Figure 17 Mean aggression by focal *P. thapsinogen* females displayed to various intruders as organised by time period with 95% confidence limits. N = 8.

### Part B.

#### Comparisons between the focal species

When comparing the two focal species, *P. thapsinogen* focal fish used significantly more aggressive behaviours towards conspecific intruders (Mann-Whitney U,  $Z = -2.526$ ,  $P = 0.012$ ) than *A. calliptera* did.

*A. calliptera* show a similar response to a distantly related heterospecifics (*O. shiranus*) over the entire brooding cycle as *P. thapsinogen* (*C. afra*) Overall the two focal types show no significant difference in levels of aggression (Mann-Whitney U,  $Z = -1.734$ ,  $p = 0.083$ ).

*P. thapsinogen* show a drop in levels of aggression directed towards *R. sp.* 'Chilingali' however *A. calliptera* show a continual rise in aggression towards the end of the brooding phase, peaking when the fry are released and guarded by the females. Overall there was no significant difference in levels of aggression directed at *R. sp.* 'Chilingali' (Mann-Whitney U,  $Z = -0.166$ ,  $p = 0.868$ ).

*A. calliptera* held on to their brood for at least an additional 6 days compared to *P. thapsinogen* during which time the fry would frequently leave the females buccal cavity and return when startled.



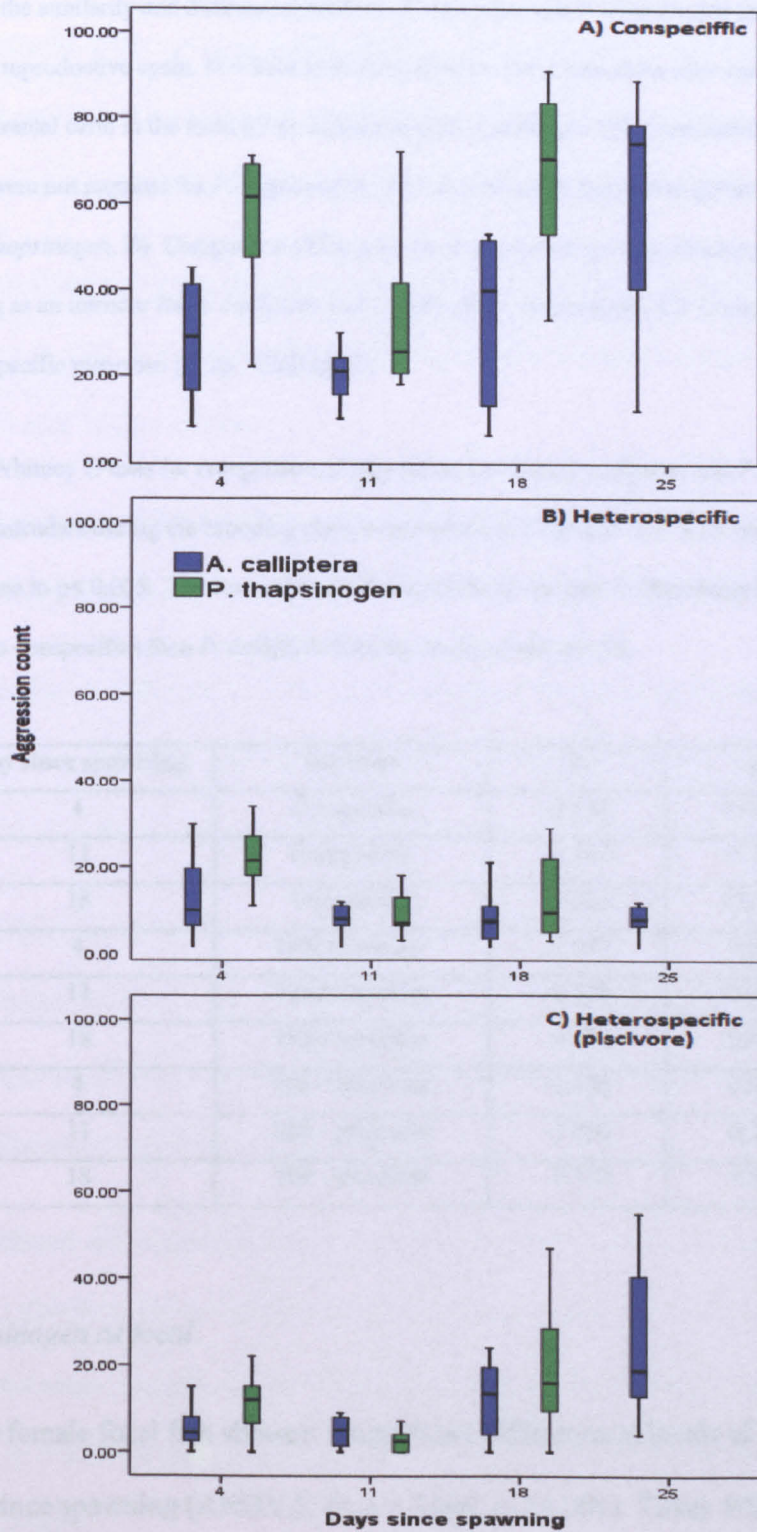


Figure 18 Shows the similarity and differences between *A. calliptera* and *P. thapsinogen* in response to various intruders over the reproductive cycle. N = 8 for both focal species. Error bars show inter-quartile ranges Note *A. calliptera* offer parental care in the form for an additional period leading to trilas conducted 25 days after spawning which were not required for *P. thapsinogen*. **A)** Comparison of aggression given to conspecifics by *A. calliptera* and *P. thapsinogen*. **B)** Comparison of the amount of aggression given to heterospecific intruders with *O. shiramus* acting as an intruder for *A. calliptera* and *C. afra* for *P. thapsinogen*. **C)** Comparison of aggression given to a heterospecific piscivore (*R. sp.* 'Chilingali').

Table 5 Mann – Whitney U tests for comparison of aggression levels in *A. calliptera* and *P. thapsinogen* females for three intruders during the brooding cycle as presented in Figure 18. N = 8 for each focal type. \* denotes significance to  $p < 0.005$ . The two only significant results reveal that *P. thapsinogen* focal females were more aggressive to conspecifics than *P. calliptera* females on day 4 and day 18.

Day since spawning	Intruder	Z	p
4	Conspecific	-2.682	0.007*
11	Conspecific	-1.367	0.172
18	Conspecific	-2.521	0.012*
4	Heterospecific	-1.947	0.052
11	Heterospecific	-0.318	0.751
18	Heterospecific	-0.842	0.400
4	Het – Piscivore	-1.738	0.082
11	Het – piscivore	-1.236	0.216
18	Het - piscivore	-0.955	0.340

#### *Part B P. thapsinogen as focal*

*P. thapsinogen* female focal fish showed a significant difference in levels of aggression between days since spawning (ANOVA;  $F_{3, 84} = 7.660$ ,  $p < 0.001$ ). Tukey HSD test revealed significant differences between day 4 and day 11 ( $p = 0.003$ ) and between days 11 and 18 ( $p < 0.001$ )

A significant difference (ANOVA;  $F_{2, 84} = 76.619$ ,  $p < 0.001$ ) between levels of aggression given to each intruder type was also found, with the post hoc Tukey HSD test showing significant differences between aggression directed at conspecifics and heterospecifics ( $p < 0.001$ ) and between conspecifics and the heterospecific piscivore ( $p < 0.001$ ).

Mean aggression significantly differed between days since spawning towards the conspecifics (ANOVA,  $F_{3, 7} = 3.240$ ,  $p = 0.037$ ), Tukey post hoc tests revealed a significant difference between days 11 and 18 ( $p = 0.038$ ).

There was no significant difference in levels of aggression given to the heterospecific intruder over the reproductive cycle (ANOVA,  $F_{3, 7} = 2.294$ ,  $p = 0.1$ ).

There was a significant differences in the levels of aggression given to the heterospecific piscivore over the reproductive cycle (ANOVA,  $F_{3, 7} = 3.905$ ,  $p = 0.019$ ). Tukey post hoc testing revealed a significant difference between days 11 and 18 ( $p = 0.017$ ).

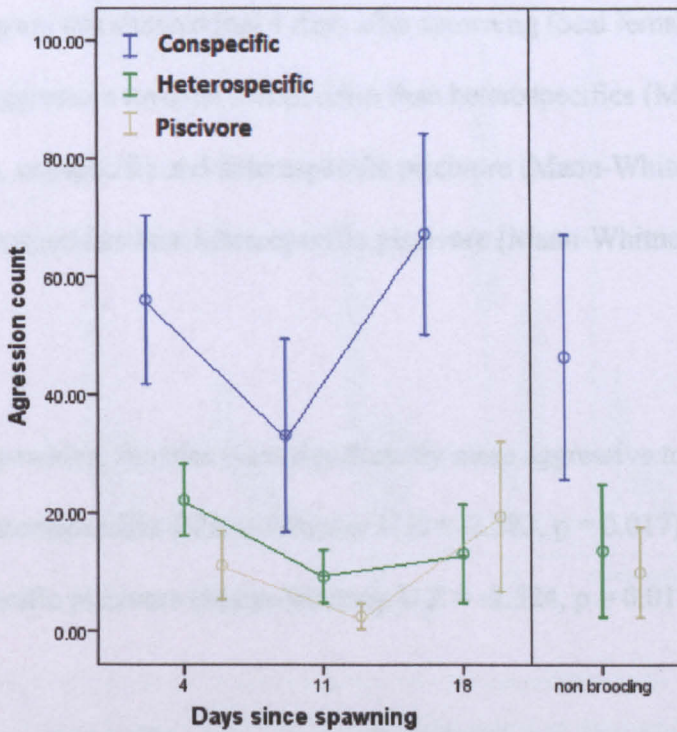
A significant differences in levels of aggression given to different intruders 4 days after spawning was found (ANOVA,  $F_{2, 7} = 12.658$ ,  $p = <0.001$ ). Tukey post hoc test revealed significant differences between the conspecific and piscivore aggression levels ( $p < 0.001$ ) and heterospecific and piscivore means ( $p = 0.04$ ).

There was significant differences in levels of aggression given to different intruders 11 days after spawning (ANOVA,  $F_{2, 7} = 26.329$ ,  $p < 0.001$ ). Tukey post hoc tests revealed the differences lay between. For day 11 the significant difference lay between conspecifics and heterospecifics ( $p = 0.009$ ), conspecifics and piscivores ( $p < 0.001$ ) and heterospecifics and piscivores ( $p = 0.002$ ).

Significant differences in levels of aggression given to different intruders 18 days after spawning was found (ANOVA,  $F_{2, 7} = 8.815$ ,  $p = 0.002$ ) Tukey post hoc tests revealed

significant differences lay between conspecifics and heterospecifics ( $p = 0.003$ ) and between conspecifics and piscivores ( $p=0.006$ ).

There was also significant differences in levels of aggression given to different intruders when females were not brooding (ANOVA,  $F_{2, 7} = 6.249$ ,  $p = 0.007$ ). Tukey post hoc tests revealed significant differences between conspecifics and heterospecifics ( $p = 0.019$  and conspecifics and piscivores ( $p=0.013$ ).



**Figure 19** Shows the mean aggression given to different intruders by *P. thapsinogen* focal females over the entire reproductive cycle. Error bars = 95% confidence limits.

*Part B A. calliptera as focal*

*A. calliptera* female focal fish showed a significant difference in aggression towards a conspecific, a heterospecific (*C. afra*) and a heterospecific piscivore (*R. SP.* 'Chilingali'), (Kruskal – Wallis, Chi-Square = 41.103, d.f. = 2,  $p < 0.001$ )

There was also a significant difference in levels aggression given over the reproductive period (Kruskal – Wallis, Chi-Square = 10.535, d.f. = 4,  $p = 0.035$ ).

Wilcoxon matched pairs test showed that 4 days after spawning focal females were significantly more aggressive towards conspecifics than heterospecifics (Mann-Whitney U Z = -2.524,  $p = 0.012$ ), conspecific and heterospecific piscivore (Mann-Whitney U Z = -2.521,  $p = 0.012$ ) and heterospecifics than heterospecific piscivore (Mann-Whitney U Z = -1.963,  $p = 0.050$ ).

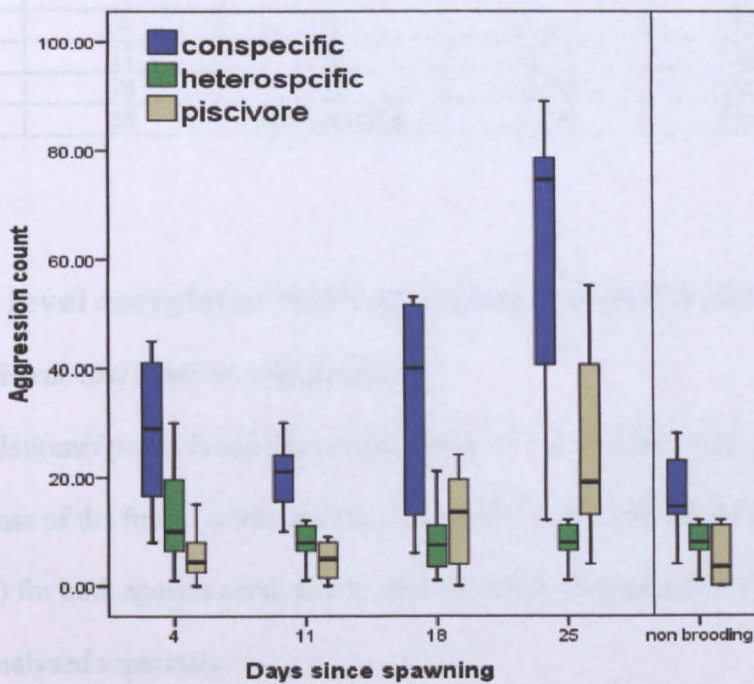
Eleven days since spawning, females were significantly more aggressive towards conspecifics than heterospecifics (Mann-Whitney U Z = -2.383,  $p = 0.017$ ) and conspecifics more than heterospecific piscivore (Mann-Whitney U Z = -2.524,  $p = 0.012$ ).

Eighteen days since spawning, females were significantly more aggressive towards conspecifics and heterospecifics (Mann-Whitney U Z = -2.103,  $p = 0.035$ ) and conspecifics than heterospecific piscivores (Z = -2.240,  $p = 0.025$ ).

Twenty five days since spawning, females were significantly more aggressive towards conspecifics than heterospecifics (Mann-Whitney U Z = -2.523,  $p = 0.0121$ ), conspecifics

than heterospecific piscivores (Mann-Whitney U  $Z = -2.521$ ,  $p = 0.012$ ) and heterospecifics piscivores more than heterospecifics ( $Z = -2.313$ ,  $p = 0.021$ ).

During the non-brooding phase focal females were more aggressive to conspecifics than heterospecific piscivores (Mann-Whitney U  $Z = -2.527$ ,  $p = 0.012$ ). Before the first of all the day 25 tests the *A. calliptera* females had released their fry.



**Figure 20** Females varied the amount of aggression given over the reproductive cycle. They also varied the intensity of aggression given to different intruders. Error bars show inter-quartile ranges.

Table 6 Wilcoxon matched pairs test results as multiple tests are made for comparisons of aggression given to intruders between each trial including the non-brooding phase when *A. calliptera* was focal. **Bold** indicates which trial showed a significantly greater amount of aggression. Non-significant results not shown see appendix. There were no significant differences between aggression for heterospecifics between any of the trials.

Intruder	Day	Day	Z	P
Conspecific	<b>4</b>	11	-2.106	0.035
	4	<b>25</b>	-2.100	0.030
	<b>4</b>	Non-Brooding	-1.960	0.05
	11	25	-2.380	0.017
	18	<b>25</b>	-2.240	0.025
	<b>25</b>	Non-Brooding	-2.240	0.025
Heterospecific Piscivore				
	4	<b>25</b>	-2.521	0.012
	11	<b>25</b>	-2.524	0.012
	18	<b>25</b>	-2.103	0.035
	<b>25</b>	Non-Brooding	-2.380	0.017

## Aggression level correlates relating to parental investment theory

### *Mass of focal female and number of fry produced*

No apparent relationship was found (Spearman's rank;  $r^2 = 0.086$ , d.f. = 23,  $p = 0.696$ )

between the mass of the focal female and the number of fry it produced Includes data for both parts (A and B) for both species used, this is also true when each part of the study (*i.e.* Part A, part Bi, ii) is analysed separately.

### *Aggression vs. number of fry*

There was no relationship between the amount of aggression given and the number of fry produced by focal females (Spearman's rank;  $r^2 = -0.153$ , d.f. = 23,  $p = 0.485$ ), this is also true when each part of the study (*i.e.* Part A, part Bi, ii) is analysed separately.

### *Aggression vs. mass of focal females*

It was found that there was no correlation between mass of the focal and mean amount of aggression given (Spearman rank order correlation,  $r^2 = 0.296$ , d.f. = 23,  $p = 0.171$ ) when all experiments and all focal fish were analysed (*P. thapsinogen* in part A and *P. thapsinogen/A. calliptera* in Part B). However, in part A only a correlation with *P. thapsinogen* was found – see below.

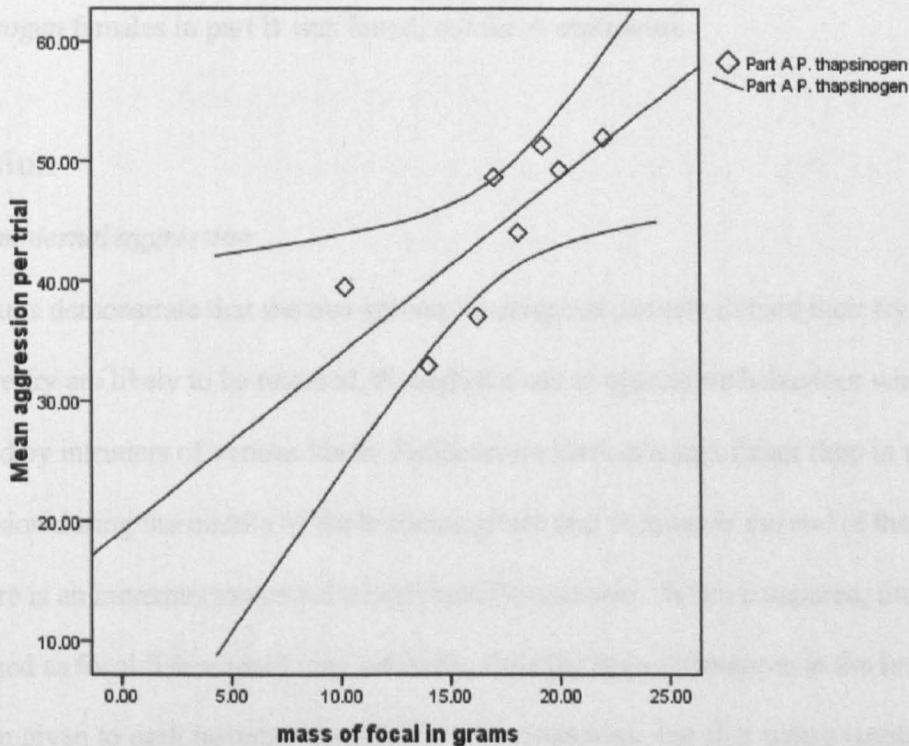


Figure 21 A Pearson product moment correlation was carried out and a significant correlation was found between mean aggression given to intruders and mass of focal for *P. thapsinogen* in part A ( $r = 0.762$ , d.f. = 7,  $p = 0.028$ ). This positive association was only found for Part A of the experiments. (See Appendix IV for scatter chart including all three data sets). 95% Confidence limits shown.  $N = 8$  over 20 trials per female focal fish.



*Aggression vs. mass of intruder***Part A**

No correlation was found between mean aggression given to any of the intruder types and mass of the intruder for *P. thapsinogen* in part A

**Part B**

No correlation between mass of any of the intruders and aggression given to the intruder for *P. thapsinogen* females in part B was found, nor for *A. calliptera*.

**Discussion***Levels of maternal aggression*

These results demonstrate that the two species investigated actively defend their fry or the area where fry are likely to be released, through the use of aggressive behaviour when confronted by intruders of various kinds. Furthermore there is a significant drop in the level of aggression during the middle of the brooding phase and in towards the end of the brooding phase there is an increase towards the heterospecific piscivore. When compared, the two species used as focal fish reacted very similarly, showing little differences in the levels of aggression given to each intruder on each day since spawning, but also with a similar drop in aggression during the middle of brooding phase. Finally, a shared rise in aggression towards the heterospecific piscivore shortly before fry release is seen. These shared characteristics of parental care are interesting as *A. calliptera* have extended fry care (fry guarding post release) and might be expected to behave differently to *P. thapsinogen* which exhibit the more derived condition of no additional care after fry release.

Aggression may carry significant physiology costs and a reduction during the brooding period may be beneficial. Brooding fry for extended periods has significant costs in itself, although some (*e.g.* mbuna) may take in small amounts of food many haplochromine cichlids

do not eat while brooding (Oppenheimer, 1970). Carrying fry in the buccal cavity may also compromise oxygen uptake (Corrie *et al.*, 2008) forcing behavioural modifications which may increase the risk of predation (Corrie *et al.*, 2008) - for example females may have to swim closer to the surface where oxygen levels may be higher (Corrie *et al.*, 2008). These factors may have significant impacts on energy reserves and the ability to be active which could explain a significant drop in level of aggression during the middle of the brooding phase in these experiments.

The observed dip in aggression levels may also correlate with a drop in testosterone during the middle of brooding; as has been demonstrated in the cichlid *O. mossambicus* (Smith and Haley, 1988), which showed one peak in testosterone just before spawning and another during the final phase of incubation. It is interesting to note that *P. thapsinogen*, although thought to offer no post release care (*e.g.* Genner and Turner, 2005), were generally more aggressive during the last experimental trial; after fry had been released into the experimental tank. Very little is known about female behaviour at the end of brooding in the wild, though it is believed that they release fry into rocky crevices or under rocks where they are left to defend themselves (Trendall 1988; Genner and Turner, 2005). However, this increase in aggression after fry release could indicate some degree of care through the defence of space in which fry will remain for some time, which could explain weak female territoriality in the wild (see Ribbink *et al.*, 1983). Fry have been observed to be aggressive from an early stage (Trendall, 1988) and this early aggression may be to secure a place within groups found in crevices or under rocks, with outside places possibly being subjected to greater predation threat. Would fry need to be this aggressive, wasting energy for growth, if the mother was still actively defending them from outside of the crevice? Possibly, as mothers who defend a crevice against fry predation are increasing the likelihood of survival for all the fry found there. Haplochromine fry in the two species used *may* be capable of independent living after

2 weeks of brooding (A. Smith pers. com) as they are developed enough for swimming and probably feeding although at this stage they very small. Females probably keep them longer to ensure they have better of survival post release. It has been observed (G. M. Cooke) that fry get bigger during the last week of brooding despite no egg/ remaining. Where they receive nourishment is unknown although it has also been observed (A. Smith pers. com.) that in aquaria females will sometimes eat flake food whilst brooding.

*P. thapsinogen* females did not bias aggression towards any of the congeneric intruders during Part of A of this investigation. This was unexpected for a number of reasons. Firstly, *P. thapsinogen* females may be in competition for fry release places. Although there are potentially thousands of crevices in any one stretch of shoreline, there may also be many brooding females (of multiple species) looking for release sites at any one time. If the focal females were in serious competition with other brooding females then it is not unreasonable to expect an aggression bias towards the brooding female intruders; this however was not the case. The male intruders were typically much larger than the focal females and it was expected that the focal females would use the refuge provided during the experimental trials with males as stimulus fish, however this was not the case. In other circumstances a female may avoid physical contact with aggressors through ritualised display which has been shown to be an effective deterrent in cichlid aggression (Keeley and Grant, 1993). However, the loss of fry has potentially higher costs and may cause a female to risk much more by taking on bigger opponents than usual. Infant mortality is widespread throughout the animal kingdom (Archer, 1988) and the results of this study suggest that males and females may pose a similar threat in this respect; the sex of the intruder did not influence the amount of aggression received from the focal fish.

When presented with different con- and heterogeneric intruders, females of both subject species were found to show higher levels of aggression towards some intruders over others.

Both *P. thapsinogen* and *A. calliptera* females consistently attacked conspecific females significantly more than the other two intruder types, which is in keeping with well documented conspecific aggression biases in male haplochromine cichlids (Pauers *et al.*, 2008). Conspecifics may gain more than just opportunistic feeding from unprotected fry; they may be removing competitors to secure resources for their own offspring, which is considered a function of infanticide (Archer, 1988; Sherman, 1981).

It is interesting to see that *A. calliptera* and *P. thapsinogen* females showed an increase in aggression towards the heterospecific piscivore at the late phase of mouth brooding, just before fry release. The females may be able to recognise that different species pose different threats at different times during the reproductive cycle. If the threat to fry from a heterospecific piscivore was not deemed to be higher around the time of fry release, then levels of aggression towards it should remain constant throughout the brooding period (as it is with the heterospecific 'non-piscivore' intruder). *R. sp.* 'Chilingali' was collected from the same satellite lake of Lake Malawi (Lake Chilingali) as the *A. calliptera* population used in these experiments. It is therefore not surprising that they should be recognised as a threat, as they may come across each other frequently in the wild. Although the *P. thapsinogen* females will not have come across this specific species other *Rhamphochromis* species occupy their habitat.

Aggression directed towards the other heterospecific stimulus fish may be the result of some general strategy to deter intruders of any kind. This could be tested with further experiments using non-cichlid stimulus fish. It is also possible that due to aggressive signalling being very conserved in cichlids (Myers 1960; Ribbink, 1990) positive feedback may drive up levels of aggression. It was observed that when *A. calliptera* females made the lateral display towards *C. afra* intruders, some *C. afra* females would seemingly recognise this signal and reciprocate with a lateral display and the same may be true for *P. thapsinogen* focal females.

*Past investment vs. future potential*

Females might be expected to gradually increase care as fry develop (Dawkins and Carlise, 1976; Pianka, 1983) as older fry are more likely to reproduce themselves and this is supported by a number of studies (e.g. Wisenden and Keenlyside, 1995) including some with cichlids (e.g. Almada and Oliveira, 1998; Taborsky and Foerster, 2003; Thünken *et al.*, 2010)). By reducing effort and the possibility of exhaustion when energy reserves are dwindling, the females may be considering future prospects *i.e.* saving themselves for future spawning. Mouth brooding in cichlids is a costly strategy; unlike many animals they may not feed during the period in which they protect developing offspring, so there may be a trade-off forcing a reduction in care (*i.e.* aggression) during the middle period of mouth brooding which is seen in this study. This allows them to have the required energy for high levels of aggression when fry are at their most developed and still in the care of the female. As stated above, it has been shown in *O. mossambicus* (Smith and Haley, 1988) that hormonal peaks of testosterone coincide with peaks of aggression found in this study which may indicate a hardwired response, one that has been strongly selected for, and one not based on information available to the female *i.e.* size of brood or quality of offspring.

An indicator of a future potential strategy might be one of a correlation between fry number (brood size) and aggression, which has been experimentally shown to be important in other cichlids (e.g. Carlisle, 1985). Here this is not found; there is no correlation for either species between brood size and aggressive behaviours directed at various predatory threats, which may suggest the alternative hypothesis of past investment (*i.e.* Trivers, 1972) or some flaw in experimental design based on order effect.

Maynard Smith (1977) was the first to point out that past investment may be a reliable indicator of future potential if brood size is likely to remain constant, as it may for *P.*

*thapsinogen* females. *A. calliptera* females may have broods reduced during the post release care stage. Parental investment theory predicts that parental care should increase with age of the parent (e.g. Williams, 1966a, b; Tallamy, 1984; Pianka, 1983), this is because as a female gets older the number of reproductive opportunities diminishes meaning that securing fry reach reproductive age themselves becomes more worthwhile. Young females can afford mistakes or less input as they have many more reproductive opportunities left. This may explain why *P. thapsinogen* females show a correlation between mass of focal and aggression in Part A, but this correlation is lost in Part B where a correlation between aggression and mass of intruder is found. In some bird species it has been shown that older birds lay earlier, enjoying a greater reproductive success (Crawford, 1977; Finney and Cooke, 1978; Middleton, 1979) and it has been shown in another cichlid (*Cichlasoma nigrofasciatum*) that more experienced parents were more aggressive than parents of the same age who were only breeding for the first time (Lavery, 1994). There was a 20 month gap between the experiments of part A and part B where females were kept in mixed sex stock tanks for long periods and allowed to breed, and this could allow sufficient time for the *P. thapsinogen* females to develop experience and adopt a different strategy.

There are possibly difference in maternal strategies even within the very recently radiated group of African haplochromine cichlids, this comes not only from the two clear behavioural differences in post release care by the two species used here but also observations of how significantly differentiated Lake Malawi cichlids behave in aquaria. Haplochromines such as *Rhamphrochromis* spp., *Lethrinops* spp. and *A. calliptera* (used as focal fish in this study) will spit out all eggs early on in fry development should they be handled, later on in fry development females will be much more likely to hold onto fry despite probably feeling under the same threat. At least some of the rock dwelling mbuna cichlids (e.g. *P. thapsinogen*, used as focal fish in this study, and *Cynotilapia* spp.) however do not do this;

females will retain fry whilst being handled at any stage after fertilisation of the eggs (G. M. Cooke pers.obs). However, other researchers with experience of catching these fish in the wild have found this not to be the case (M. Knight pers.com)

#### *Order effect within the experiments*

As the order of intruders was not randomised with respect to each trial for both parts of this study a number of potential confounding effects must be addressed.

Martin and Bateson (2002) list the following as potential problems when order of intruders is not randomised: arousal, sensitisation, conditioning, fatigue, and habituation. These are undoubtedly impossible to untangle and it is not possible to be absolutely confident that they had no effect on the outcome of the results regarding aggression to intruder type and conclusions regarding aggression biases must be cautiously accepted.

Part B shows a change in aggression towards intruder type over the time period (*e.g.* relative changes towards the distantly related heterospecific intruder and the heterospecific piscivore towards the end of the brooding period). If order effect was influencing aggression biases, for example if habituation was important a change in aggression might not go up but rather go down. If arousal was a factor then one might expect the last of the three experiments for each focal would produce the most aggressive behaviours which was never seen. Habituation may be a problem with respect to the focal fish receiving the same intruders each trial. This may be a necessary evil as presenting different intruders (of each type) may produce different responses *i.e.* if the size of the intruder, the motivation state of the intruder or other uncontrollable features varies for each focal then a varying response might be produced that is in addition to the time variable. Territorial aggression and habituation has been shown in a number of species, including cichlids (*e.g.* Peeke and Peeke, 1982) and sticklebacks (Peeke and Veno, 1973) which is probably an adaptive function. Although this was specifically the case between permanently adjacent territorial neighbours and not intruders that were

presented at least 5 days apart as in this study). Furthermore, Peeke and Veno (1973) showed that sticklebacks are highly specific in the stimulus they become habituated too suggesting a serious but repeating threat (to fry for *e.g.*) may not result in a diminished response.

It may be that confidence of the focal female increases and this altered how they treated a specific threat but if females were remembering intruders or types of intruders the order they were presented would not matter.

Given the nature of the experimental issue within this study any conclusions must be very limited. Females of both species are clearly aggressive and future work with better experimental rigour may reveal some of what is found here to be consistent with what females do in the wild.

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## Appendix

### Statistics and data analysis

#### *Part A.*

As there was no significant differences in variances (Levene's test,  $p = 0.669$ ) and the data normally distributed (Kolmogorov – Smirnov test,  $Z = 1.040$ ,  $p = 0.569$ ), ANOVA was used to look for differences in aggression directed at congeners over the reproductive cycle.

#### *Part B.*

##### *P. thapsinogen as focal*

No significant differences in variance (Levene's,  $p = 0.458$ ) and a normal distribution (Kolmogorov – Smirnov test,  $Z = 2.001$ ,  $p = 0.230$ ) allowed for the use of parametric statistics (ANOVA) for the *P. thapsinogen* data set.

##### *A. calliptera as focal*

*A. calliptera* focal data was found to deviate significantly from a normal distribution (Kolmogorov – Smirnov test,  $p = 0.001$ ). Data were arcsine transformed but this did not alter the distribution with respect to normality. The Levene test of equality of variances produced a probability value suggesting variances were not equal ( $F = 7.321$ ,  $d.f. = 105$ ,  $p < 0.001$ ). Having failed two assumptions of parametric tests, non-parametric equivalents were used for subsequent data analysis.

## Chapter 5: Female aggression in Malawian mbuna cichlid fish

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### Abstract

Males are more aggressive than females in many species, but female aggression may aid foraging success, predator avoidance (*e.g.* through access to refuge) or defence of offspring (maternal aggression). Little work has gone into exploring female aggression and this is no less true than for haplochromine cichlid fish where recent work has suggested aggression (and territoriality) may aid coexistence of a remarkable number of species. Female haplochromine cichlid fish appear relatively unaggressive compared to males, particularly those that do not care for free swimming young, such as *Pseudotropheus* spp. However, in controlled experimental conditions, male and female *Pseudotropheus zebra* showed no difference in their rates of performance of aggressive behaviours although the frequencies of behavioural types differed significantly, with males performing more non-contact visual displays and females more direct attacks. *Pseudotropheus thapsinogen* females were found to be more aggressive to conspecific female intruders confined near to a refuge than those in proximity to a conspecific male or a bare tank. Levels of aggression by *P. thapsinogen* females towards conspecifics varied through the day, and were significantly higher in the morning than in mid-afternoon or evening.

## Introduction

### *Aggression*

Aggression is widespread in the animal kingdom, found in a wide variety of taxa (Archer, 1988) from sessile invertebrates (*e.g.* the sea anemone *Actinia equina*, Brace *et al.*, 1979) to more complex organisms with sophisticated social systems. Aggression can be classified into three functional aspects; protective, parental and competitive (Archer, 1988). Aggression can carry significant costs, increasing chance of injury, energy requirements, risk of predation and time spent away from foraging or reproducing. Simply having elevated levels of testosterone may be metabolically expensive - male Yarrow's spiny lizards *Sceloporus jarrovi* with higher levels of testosterone are more energetic than those with lower levels (Marler and Moore 1991). We are just beginning to understand the molecular and genetic basis of aggression (Nelson and Chiavegatto, 2001, Gill *et al.*, 2007 and see Chapter 1).

Male aggression is often explained by sexual selection (*i.e.* during competition for females). Male territorial aggression may be to defend resources that attract females; territories may provide benefits to females in the form of additional resources (*e.g.* food or a refuge). Where there are no apparent direct benefits, male competition is proposed to provide indirect benefit in the form of superior genes, as strong and fit males are more able to hold territories or win physical contests (Zahavi, 1975; Wilson, 1975).

### *Female aggression*

Female aggression in the animal kingdom is common though relatively understudied (Archer, 1988). We are just beginning to untangle the molecular basis of female aggression and whether phenotypic convergence between males and females is common or whether females and males use the same genes/ hormonal pathways (*e.g.* Davis and Marler, 2003; Rubenstein and Wikelski, 2005; West-Eberhard, 2003; Aubin-Horth *et al.*, 2007). Most studies of female aggression relate to parental care (*i.e.* maternal aggression) which has been shown in a wide variety of taxa (see Archer, 1988). Female territoriality has been proposed to have originally evolved as a method for reducing infanticide; females may occupy territories for the sole

purpose of settling contests with conspecifics before offspring are raised (Sherman, 1981; Wolff, 1993; 1997). There are far fewer studies focusing on female aggression where parental care is not invoked as a functional explanation.

### *Female – female competition*

There are a few examples which suggest that in some specific cases aggression may actually be used by females to gain access to mates. The conditions may be relatively rare as it has been proposed that similar parental roles, a female biased operational sex ratio (OSR) and variation in male quality are required (Berglund *et al.*, 1993). Yet despite these relatively rare conditions female competition for males has been shown. For example, in the red deer (*Cervus elaphus*) females are more aggressive in harems where mating order is at stake (Bebie and McElligott, 2006).

Mating order may matter, it has been suggested that in Topi (*Damaliscus lunatus*), dominant males suffer sperm depletion (Bro-Jorgensen, 2002) and this may be the case in other ungulates too (*e.g.* Preston *et al.*, 2001). Although sperm are considered cheaper to make than ova only so much may be stored and more will have to be produced in a males lifetime, selection should favour males that allocate their limited ejaculates prudently (Parker, 1990). A lekking haplochromine cichlid (*Astatotilapia flavijosephi*) has been shown to exhibit male choosiness for larger females (Werner *et al.*, 20023). This may mean males with similar reproductive strategies (*e.g.* territorial holding) may choose to mate with some females ahead of others leading to sperm depletion and ultimately female competition for dominant males.

Female birds have also been shown to use aggression in competition for males (*e.g.* Lefelaar & Robertson, 1985). Female ornaments have also been shown experimentally, to be used by males in mate choice decisions and sexual selection through male choice may be driving female signals (Amundsen and Forsgren, 2001). Although rare, sexually selected female aggression is conceivable given the right conditions.

Females may be competing for refuge from predators or food. However, aggression to obtain such resources may not specifically be a female trait; rather a trait found in the species as a whole. Where males and females are both aggressive over resources there is a propensity to focus on one sex or the other (Amott and Elwood, 2009). Where there is strong dimorphism between sexes (for example, males have weaponry and females do not) and/or differences in the frequency of aggressive encounters (where males compete for females *and* more typical resources such as food), natural selection may shape male and female behaviours separately (Archer, 1988).

### *Cichlids and aggression*

Cichlid fish have long been used to test ideas relating to aggression in animal behaviour and have helped substantiate the sequential assessment model (SAM) (Enquist and Leimar, 1983). For example; *Oreochromis mossambicus* has been used to investigate asymmetries in resident and intruder contests (Turner, 1994). Cichlids have also been used to test ideas and theories for social hierarchies (e.g. Oliveira, 1998; Chase *et al.*, 2002; 2003). *Amatitlania (Cichlasoma) nigrofasciatum* has been used to test escalation relating to visual signalling (Keeley and Grant, 1993). *Amatitlania (Cichlasoma) nigrofasciatum* has also been used to show that habitat complexity directly affects levels of aggression in females (Barley and Coleman, 2010) and used again experimentally, to compare male and female aggressive behaviour (Amott and Elwood, 2009). The results suggested that females used more biting behaviours than males and spent more time in close proximity to opponents. Males however used more lateral displays and tail beating. A study comparing aggression in males and females in African cichlids was carried out on the black-chinned tilapia (*Sarotherodon melanotheron*): Females performed more bites, chases and mouth locking than males (Balshine-Earn & McAndrew, 1995). In this species males exhibit more care for offspring than females do. Selection has probably favoured female aggression as males have lower potential reproductive rates than females; they take longer to brood eggs than females take to lay another clutch (Keenleyside, 1991) leading to female–female contests for males. What is revealing is that in both studies females and males used different behaviours. Females

generally used more risky behaviours (biting increases the chance of injury to oneself) while males initially relied on non-contact signalling to settle contests. Arnott and Elwood (2009) argue that males may be using non-contact signalling to reduce the likelihood of injury (supporting SAM theory) but as females are aggressive less frequently they may have lower overall costs for aggression and can risk escalating immediately. This would explain why males use lateral displays more often, which are ideal for a visual assessment of an opponent and are both energetically inexpensive and less risky than behaviours associated with immediate escalation such as physical combat.

Much of the African cichlid aggression literature has focused on male aggression, in particular male territoriality, and its consequences for speciation via sexual selection have been frequently discussed (*e.g.* Dijkstra *et al.*, 2005; 2006). However, females have been used to test hypotheses proposing frequency dependant selection of novel colour morphs and its potential consequences for divergence in the Lake Victoria cichlid *Neochromis omnicaeruleus* (*e.g.* Dijkstra *et al.*, 2008; 2009). Numerous studies (*e.g.* Fryer and Iles, 1972; Ribbink, *et al.*, 1983; Naish and Ribbink, 1990; Genner and Turner 2005; Dijkstra *et al.*, 2008; 2009) state that some female haplochromine cichlids are aggressive or weakly territorial in the wild but there has been no in-depth work to determine when or how females are aggressive in this very important group of teleost fish. There are also no studies comparing male and female aggression in the haplochromine cichlids apart from Naish and Ribbink (1990) which investigated male like behaviour in females and sex reversal rather than an adaptive function for female aggression.

Food is probably abundant all year round in Lake Malawi, however there is believed to be a plankton bloom and a peak in epilithic algae each year (Marsh *et al.*, 1986) which coincides with two breeding peaks found in closely related rock dwelling cichlids (*e.g.* *Pseudotropheus zebra*, *Labeotropheus fuelleborni* and *Petrotilapia* sp.) at the same location (Monkey Bay) (Marsh *et al.*, 1986). If breeding is partly synchronised by females due to an abundance of food at specific times of the year then territory occupying males may be a limiting factor. Naish and Ribbink (1990) very briefly discussed female aggression as a “species-specific



means of ensuring female mating success since dominance establishes the right of an individual to breed before subordinate females". Males and females are strongly philopatric (Genner and Turner, 2005, although some evidence suggest males will disperse further – Knight *et al.*, 1999) and this may also mean they have to avoid mating with closely related males to avoid inbreeding depression or to increase heterozygosity in offspring. If this further reduces the number of males available to females then competition for males may exist. If a limiting male hypothesis was true one might predict that available females were fathered by a disproportionate small number of males which might be revealed by analysing the relatedness of females. As such females are potentially likely to be aggressive for a number of reasons (trophic, anti-predator benefits and possibly access to males).



**Figure 22** Female colouration (left) compared to male (right) *P. thapsinogen*. Photographs taken by G. M. Cooke (left) and A. M. Tyers (right).

## Aim

The primary aim of this chapter was to investigate the behavioural ecology of female aggression in a group of haplochromine cichlids. Two questions are considered relating to female aggression:

- Do males and females differ in the frequency and type of aggressive behaviours employed?

- What are females being aggressive over?

## Materials and Methods

### *Part A: Comparison of male and female aggression in controlled laboratory experiments.*

*P. zebra* focal males were initially tested with regard to aggression biases when presented with 2 different colour morphs from the same population (Chapter 2). Here the data was re-analysed with respect to the *types* of behaviour used and not aggression biases.

In comparing data between males and females only aggressive behaviours conducted in the first five minutes were compared. Aggression displayed to both types of stimulus fish (for male and female focals) were recorded every minute and pooled for each trial. The types of behaviour recorded were; lateral display, frontal display, lunge, quiver and bite. There were 63 trials involving male ( $n = 9$ ) focal fish and 48 trials involving female ( $n = 16$ ) focal fish. For statistical comparison, means were calculated for each focal fish and behaviour type for males and females.

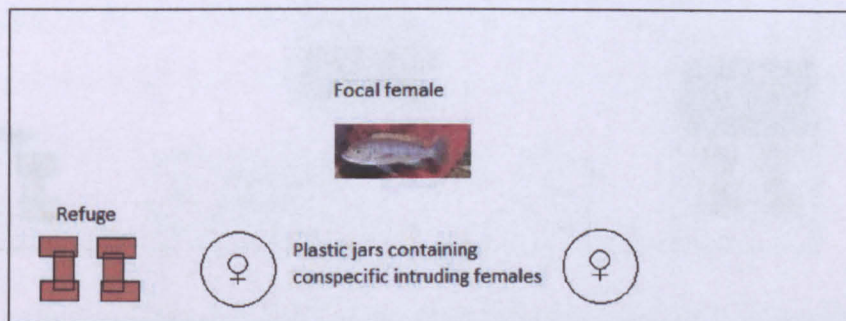
### *Part Bi: Aggression biases and potential resources*

Part B of this investigation was designed to elucidate what females might be aggressive over. The investigation was comprised of three similar experiments.

The same 12 wild caught *P. thapsinogen* females were used as focal fish in all three experiments. Each experiment used two sized matched ( $\pm 5g$ , 5mm) intruding conspecific females. Some focal fish were also used as stimulus fish. Before the first experiment each focal was assigned a pair of sized matched conspecific stimulus fish. Each trial in all three experiments consisted of introducing the two stimulus females simultaneously into transparent plastic jars, at which point recording of all aggressive behaviours began. The transparent jars were placed in the experimental tank next to either a potential resource or no resource. Focal fish were allowed to acclimatise for three days before trials began. Trials lasted for 5 minutes after which the focal fish was allowed to rest for at least 24 hours. Previous studies in the literature (e.g. Dijkstra *et al.*, 2008) and within this thesis showed that

5 minutes was sufficiently long enough to for females to engage in enough aggressive behaviours for adequate statistical analysis. After this rest period the focal fish were tested again but this time the two stimulus fish were switched to remove any potential bias aimed at specific stimulus fish. The data collected for each trial for a focal were then combined and an average produced. In all three experimental tanks a heater and air driven filter were present and located centrally against the back wall.

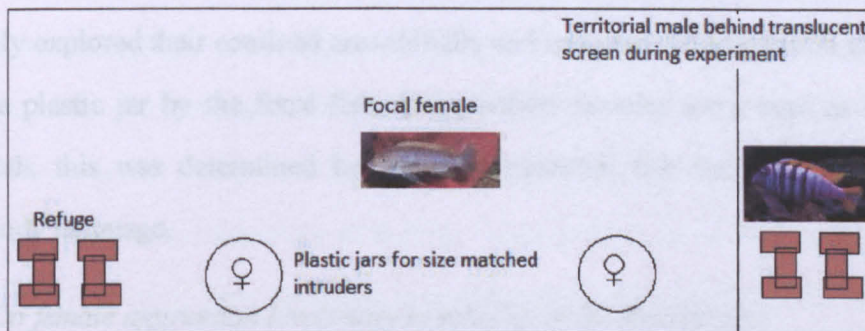
Experiment 1 consisted of a tank containing a refuge (made of house bricks) at one end, where a stimulus jar was also placed. A second transparent jar was placed at the same distance from the middle as the first but at the other end of the tank (see figure 5.2). So, the stimulus fish placed on the refuge side represented an intruder or potential competitor for the refuge. If the focal fish did indeed use the refuge then this might affect the encounter rate of each stimulus fish and this limitation in experimental design is discussed.



**Figure 23** Experimental setup where the focal was given a choice to attack an intruder next to a refuge or a female not associated with any resource

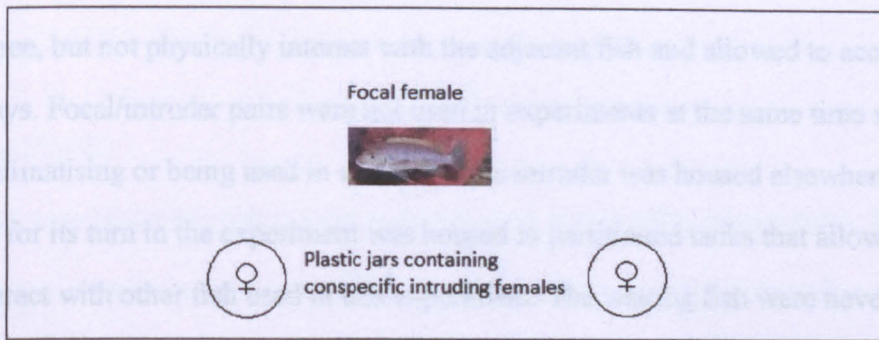
Experiment 2 was similarly set up except that the opposite side to the refuge contained a partitioned off territorial conspecific male (see Figure 5.3). The male was given a house brick refuge to encourage territoriality and to promote breeding behaviour (*e.g.* colouring up). During the acclimatisation period (three days) the partition containing the male was opaque but contained sufficient small holes to allow mixing of water between the main chamber and the male chamber. All females used (focal and stimulus fish) had not been in contact with a male for at least 6 months nor had any been brooding, it is possible then that all the females were gravid and may therefore see the male as resource. Females used appeared gravid (*i.e.*

swollen stomach, opened genital pore). The same male was used for all 12 focal fish so the value of this potential resource did not vary for each female. Twenty minutes before each trial the opaque partition separating the stimulus male and focal fish was replaced with a transparent one to allow visual and olfactory contact between male and female. It was noted that all 12 focal fish appeared to be aware of the males presence during the three days acclimatisation as they routinely investigated the opaque partition, as would the stimulus male. After the change of partition both stimulus females were added to the experiment simultaneously and all subsequent aggressive behaviours directed towards them were recorded. If the focal fish did use the refuge or frequent the males territory then this might affect the encounter rate of each stimulus fish and this limitation in experimental design is discussed.



**Figure 24** Experimental set up when focal females had the choice to attack intruding conspecific females next to a refuge or a territorial male

Experiment three contained none of the preceding resources; the tank was bare except for the centrally placed heater and air driven filter. This set-up was intended to reveal any side biases by the focal females due to unknown factors within the room and therefore was a control for the preceding two experiments. It would also reveal whether environmental complexity has an effect on levels of aggression when overall levels of aggression are compared to the two previous experiments. Both intruder jars were placed equidistant from the centre. After three days acclimatisation the two intruding stimulus fish were added and recording of aggressive behaviours was began.



**Figure 25** Experimental setup when focal was presented with two intruders in a tank containing no obvious resource

In all three experiments the fish were fed flake food which was deposited centrally by a battery powered food dispenser. Water temperatures were maintained between 24 and 26 degrees Celsius with a 12L12D light cycle. These experiments took place in February/March 2010 and data was collected by a volunteer who was unaware of the hypotheses. Stimulus fish typically explored their confined area initially and reciprocated aggression when attacked through the plastic jar by the focal fish. Only healthy females were used as focal fish or stimulus fish: this was determined by watching potential fish for signs of ill health or worrying body language.

*Part B ii: Do female aggression levels vary in relation to the time of day?*

The third aspect of this investigation into the behavioural ecology of non-brooding female aggression looked at variation in female aggression in relation to time of day.

12 wild caught *P. thapsinogen* females were tested against a conspecific intruder ( $n = 12$ ; each focal fish was size matched with its intruder) at three different times of the day in a tank containing a refuge and an air driven filter. Focal fish were re-used as intruders. The same sized matched intruder pair was given to each focal for all three trials (morning, noon and night). This was to provide consistency between trials with only time of day varying. All 12 focal fish were weighed and their standard length measured (mean 23.63g, 87.22mm), a table was created pairing size matched ( $\pm 1.75g$ , 5mm) females. Six identical experimental tanks were created within three 2 metre tanks. The heaters for this experiment were housed centrally and partitioned off from the fish. The fish in each experimental compartment were

able to see, but not physically interact with the adjacent fish and allowed to acclimatise for three days. Focal/intruder pairs were not used in experiments at the same time so if a focal was acclimatising or being used in a trial then its intruder was housed elsewhere. Each fish waiting for its turn in the experiment was housed in partitioned tanks that allowed them to see and interact with other fish used in this experiment. The waiting fish were never housed next to or in sight of its partner. Social interaction has been shown to be important to these fish (Earley *et al.*, 2006). Psychological health increases reliability of behavioural observations as isolation can cause a raise in cortisol levels which are an indicator of stress (Earley *et al.*, 2006).

The experiment took place during August/September 2010 and whilst the lights in the room were on a 12:12 cycle some daylight was able to enter the room through gaps in the material used to block out daylight. After lights out the room would be dark with just enough light allowed in to make the last trial of each day just visible for data to be collected. However, it required observations to be made at a distance of approximately only 0.5 metres away. This did not appear to affect the behaviour of the fish. Subsequently, in all trials conducted (after a pilot trial), irrespective of light available to the observer observations were made from this distance. Stimulus fish typically explored their confined area initially and reciprocated aggression when attacked through the plastic jar by the focal fish.

For each focal all three trials were conducted on the same day. The first trial in the first tank began at between 0830 and 0915. The second time of the day trial was conducted between 1300 and 1345. The first of the night trials was conducted as soon as lights went out at 1800. After all the trials each focal was replaced and the experiment repeated until all 12 focal fish had been tested at three times during the day. This approach may lead to habituation of the focal fish as they might become desensitised to the same intruder used as a stimulus at predictable time. This limitation is discussed.

## Data analysis

Data sets were analysed with respect to distribution and equality of variances. If assumptions for parametric testing were not met transformation attempts were made before parametric tests were used. See Appendix for analysis of data sets.

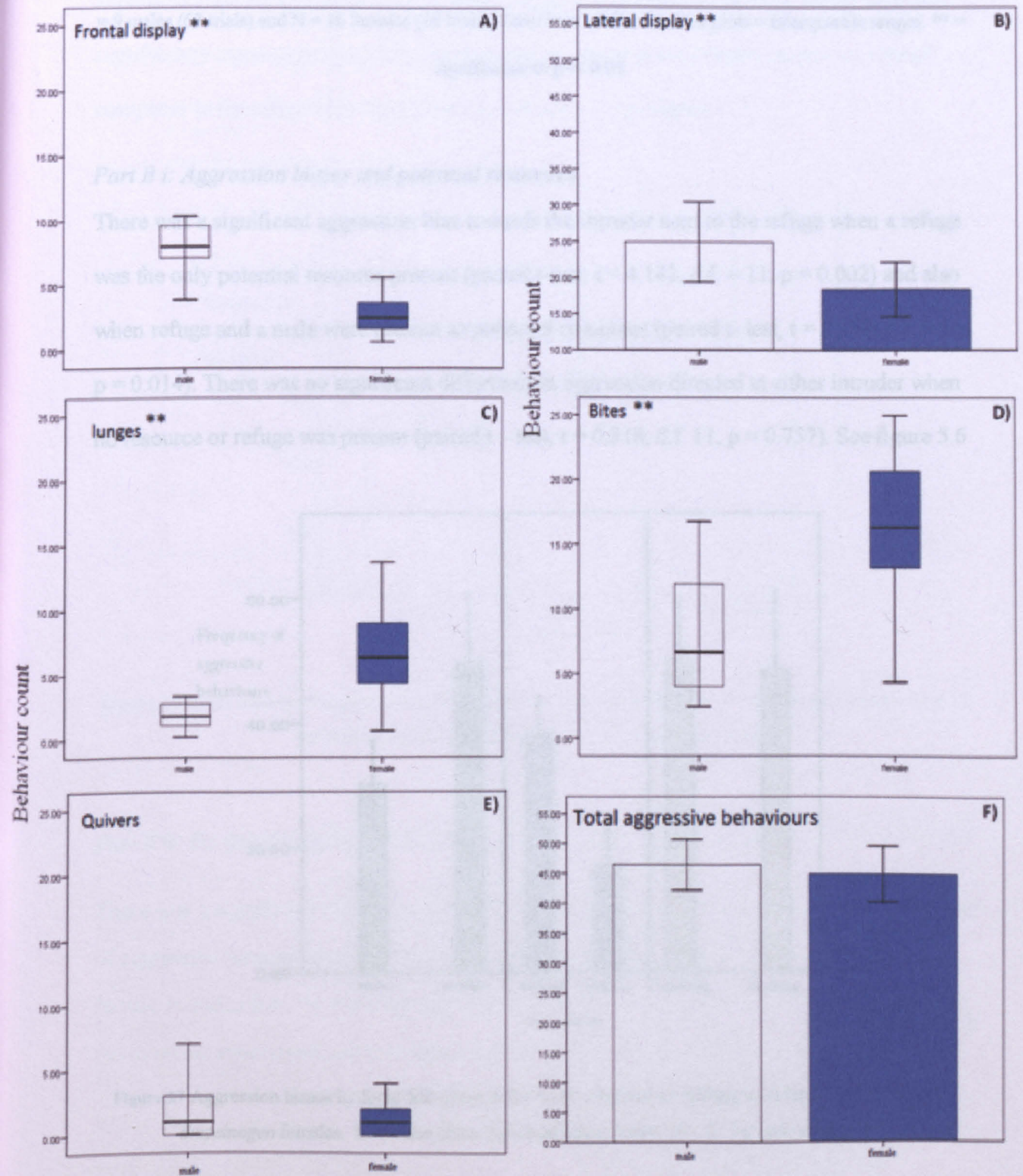
## Results

### *Part A: Comparison of male and female aggression in controlled laboratory experiments.*

The results from Chapter 1 performed to determine aggression biases by male and female *P. zebra* were re-analysed with the aim of comparing the *types* of aggression used by males vs. females. It was found that males use significantly more frontal displays than females (Mann-Whitney U,  $Z = -3.479$ , d.f. = 24,  $p = 0.001$  – Graph A figure 5.5). It was also found that on average males used significantly lateral displays than females (t-test,  $t = 2.137$ , d.f. = 24,  $p = 0.022$  – Graph B Figure 5.5.)

However, females used significantly more lunges than males (Mann – Whitney U,  $Z = -2.443$ , d.f. = 24,  $p = 0.012$  Graph C figure 5.5). Females also used significantly more bites than males (Mann- Whitney U,  $Z = -2.005$ , d.f. = 24,  $p = 0.005$  – Graph D Figure 5.5)

There were no significant differences in the number of quivers performed by males and females (Mann- Whitney U,  $Z = -0.243$ , d.f. = 24,  $p = 0.808$  – Graph E Figure 5.5). Nor were there any significant differences in the total aggression by males and females towards two intruders over the same time period in controlled experiments (t-test,  $t = 0.517$ , d.f. = 24,  $p = 0.606$  – Graph F figure 5.5)



**Figure 26** Comparison of the types of aggression used by *P. zebra* males and females in Chapter 1. Male = White, Females = Blue. Focal fish presented with two intruders, one same morph the other a different morph. N revealed that there was significant differences in aggression between males and females for Frontal display, Lateral display, lunges, Bites and Total aggressive behaviours.



= 9 males (63 trials) and N = 16 females (48 trials). Error bars = 95% C.L. Box plots = Interquartile ranges. \*\* = significance to  $p < 0.05$ .

### Part B i: Aggression biases and potential resources

There was a significant aggression bias towards the intruder next to the refuge when a refuge was the only potential resource present (paired t-test:  $t = 4.142$ , d.f. = 11,  $p = 0.002$ ) and also when refuge and a male were present as potential resources (paired t-test,  $t = 2.196$ , d.f. = 11,  $p = 0.014$ ). There was no significant difference in aggression directed at either intruder when no resource or refuge was present (paired t-test,  $t = 0.318$ , d.f. 11,  $p = 0.757$ ). See figure 5.6

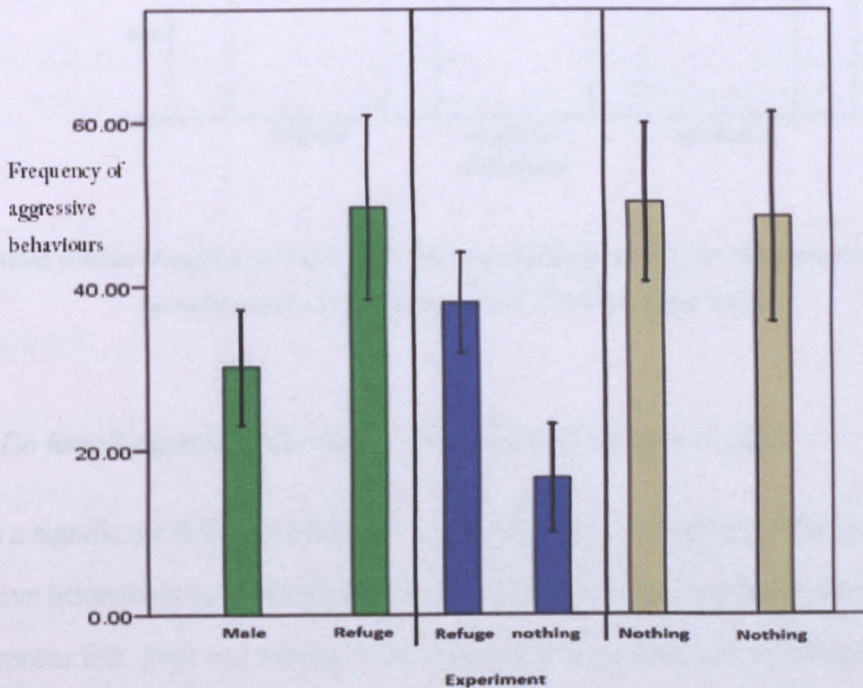


Figure 27 Aggression biases by focal fish given in the three experiments relating to different resources by *P. thapsinogen* females. Error bars show 95% confidence limits. N = 12 for each experiment.

There were significant differences in the mean number of aggressive behaviours between the three experiments (One way ANOVA,  $F_{2,33} = 12.443$ ,  $p < 0.001$ ). Tukey post hoc testing revealed that there were significantly more aggressive behaviours between the refuge only

experiment and the experiment containing a refuge and a male ( $p = 0.012$ ). There were also significantly more aggressive behaviours in the no resource experiment (empty vs. empty) compared to the refuge only experiment ( $p < 0.001$ ). – see Figure 5.7

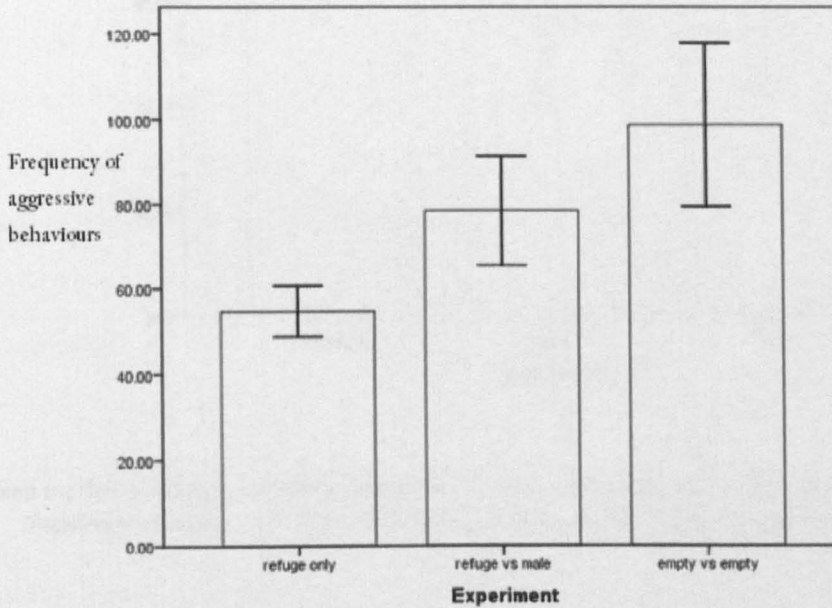


Figure 28 Mean number of aggressive behaviours in each experiment given by *P. thapsinogen* females.  $N = 12$  for each experiment. Error bars show 95% Confidence limits.

*Part B ii: Do female aggression levels vary in relation to the time of day?*

There was a significant difference (One way ANOVA,  $F_{2,33} = 3.743$   $p = 0.034$ ) in the number of aggressive behaviours given by focal females at different times of day to a conspecific female stimulus fish. Post hoc testing (Turkey) revealed the significant difference lay between the morning experiment and the night experiment ( $p = 0.026$ ). – see Figure 5.8

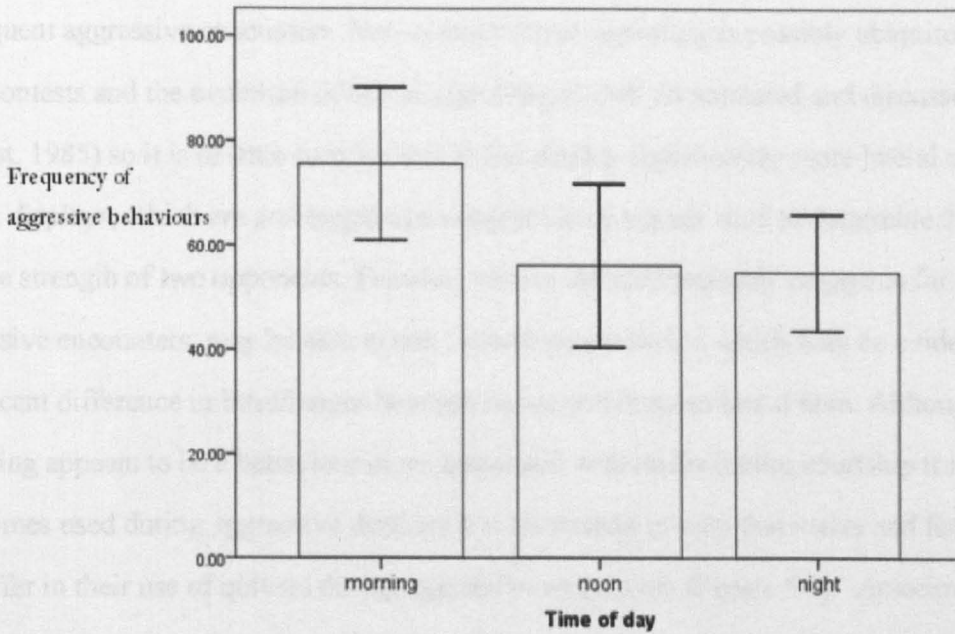


Figure 29 Mean number of aggressive behaviours given to a single intruder at three different times of day by *P. thapsinogen* females. N = 12 for each trial, error bars show 95% confidence limits.

## Discussion

### *Males vs. females*

In controlled conditions female *P. zebra* are as aggressive as males. Males and females might be expected to behave differently due to differences in sexually selected character traits, for example sexual dimorphism influences the ability to be aggressive and different motives for aggressive behaviour may lead to asymmetries in the frequency at which the different sexes are aggressive. These factors might lead to the prediction that natural selection will shape the types of aggressive behaviour used by males and females differently; as shown in this study. Much like two previous studies comparing male and female aggression in cichlids (Balshine-Earn and McAndrew, 1995; Arnott and Elwood, 2009) females employed different behaviours in aggressive encounters (figure 5.6), however in these two examples males and females employ different parental care to the species used in this study. Males are expected to follow variations of the SAM model (Enquist and Leimar, 1983) to reduce the overall costs

of frequent aggressive encounters. Non-contact visual signalling is possibly ubiquitous in male contests and the evolution of honest signalling is well documented and discussed (*e.g.* Enquist, 1985) so it is of little surprise that males employ significantly more lateral and frontal displays, which are archetypal non-contact visual signals used to determine the relative strength of two opponents. Females, who in the wild probably engage in far fewer aggressive encounters, may be able to risk immediate escalation which may be evident in the significant difference in bites/lunges between males and females found here. Although quivering appears to be a behaviour more associated with males during courtship it is sometimes used during aggressive displays it is interesting to note that males and female did not differ in their use of quivers during aggressive encounters (Figure 5.5). Amorim *et al.*, (2004) have shown, in *Pseudotropheus* spp., that quivering is correlated with sound production. Low frequency sounds are used frequently in the animal kingdom to intimidate opponents, which may explain the appearance of quivering in aggressive contests and why males and females use it at similar frequencies. The differences in types of aggressive behaviours employed by male and female cichlids appear to be conserved in relatively distant groups (the Central American cichlid *Amatitlania (Cichlasoma) nigrofasciatum* (Arnott and Elwood, 2009), a member of the African tilapiae tribe (*Sarotherodon melanotheron* – Balshine and McAndrew, 1995) and now possibly the haplochromine lineage, suggesting that in different lineages, in potentially different conditions and sex roles, female behaviour is being shaped similarly by natural selection as costs associated with aggressive behaviour make it unlikely to be selectively neutral.

#### *Aggressive over what?*

When given the choice to attack two intruders, placed near different potential resources, females biased aggression towards intruders positioned near a refuge. The refuge in this experiment potentially provides two benefits. ‘Mbuna’ females sometimes graze on rocks and the wild caught females used in this study possibly associated the brick refuge as not only as a safe place but a possible food source. This may actually reflect what females are being aggressive over in the wild. Females may enjoy better grazing opportunities by being

aggressive towards other fish and at the same time being in very close proximity to a refuge should a predator attack (*e.g.* easy escape from an avian threat, see Mann *et al.*, (2008) for evidence of avian predation on haplochromine cichlids in Lake Victoria). There is a design flaw within this experiment however that affects the first two experiments in this part of the chapter. Encounter rates of stimulus fish may well be skewed by the focal females actually using the refuge as designed. This was not controlled for. The females may simply not see the other stimulus fish. As a result the conclusions, if any are possible, should be tentatively accepted.

The evidence presented in this study does not appear to strongly support a hypothesis based on female competition for males. Although overall, aggression was greater in the presence of a territorial male compared to when there was just a refuge. Too completely rule out female competition for males would require a study involving quantifying female aggression at different times of the year within Lake Malawi alongside breeding observations and trophic conditions.

The overall levels of aggression given under the three conditions support other experiments on cichlids (Barley and Coleman, 2010) and zebra fish, *Danio* spp. (Carfarigain *et al.*, 2009), which suggest that reduced habitat complexity can increase aggression.

The results from the time of day experiments again may suggest that females are at least partly aggressive for trophic reasons. The fish were significantly more aggressive during the morning trials just before feeding with which they are likely to have become habituated too.

Females may well be defending a refuge for protection against night predators (*e.g.* the cat fish *Bagrus meridionalis*) with this weak territoriality continuing throughout the day; perhaps with more emphasis on trophic defence before feeding. The fact that each female was tested sequentially with respect to the time of day, all in one single day, may lead to habituation (Martin and Bateson, 2002).

In conclusion, this study suggests that aggression in females may be selected for. This is evident in the different types of aggression used by males and females. Females are likely to be competing for refuge acquisition or feeding opportunities. However, much more work, particularly in natural settings needs to be done until we understand the nature and significance of aggression in female haplochromine cichlids.

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## Appendix

### *Data Analysis*

#### *Part A: Comparison of male and female aggression in controlled laboratory experiments.*

An initial comparison of the total and types of behaviour given by males and females (*P. zebra*) to intruders was carried out to determine how males and females compare in both of levels of aggression and types of aggressive behaviour used in controlled experiments. Each data set for types of behaviour, for males and females, was tested for normality. Where a data set failed the assumptions required for parametric tests non-parametric equivalents were used only if transformation attempts failed to rectify the assumption.

**Table A5.1** shows the results of the Kolmogorov-Smirnov test for normality and whether parametric or non-parametric tests were subsequently used for analysis. Where  $p < 0.05$  for either data set (male or female) transformation was used and if unsuccessful then a non-parametric test was employed. N = 9 (63 trials) for males and 16 (48 trials) for females.

<b>Behaviour type</b>	<b>Male p =</b>	<b>Female p =</b>	<b>Subsequent approach to data analysis</b>
<b>Lateral display</b>	0.192	0.076	Parametric
<b>Frontal display</b>	0.200	< 0.001	Non-parametric
<b>Quiver</b>	< 0.001	< 0.001	Non – parametric
<b>Lunge</b>	< 0.001	0.031	Non- parametric
<b>Bite</b>	< 0.001	0.049	Non – parametric
<b>Combined aggressive behaviours</b>	0.193	0.200	Parametric

*Part B**Aggression biases and potential resources*

The data collected for part B were tested using the Kolmogorov-Smirnov test, as all three data sets showed no significant deviation from normality (refuge vs. nothing  $Z = 0.488$ ,  $p = 0.600$ , refuge vs. male  $Z = 0.766$ ,  $p = 0.971$  and lastly empty vs. empty  $Z = 0.349$ ,  $p = 0.998$ ). Levene's test for homogeneity of variances showed no significant differences in variance ( $p = 0.065$ ). As intruder types were both conspecifics all aggressive behaviours were pooled and analysed as total aggression. Paired T-tests were used to compare mean aggression (all aggressive behaviours) directed at intruders. A one way ANOVA was used to compare overall levels of aggression between experiments.

*Do female aggression levels vary in relation to the time of day?*

As the focus of this experiment lay in overall levels of aggression relating to time of day and not intruder type all types (attack or display) were pooled and total levels of aggression analysed. The Kolmogorov-Smirnov test for normality found the data did not differ significantly from a normal distribution ( $Z = 0.819$ ,  $p = 0.514$ ). Levene's test for homogeneity of variances showed no significant differences in variance ( $p = 0.552$ ). A one way ANOVA was used to compare mean aggression given at the three different times of day.

# Chapter 6

## General discussion, main findings and ideas for future research

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### General discussion

This thesis has investigated aggression in Malawian haplochromine cichlid fish. Cichlid fish, and in particular the haplochromine cichlid fish, are ideal for such investigations because of their aggressive nature and highly territorial males. This thesis is split into two parts; aggression between colour morphs and aggression between species followed by work on the behavioural ecology of female aggression, including female parental care and aggression.

### *Haplochromine cichlid fish - aggression and evolution*

Much work has focused on the remarkable radiations seen in the African Great Lakes. Whilst much of the diversity seen is likely to be due to adaptive radiations (*e.g.* Hunter, 1998) some important and interesting work remains in explaining how so many similar species are able to coexist sympatrically. Sexual selection causing divergence of male traits has been suggested as a mechanism for speciation (*e.g.* Knight & Turner, 2004; Seehausen *et al.*, 1998; Dijkstra *et al.* 2007; Dijkstra *et al.*, 2009) and the coexistence of similar ecological forms. Initially female choice on male nuptial signals was studied (*e.g.* Couldridge, 2002) but more recently male – male competition and aggression has received attention (*e.g.* Dijkstra *et al.*, 2006, 2007).

The results of **Chapter 2** of this study concur with previous work (Dijkstra *et al.*, 2005) that overall a common morph aggression bias may exist, at least for females, in some haplochromine cichlid fish. Recent studies with haplochromine cichlids (*e.g.* Dijkstra *et al.*, 2009) have proposed that a rare morph advantage may exist. The results from **Chapter 2**

provide evidence for this although lifetime fitness increases for rare morphs has not been shown. At Thumbi West Island, Lake Malawi, the rarer *P. callainos* W morph appears to have an advantage which could be due to reduced aggression (in comparison with the more common B morph) from both conspecifics and heterospecifics, which was found during field observations carried out for this study. However, W only populations do exist suggesting that frequency dependent advantage may not be the full story; W males may possess some other unknown advantage. Conversely, W males may suffer costs that B males do not (*e.g.* higher frequency of predation) and therefore may always remain rare where populations are polymorphic. Ideally relative fitness levels of both morphs need to be calculated over sufficient time (*i.e.* the likely lifespan of *P. callainos* males) which was unfortunately out of the scope of this study.

Male – male competition may promote stable polymorphism in some species/populations which has been discussed previously (Mikami *et al.*, 2004; Seehausen & Schluter, 2004; Dijkstra *et al.*, 2007; Dijkstra *et al.*, 2009). This is probably due to a potential negative frequency dependant advantage for rare morphs, an advantage that may reduce as numbers of a ‘rare’ morph increase. This mechanism, allowing stable polymorphisms to be maintained in a population, is a relatively rare one (when considering the wide animal kingdom). More typically, predator avoidance (*e.g.* Hall *et al.*, 1966; Owen, 1967), physiological benefits (*e.g.* Huxely, 1942; Braendle *et al.*, 2006) or intraspecific predatory competitive benefits (*e.g.* Galeotti *et al.*, 2003) are reported.

The frequency of rare morphs at any given location has been shown to vary. Holzberg (1978) reports that some populations of *P. callainos* are either all blue or all white at specific points in Nkhata Bay. Furthermore, Knight and Turner (1999) report that O morph females may make up between 4 and 8% of the overall population in *P. zebra*, whereas at Chiofu Bay there are no O individuals of either sex (Ad Konings pers.comm). These differences suggest

that other factors (predation for *e.g.*) may have a stronger influence on frequencies than any advantage of being rare can make up for. No matter how much of an advantage by being very rare an O morph may have, if it is disproportionately conspicuous to predators numbers may dwindle to zero as found at Chiofu Bay. Alternatively, if there are all W populations and they are indeed *P. callainos* and not some other un-described Mbuna species, then a W morph at some locations maybe have advantages that are not frequency dependant related, allowing W to proliferate to fixation despite the potential drawbacks of becoming common.

**Chapter 3** of this study adds evidence to the hypothesis that mbuna cichlids are more likely to attack similar looking heterospecifics than (relatively) dissimilar ones (Pauers *et al.*, 2008). Whereas Pauers *et al.*, (2008) investigated sympatric species this study investigates allopatric populations. The results suggest that recently diverged species that differ only subtly may not discriminate although some may. Strictly speaking it is perhaps unlikely that *P. emmiltos* and *P. thapsinogen* would ever come into contact. If lake levels do indeed drop again their geographical distance probably means they will not meet. However, this may not be the case for geographically proximate yet presently separated species that are also recently diverged and only differ subtly in secondary sexual characteristics. How species interact on secondary contact may affect levels of diversity in the future; the remarkable diversity we see today may just be snapshot of a repeating process where diversity waxes and wanes with lake levels.

Whilst the first part of **Chapter 3** suggests that dorsal fin colouration may be enough for discrimination (monochromatic light treatment vs. full light treatment) the second part (female *P. thapsinogen* as focal fish only) appears to show that even more subtle patches of pigmentation may be enough for some populations to bias aggression towards similar fish. Furthermore the second part of Chapter 3 appears to show that olfactory cues are not a significant sensory modality when females are being aggressive and may only be important for females in mate choice (see Plenderleith *et al.*, 2005).

The literature search for information regarding patches of pigmentation in relation to congeners at several locations suggest that future work, quantifying the part of the body a novel patch is found and its colour, may add to the growing evidence that aggression and territoriality may play a role in community composition and might have consequences for reproductive isolation on secondary contact.

It is also interesting to find in this chapter that *P. thapsinogen* females were significantly more aggressive than *P. emmiltos* females. Aquaria observations suggest that *P. thapsinogen* breed at a higher frequency (that is females are ready to spawn again sooner than *P. emmiltos* females - G. M. Cooke pers.ob). If this is true in the wild, and not an artefact of captive conditions, which may have resulted in different conditions for the two species held, then a correlation between increased fecundity and higher baseline levels of aggression may represent a different life history. The two features (increased aggression and increased reproductive output) that differ between the two recently diverged species could make up a behavioural syndrome. A behavioural syndrome is described as a single or set of behaviour that is consistently carried out across contexts, even if this results in occasionally sub-optimal fitness (Sih *et al.*, 2004). Aggression, or rather consistent aggression, is frequently investigated in research on behavioural syndromes (*e.g.* Riechert and Hedrick, 1993). For example the funnel web spider (*Agelenopsis*) has been shown to be much aggressive than closely related spiders. This increased aggression results in killing unnecessary prey which may waste time/energy and increase the likelihood of it being predated (Riechert and Hedrick, 1993). Although typically considered within species behavioural syndromes can be considered between species (Sih *et al.*, 2004). *P. thapsinogen* may well need to be highly aggressive if competition is relatively greater for some unknown reason in their location but this may result in occasional unnecessary aggression.

*Female aggression in cichlids*

The second half of this thesis focuses on female aggression and females are a relatively understudied group when considering aggression (Archer, 1988.).

**Chapter 4** deals with maternal aggression which has received more attention than non-maternal aggression (Archer, 1988.). The results of **chapter 4** are consistent with studies using other organisms so far as females will use aggression during the parental care phase of reproduction. This is also seen in many other organisms and is quite easy to understand. The cost of reproduction on present and future inclusive fitness is high and aggression, although costly, can be an effective method for defending fry from predatory threats.

Very little is known about the behaviour of brooding haplochromine cichlids in the wild and this study may provide an insight into how they are likely to respond at different times throughout the brooding cycle. Mbuna females showed no difference in levels of aggression directed at congeneric males or females in the first part of the maternal aggression investigation, which suggest that opportunistic predation on fry may be common, forcing females to be aggressive towards many sympatric species. However, the second half of the investigation showed that females may vary aggression to less related sympatric taxa and that aggression can significantly change towards the end of the brooding process. Mbuna females surprisingly exhibited high levels of aggression after fry had been released, much like the *A. calliptera* females which exhibit the ancestral condition of fry guarding. The aggressiveness of mbuna females might be due to a hormonal 'hangover' in that levels of testosterone had yet to subside. It may also be true that the more derived state of no post release care still carries ancestral behaviours of increased aggression that is selectively neutral. Conversely it may be that mbuna females do offer post release care in the form of weak territoriality of places where fry are released.

Haplochromine cichlid females appear to differ from other cichlid groups in that they do not show a steady rise in aggression over the reproductive cycle (*e.g.* Wisenden *et al.*, 2008), rather a significant dip is seen which probably relates to the mouth brooding strategy. The dip in aggression may be common to mouthbrooders but unseen in other organisms that do not carry the cost of reduced feeding.

**Chapter 5** deals with non-maternal aggression in female cichlids. There have been few studies on non-maternal female aggression. This chapter shows that females, in controlled (and admittedly unnatural) conditions may be as aggressive as males. This is unlikely to reflect what happens on a day to day basis in the wild. However, what is revealing is that females use aggression differently to males. Males have been frequently used to explain aggressive contests (*e.g.* Austad, 1984; Mosler, 1985; Keeley and Grant, 1993; Turner, 1993). The sequential assessment model (SAM) has been useful tool in predicting not only the outcome of male-male contests but when individuals will escalate from visual displays into physical combat ((Enquist and Leimar, 1983). However it appears that female competition (for whatever reason) may not be able to use the SAM model in the same way. This study suggests that females may escalate sooner than a male would when confronted with an intruder and supports a more complex approach to animal conflicts (Arnott and Elwood, 2009).

#### *Limitations of the approaches used in this thesis*

Limitations to this study include observer bias which cannot adequately be removed in all chapters. In future and if possible methods should include focal watches by observers unaware of the hypothesis (achieved in some experiments of Chapter 5). Filming of trials and experiments in behaviour would allow the analysis of stimulus behaviour which then could be controlled for and ruled out as a significant factor in focal fish response. The intensity of



colour change by focal fish and stimulus fish may reveal the motivational state of intruders, again allowing more concrete conclusions and the importance of results collected.

Randomising intruders is an issue in some experiments and discussed where appropriate.

## **Main findings and ideas for future research**

### **Chapter 2 Main findings**

- Male mbuna common (BB) morph cichlid fish may bias aggression to their own morph.
- Experiments with females using both morphs suggest that overall a common morph bias may exist.
- A common morph aggression bias might lead to a rare morph advantage in natural settings
- The advantage might be manifested in a number of ways: A greater frequency of territorial rare morphs males, greater feeding rates (males and females), and a reduction in aggression received (from conspecifics and heterospecifics) which is shown here through field work.

### **Future research**

To confirm that overall a common morph bias exists in male mbuna cichlids, rare morph males ought to be tested in a similar fashion as the common morphs. Field work focusing on territory sizes of males from either morph may add to the results here and further show an advantage to rare males who may be able to hold larger territories which may amplify the benefits of being rare.

It would also be ideal to actually measure any fitness advantage W morphs may have. To test fitness you would need to get a lifetime fitness estimates for both B and W males (*e.g.* through offspring sired over a long period). To test a rare male advantage, you would need to

compare the fitness differentials at a range of frequencies of the two morphs, perhaps by removing males of a particular morph from patches of the habitat to adjust relative numbers, and replicating this.

Aggression biases may be learned; testing fish in controlled aggression bias experiments on naïve and experienced fish may reveal what happens if negatively frequency dependence selection increases the frequency of rare morphs. Naïve fish may learn to attack rare morphs more frequently if rare morph fish numbers are artificially increased.

It might also be interesting to compare congeneric and heterospecific aggression biases towards rare morphs of one species in controlled laboratory experiments too. This may reveal what may happen when or if secondary contact occurs. Does an innate tendency to attack a certain coloured fish promote a particular morph advantage on secondary contact?

### Chapter 3 Main findings

- Mbuna male's bias aggression towards more similar looking heterospecifics and this choice appears to be based on dorsal fin colouration.
- Although possibly important in mate choice, olfactory cues are not essential in aggression biases
- Some populations of mbuna may be capable of discriminating between very similar looking populations/species based on very subtle colour differences whilst other populations/species may not.
- Initial reviews of the available information suggest that mbuna cichlids may be evolving to look different to other sympatric species

### Future research

This study suggests that some populations may already be cable of fine scale discrimination. Why does this happen in some species but not others? It would be interesting to survey where *P. thapsinogen* and *P. emmiltos* were collected and quantify the nature of colour patches in sympatric species. Although females are clearly aggressive and may well hold small territories males are affected more significantly by aggression in competition for territory acquisition leading to reproduction; therefore they ought to be investigated in future aggression studies.

To ascertain whether disruptive selection (to reduce unnecessary heterospecific aggression) is indeed driving changes in phenotype at a community scale further work would be needed. Although useful the two sources (Ribbink *et al.*, 1983; Konings, 2007) are incomplete for this job. Ideally a rocky shore in Lake Malawi would be extensively surveyed for which species significantly overlap and then quantifying traits which may reduce aggression. Other locations could similarly be analysed to prove that aggression consistently drives some phenotypic divergence. Fortunately there are two locations (Thumbi Island West and Nkahata Bay) where translocations 30 years ago may allow further predictions to be tested. If species are evolving novel patches of pigmentation to avoid aggression then one might predict that if some translocated species look similar to incumbent ones then they may have to evolve differences to remain extant at their new location. It appears (Young *et al.*, 2009) that translocated species are able to coexist with the incumbents so looking for divergence in the traits outlined in chapter 3 may prove revealing (although it has only been 30 years, 10-15 mbuna generations since the introductions at Thumbi Island West.

Why are *P. thapsinogen* females significantly more aggressive than *P. emmiltos* females? Controlled aquaria work could easily determine rates of breeding by various species and

whether or not they tie in with aggression levels. Field work would be required to investigate why such difference may evolve. Are conditions different between locations and how might this affect secondary contact scenarios?

#### Chapter 4 main findings

- Part of female care in mbuna female cichlids is aggressive behaviour towards individuals who may be a threat to fry.
- Haplochromine cichlid species that may represent the ancestral form are also actively aggressive towards threats.
- In laboratory trials mbuna females show similar levels of aggression towards conspecific/congeneric and males/ females.
- Two distantly related haplochromine cichlid species appear to show a consistent drop in levels of aggression during the middle of the mouthbrooding period.
- Haplochromine cichlids may increase levels of aggression to some threats towards the end of the brooding period when fry are about to be released
- Order effects via experimental design may have confounded results and as such caution must be exercised in interpreting these findings.

#### Future research

To untangle the order effects that may have affected this study it would be essential to randomise the order of intruders.

Laboratory measures of mass loss over the brooding cycle may reveal the calorific cost of mouthbrooding, as would a study of aggression levels related to body fat percentage – can females know their own condition and adjust their behaviour as appropriate?

### Chapter 5 Main findings

- In controlled laboratory conditions females showed similar levels of aggressive behaviour as males.
- But, males and females differ in the types of behaviours used, with males using aggressive visual signalling and females using behaviours associated with physical combat.
- In the experiments performed in this study it appears females choose to defend areas where a male might be compared to a refuge area
- This study suggests females are more likely to see a refuge or trophic resources as worth being aggressive over although there may be confounding factors
- Females might be aggressive in the wild at frequencies that may carry significant costs

### Future research

To rule out female – female competition for males field work would be more useful than the laboratory experiments performed here. By observing females at locations that have breeding peaks, determining if there aggressive encounters correlate with breeding seasons, it might be possible to rule out female – female competition. Furthermore observing males at these locations/peak times to determine if there is even a shortage of territorial males would be a critical for a female competition hypothesis.

Field work may also corroborate the hypothesis that at least some female aggression related to refuge acquisition or trophic competition by using focal watches to observe when females are more aggressive and to whom they are aggressive towards.

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