BEHAVIOURAL AND PHYSIOLOGICAL STUDIES OF FIGHTING IN MALE <u>TILAPIA ZILLII</u> (CICHLIDAE).

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I would like to dedicate this thesis to my family, friends and teachers.

Declaration

I declare that the work described in this thesis has been carried out by myself unless otherwise cited or acknowledged. It is entirely of my own composition and has not, in whole or in part, been submitted for any other degree.

Francis C. Neat, September 1996.

Acknowledgements

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SUMMARY

This thesis is a compilation of several behavioural and physiological studies of fighting in a species of African cichlid fish, *Tilapia zillii*. The behavioural studies are concerned with explaining the functional significance and behavioural organisation of fighting. The physiological studies are concerned with attempting to elucidate the mechanisms that underlie the expression of aggressive behaviour. My aim is show how these two levels of study are related and why it is important to maintain a balance between them in the study of animal behaviour.

Chapter 2 reports a series of laboratory experiments that investigated how asymmetries in body size and gonadal state influence the fighting strategies of male *T. zillii*. Fights between animals over limited resources often end in victory to the larger contestant. Game theory predicts that relative body size is assessed during the fight and thus also determines fight duration and intensity. However, if the contestants differ in the value they place on the disputed resource, this can override the effects of relative body size. In the experiment described here, relative gonad weight was a much stronger predictor of fight outcome than relative body size. This suggests that males with large gonads fight harder to defend their territory, implying that the value of a territory is a function of the gonadal state of the individual. Despite this, a detailed behavioural analysis suggested that relative body size is assessed through a behaviour termed 'mouth wrestling'. In addition to persisting in the fight, smaller contestants escalated to a higher degree (in terms of biting), especially if they went on to win the fight. Winners and losers differed consistently with regard to a behaviour

termed mouth locking, suggesting that through this behaviour the fish were assessing an asymmetry related to the intention to persist with the fight, although I could not demonstrate that this was related to relative gonad weight.

Chapter 3 was undertaken with the aim of gaining information on the social structure and occurrence of aggressive behaviours in a semi-natural environment. To this end, a study was made of the social interactions of a group of male fish allowed free range over approximately ten metres of artificial 'river bed' during a four week period. Under these conditions, the social structure of *T. zillii* may be described as a loose group with an unstable dominance hierarchy. Aggression is a major component of this species' time budget, with different acts and degrees of escalation probably functioning to establish, challenge and maintain dominance relationships within the hierarchy. The expression of territorial behaviour appeared to be conditional upon social status (only the two top ranked males were seen to dig nests and consistently court females). One escalated fight was observed between the two highest ranked fish, but it was not clear whether this was a territorial dispute, a challenge for the top position, or a dispute over both. Thus, while the immediate reasons for escalated fighting between male *T. zillii* depends upon the social and physical environment, it is probably ultimately caused by competition for mating opportunities.

Chapter 4 deals with the issue of how body size and gonadal state relate to life-history aspects of the animal by means of a morphometric analysis. The analysis provided evidence to suggest that there was a trade off between gonad size and the storage of fat. It is not clear whether this relationship arises from a direct energetic trade-off or

is a result of adopting different behavioural strategies. I also explored the relationship between behaviour, morphology and somatic and reproductive condition. It was possible to predict gonadal state on the basis of mouth morphology which may explain why so much fighting involves use of the mouth in this species.

In Chapter 5 I studied the proximate costs of fighting in terms of physical injury and the metabolic consequences of engaging in a energetically demanding activity. In relation to injuries incurred during fights, losers suffered greater scale loss than winners, especially if the loser was larger than its opponent. In relation to energy metabolism, fighting resulted in significant depletion of total sugar reserves from the muscle and the liver (compared to unfought controls). It appears that the muscle energy reserves are respired anaerobically, as was evident from the accumulation of lactate in the muscle. Interestingly, losers had significantly higher levels of lactate than winners. Together, the data on injury and metabolic state suggest that fighting is costly for both winners and losers, but that this is especially marked for losers. These data are discussed in relation to models of animal decision-making and it is concluded that the summation of different proximate costs incurred during fighting is likely to underlie the making of decisions such as continuing, giving up or escalating the fight.

In Chapter 6 I report a study of plasma concentrations of gonadal steroids in *T. zillii* in relation to fighting and gonadal state. The gonadal steroids, particularly the androgens, have been shown to be associated with aggression in a wide range of species and seemed like obvious candidates for the mechanism by which gonadal state influences behaviour. Blood samples were taken from fish immediately after fighting

and the plasma concentrations of the following gonadal steroids were then determined by radioimmunoassay: testosterone (T), 11-ketotestosterone (11kT) and $17\alpha,20\beta$-Dihydroxy-4-pregnen-3-one (17,20-P) and estradiol (E_2). T concentrations did not correlate with GSI and no differences were detected between winners, losers and unfought controls. 11kT concentrations were on average twice those of T and negatively correlated to GSI, although winners, losers and controls were not significantly different. T and 11kT concentrations were positively correlated with each other but 17,20-P and E_2 levels were too low to be accurately measured. It appears that in *T. zillii*, 11 kT is the major androgen as it is in most other mature male teleosts.While the present results suggest that these sex steroids may play a role in physiological regulation of testicular maturation, they do not support the idea that these steroids are the mechanism by which GSI influences aggressive behaviour.

In Chapter 7 I review the main conclusions of the previous chapters. I then offer my personal opinion on how the different levels and approaches taken throughout the study interrelate and collectively reinforce each other and why integration between disciplines is important in the study of animal behaviour.

CHAPTER 1

GENERAL INTRODUCTION, AIMS AND ORGANISATION

1.1 Animal aggression

1.1.1 *The definition of animal aggression*

There has been much debate over the definition of what constitutes an aggressive behaviour (see, for example, Archer, 1988; Huntingford & Turner, 1987). However, in the context of this project, I consider a functional definition axiomatic. The function of aggressive behaviour is to win or defend resources that are important for the survival and reproduction of the individual. Resources are won or successfully defended by imposing costs upon the competitor to such a degree that it withdraws from the conflict. Therefore, the definition used throughout this thesis of an aggressive behaviour is; any act from one individual toward a conspecific that either inflicts cost or carries information about the intention to inflict cost.

1.1.2 *Perspectives on animal aggression*

There can be few aspects of behavioural biology that have attracted as much study from so many different angles as animal aggression has done. Behavioural ecologists have widely explored the functional significance of animal aggression and the cost-benefit approach (particularly in the form of evolutionary game theory) has provided a strong unifying framework. The more causal aspects of the study of animal

aggression have yet to reach a similar level of unification. Since the early days of ethology, a central issue has been to understand the motivational organisation of the spectacular nature and intensity of aggressive interaction. Ideas based on motivational theory have, to some extent, been replaced by models of physiological control of the expression of aggressive behaviour. Understanding of the physiology of aggression has been making rapid progress with the development of techniques to measure biochemical responses such as the release of hormones and neurotransmitters.

While this intense research effort has generated a vast literature, a synthesis between disciplines has been slow to emerge. Functional, motivational and physiological aspects of aggression are considered throughout this thesis and the emphasis of the research reported here is on the integration and synthesis of the different approaches and levels of study.

In this chapter, I review the general literature surrounding the study of aggression and the different levels of approach taken to study it. I then introduce the study species, with particular reference to its aggressive behaviour. Finally, I discuss the general approach taken in this project and the specific aims of the individual chapters.

1.2 Levels of study of animal behaviour

1.2.1 *Tinbergen's four questions*

There are four different levels of biological enquiry that are essential to the study of behaviour (Tinbergen, 1964). The first level is causal analysis, in terms of the

proximate mechanisms and physiological control of the expression of behaviour. The second is functional analysis, in terms of the survival or reproductive value the behaviour confers to the individual. The third is developmental analysis, in terms of the behavioural changes associated with the process of learning and changes in the animal's life history. The final level is phylogenetic (evolutionary history) analysis, in terms of the patterns of behavioural similarity between species that arise as a consequence of their common descent. Tinbergen considered all four levels as equally important and as mutually reinforcing, but in the scope of the present project, only causal and functional approaches could be addressed.

1.2.2 Causal questions about behaviour

One of the main challenges of ethology has been to understand how animals are organised so that they are able to make the appropriate choice of behaviour in different circumstances. According to the classical ideas about the proximate causation of behaviour, behaviour was thought to be expressed as the result of specific internal and external stimuli (Tinbergen, 1951). These stimuli contributed toward motivational drives such hunger, sex and aggression. As only one behaviour can be expressed at any one time, the tendencies were thought to compete for expression; the motivational drive with the most stimulation gained expression at the expense of the others. These ideas are still discussed (for example, Baerends, 1993; Tyrrell, 1993) and one model serves to illustrate the approach with specific reference to aggression. Fighting is modelled as a competition between the conflicting motivational tendencies to attack (aggression) and to flee (fear). Both tendencies arise during a fight but vary independently of each other. The absolute levels and the

balance between the tendencies to attack and flee determine which animal eventually flees and how escalated the fight becomes (Baerends, 1975). Maynard Smith & Reichert (1984) applied such a model to their observations of conflict between spiders and achieved a good qualitative fit with the empirical data. Figure 1.1 is a graphical representation of the model. The spider has six behavioural options; Locate, Signal, Threaten, Contact, Retreat and Withdraw permanently. The expression of Locate, Signal, Threaten and Contact depend upon the absolute level of aggression, whereas the expression of Retreat and Withdraw permanently depend upon the balance of aggression and fear; when fear is just greater than aggression, the spider Retreats, and if this difference increases beyond a certain threshold it Withdraws permanently.
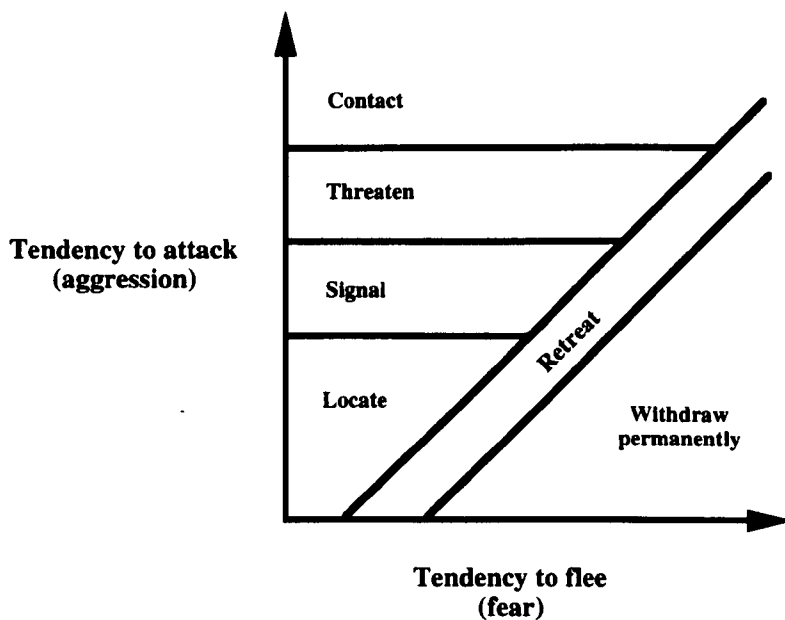
Figure 1.1 Graphical representation of Maynard Smith & Reichert's (1984) conflicting tendency model of fighting (after Huntingford & Turner, 1987). The balance of A of F determine whether the animal fights or escapes and, provided A > F, the absolute level of A determines the level of escalation (Contact being the most escalated).

The idea that motivational tendencies for different behaviours competed for expression received a quantitative revision by McFarland & Houston (1981). Their 'state-space' approach set ideas about motivation within a functional framework. Incorporating the element of functional significance was a major conceptual advance, for it enabled one to see how ultimate goals are reached by proximate means. McFarland & Houston's ideas can be summarised as follows; causal factors are variables resulting from the animal's perception of the external environment and variables relevant to the animal's internal environment. These combine to form a motivational state variable, which provides the animal with information relevant for making functional decisions. Decision rules are specific responses to a given level of a motivational state variable. For example, low blood sugar and the sight of food are causal factors that combine to increase the motivational state variable 'hunger' and the decision rule 'feed' is executed provided that feeding is the animal's best option for the current level of hunger. McFarland & Houston were able to offer some empirical support for their theories, but only for easily defined and measured motivations such as hunger and thirst.

Elwood & Neil (1992) extended the state-space approach to fighting, where the immediate cost associated with fighting is the relevant motivational state variable for making decisions on whether to continue, escalate or flee from the fight. They tested their ideas with data from hermit crabs fighting over the shells in which they live. The two dimensions of the model are the crab's estimate of the costs of fighting and its estimate of the value of disputed shell. Elwood and Neil manipulated costs by varying the degree in size asymmetry, and resource value by manipulating the size of the shell

being disputed. Fight outcomes and durations predicted by the model were similar to those observed in experiments.

As techniques for the measurement of physiological parameters became available, the causal analysis of behaviour started to make greater headway. Much success was gained at demonstrating the relationship between the endocrine system and the expression of behaviour (see Nelson, 1995 for review). The role of the hormones, most notably the androgen steroids, in the mediation and control of aggressive behaviour has long been established, but it is only in recent years that the subtleties and complexities of androgen action have been appreciated (see general view by Wingfield *et al.*, 1994).

A perhaps more neglected area in the physiological control of aggression is the role of energy metabolism. Escalated fighting is an energetically intensive activity and little is known about how strategic decision-making during fighting is influenced by energetic exhaustion. However, in the last few years there have been a number of studies directed specifically at determining the energetic consequences of fighting in a broad range of taxa (see reviews by Haller, 1995 and Huntingford, *et al.*, 1995). In addition to suggesting that energy metabolism is a significant component of the cost of fighting, there is evidence that visual contact with an opponent and aggressive display may serve to prepare the body for fighting by mobilising energy reserves.

Neural approaches to unravelling the mechanisms underlying behavioural responses have been successful in a number of invertebrates. For example, in crayfish it has

been shown how the giant lateral neurone (which controls the expression of behaviours seen in aggressive contexts) is modulated by the neurotransmitter serotonin in relation to the outcome of aggressive interactions (Edwards, 1995). Experimentally increasing serotonin causes adoption of aggressive postures and inhibition of the tail flip escape response in subordinate individuals (Huber, 1995; Yeh *et al.*, 1996). In fish, serotonin has also been linked with the modulation of aggressive behaviours (Winberg & Nilsson, 1993), but its exact mechanism of action is not known.

One rarely studied aspect of the causal control of behaviour is its functional morphology, that is to say, its anatomical basis. The musculoskeletal and neural organisation of the opercular flaring display of Siamese fighting fish (*Betta splendens*) has been investigated by Ma (1995). This study suggests that the bones involved in generating the display have undergone extreme modification from their derived structure (in comparison to other fish that do not gill-flare) in order to facilitate the display. In addition to understanding the proximate control of behaviour, such an approach gives insight into how morphologically constrained or plastic a behaviour is. This can be very important for understanding the potential for modification of behaviour by selection, as well as suggesting why certain behaviours are associated with particular parts of the body.

1.2.3 *Functional questions about animal aggression*

Evolutionary change of behaviour by natural selection will occur provided there is heritable genetic variation for the behavioural trait and that this variation gives rise to

differential reproductive success. It is in relation to evolution by natural selection that the functional significance of behaviour is analysed. Behavioural Ecology studies what it is about behaviour that confers an advantage to the survival or reproduction of the individual. The concept of behavioural adaptation assumes that natural selection favours behaviour patterns that promote reproductive success, given the various constraints contingent with the animal's biology (Krebs & Davies, 1991).

The acquisition of resources such as food, territories and mates have important consequences for the reproductive success of individuals. In ecological circumstances where such resources are limited in space or time, competition between individuals inevitably arises and often leads to aggressive interactions (Grant, 1993). Much of the variation in individual reproductive success in the population is thus determined by the outcome of aggressive interactions. Aggressive behaviour and the morphologies associated with it are, therefore, likely to be under strong selection pressures.

It is not, however, always obvious what is the best aggressive behavioural option in conflicts, because the costs and benefits of behaving in a particular way depend upon how other individuals in the population are behaving (Archer & Huntingford, 1993). The more aggressive an individual is, the greater chance it has of defeating a rival, but if all rivals are equally aggressive then an aggressive strategy may incur unacceptably high costs. Such situations lead to frequency dependent selection pressures and to analyse such situations it is necessary to employ evolutionary game theory (Maynard Smith, 1982). The key concept behind this kind of modelling is the evolutionarily stable strategy (ESS). An ESS has the property such that, if all

members of the population adopt it, then no 'mutant' strategy can invade the population through the action of natural selection.

The original model of Maynard Smith & Price (1973) - the hawk-dove game - is still the best illustration of the principles of an ESS. Consider a population in which there are two different fighting strategies in equal numbers, one that always attacks and does not withdraw unless injured - the hawks, and one which only displays and withdraws if attacked - the doves. Pairs of the population then meet at random to contest resources. If a hawk meets a dove, the dove withdraws uninjured and the hawk wins the resource. If a dove meets a dove, they display at a low cost and there is a 50 / 50 chance for each contestant to win the resource. If a hawk meets another hawk, there is a 50 / 50 chance of injuring the opponent and winning the resource or getting injured and losing the resource. Clearly, hawk always beats dove, so dove cannot be an pure ESS, but equally hawk cannot be an pure ESS either, because if most of the population are hawks then the dove strategy does better because it avoids the high costs of injury. The population will eventually come to rest at a ratio of hawks to doves where the net benefits of playing each strategy are equal. This ratio of strategies is then the ESS, rather than any one strategy.

The early game theory models were invaluable in showing why ESS modelling is essential for understanding frequency dependent behaviour, but the correspondence between the model strategies and the behavioural complexities of real animal fights was weak. A step in bringing game theory further into line with the behavioural complexity that ethologists had characterised was first taken by Parker (1974). It was

well known that contestants usually differ in their ability to defend the resource or their 'resource holding potential' (RHP) and that this is often closely coupled with body size and strength. Parker proposed that in an asymmetric fight, an individual would benefit if it could estimate its RHP relative to its opponent, thus allowing it to withdraw from the fight early on if its RHP is lower (and avoid paying the extra costs of continuing to fight in vain), or attack with a high probability of winning when its RHP is greater.

Enquist & Leimer (1983) expanded the general idea in a model called the Sequential Assessment Game. They hypothesised that the process of information acquisition takes time because there is a certain amount of error in displays or aggressive behaviours as these are imperfectly correlated with fighting ability. The acquisition of information is modelled in a way similar to statistical sampling, such that the estimate becomes better with each repetition of the behaviour.

The Sequential Assessment Game is the game theory model that goes furthest toward a functional explanation of the complexity of animal fights. The model is attractive because it can also accommodate other factors, for instance, asymmetries in the value the contestants place on the resource (Enquist & Leimer, 1987). However, what is most important about this model is that it invokes a causal mechanism. The estimate of RHP is a dynamic state variable that determines a contestant's choice among aggressive actions. The model, therefore, also makes predictions about the mechanisms underlying strategic decision-making.

1.2.4 Linking causal and functional questions

So far, causal and functional questions have been considered separately and many researchers are content to work either at the functional or causal level. However, some argue that such a dichotomy is artificial and emphasise the importance of integration between the two approaches (Davies, 1991; Huntingford, 1991). Whatever adaptive features a functional analysis of behaviour can identify must be realised by a mechanism that ensures the execution of the appropriate behavioural action. This argument applies with equal force in reverse; no matter how well a physiological analysis can characterise a behavioural control mechanism, the student of animal behaviour can gain little from this understanding if the outcome of the mechanism cannot be related to the functional biology of the animal.

1.2.5 How functional studies can gain insight from causal analysis

There is a growing awareness of the benefits to be reaped from the integration of causal and functional approaches to animal behaviour (Huntingford, 1993). In recent years behavioural ecologists have found it necessary to turn to the mechanisms underlying behaviour in order to understand its functional significance. An example may make this point clearer. In the field of sexual selection, it is common for researchers to demonstrate females preference for males with the most exaggerated secondary sexual traits. It is argued that these traits are honest signals of male quality, because they are somehow costly and thereby confer a handicap upon the bearer, leading to only the fittest males being able to incur such a handicap (Harvey & Bradbury, 1991). However, sometimes it is not obvious why a trait should be costly and, therefore, honest. For instance, female three spined sticklebacks prefer males

with brighter red throats (Millinski & Bakker, 1990). Why should a red throat be honestly indicative of a males fitness ? It has recently been discovered that the red throat of the male stickleback is expressed under the control of androgen steroids (Borg & Mayer, 1995). It has also been demonstrated that high androgen levels have a detrimental effect on immunocompetance, that is to say the individual's resistance to parasites and disease (Møller & Saino, 1994). Thus, males in poor condition and low immunocompetance cannot afford a bright red throat and the red throat becomes an honest signal of male fitness because only the fittest can afford to bear them. Hence, we can see why red throats should have evolved in the first place. In this case, causal reasoning was essential in the functional interpretation of the problem.

1.2.6 *Why behavioural mechanisms are the logical level to study the evolution of behaviour*

Provided a behavioural phenotype is variable and has a heritable basis, natural selection will act upon its functional consequences. However, from this argument it does not follow that that we can predict how the behaviour will evolve. Behaviour is modified by selection through a parallel modification of the underlying morphology and physiological mechanisms (Cosmides & Tooby, 1987). Although, we are long way from a complete understanding of the mechanisms that control behaviour, it is clear that some functionally distinct behaviours can be interrelated on a physiological level. Thus, modifying the mechanistic basis to attain a change in one behaviour may concomitantly modify another behaviour. The functional significance of the original behavioural change must be revised to take into account the changes in the correlated behaviour. Studying behavioural evolution at the mechanistic level makes explicit the

constraints and hence selection potential of behavioural strategies. Constraints and adaptation should be considered with equal importance in our attempts to understand the evolution of behaviour. This is the approach I have endeavoured to follow in the present study of fighting in male *Tilapia zillii*.

1.3 Aggression in Cichlid fish

The family of teleost fish, the Cichlidae, are a good group of animals to study in relation to aggressive behaviour. The family is subdivided into the 'Old World' cichlids that are found throughout Africa, the Middle East and parts of Asia, and the 'New World' cichlids that are found throughout Central and South America. The astonishing interspecific diversity in social organisation and behaviours such as courtship, parental care and aggressive interaction make them an interesting and popular family of fish to study. Since the seminal studies of Baerends & Baerends-Van Roon (1951), aggression research has been, and still is being, carried out on many species of Old and New World cichlids (see Keenleyside, 1991, for review).

The Tilapias are an Old World group of cichlids comprising three genera, *Tilapia*, *Sarotheradon* and *Oreochromis* (Trewavas, 1983). The *Tilapia* sp. are substrate spawners, whereas the *Sarotherodon* and *Oreochromis* sp. are mouth brooders (Lowe-McConnell, 1959). My study species, *Tilapia zillii* (Gervais, 1848) is naturally distributed throughout the lake systems of the Northern and Western Africa (Fryer & Iles, 1972; Philippart & Ruwet, 1982). When sexually mature, males establish and aggressively defend territories along the lake side in which they dig (with their mouths) spawning nests. Females are attracted to spawn in the pit and there is often a

prolonged period of courtship involving continued excavation of the nest (Bruton &

Gophen, 1991). It is a monogamous species, with the female laying her eggs on a

carefully cleaned area or stone in the pit. Both parents then guard and fan the eggs.

Once the eggs have hatched, the parents guard the fry for a period of at least three

weeks (in captivity, pers. obs.). Breeding occurs all year round in equatorial regions,

but may be arrested during cooler months in more Northerly latitudes (R. H. Lowe

McConnell, pers. comm.). In equatorial regions, reproduction peaks during the wet

season, but no information is available as to the length of time between broods

(Siddiqui, 1979).

Territories are likely to be very important to reproduction and because of the

investment in lengthy periods of courtship, nest digging and parental care, the

territory is likely to be keenly defended against any intruders. It should be noted that

most of the information on the social system and breeding biology of this species

comes exclusively from Bruton & Gophen and Siddiqui's field studies and these were

by no means exhaustive in their investigations. Thus, there are undoubtedly many

unknown aspects of the behavioural biology of *T. zillii*.

T. zillii exhibits dramatic and variable colour patterns that become particularly

striking during breeding and aggressive interactions. The causation and function of

these patterns has been linked to multiple aspects of social interaction (Hulscheimer-

Emeis, 1992), although it remains to be seen whether the changes observed during

fighting function in the assessment of asymmetries.

There has not been a systematic study of aggressive behaviour in *T. zillii* either in the laboratory or in the wild. However, it is likely that, as with other cichlids (reviewed by Turner & Robinson, in prep.), most aggressive interactions are rapid chases or threat displays, with occasional escalated fights occurring when the ownership of a territory is disputed. Aggressive interactions probably also occur in non-territorial contexts, particularly in relation to social hierarchies and over competition for food.

There is a high degree of interspecific conservatism in cichlid fighting behaviour, even between the Old world and New world genera. Cichlids in which fighting has been studied in detail include *Oreochromis mossambicus* (Turner & Huntingford, 1986; Turner, 1994), *Hemichromis bimaculatus* (Baerends & Baerends Van Roon, 1950) and a New world species, *Nannacara anomala* (Jakobssen *et al.*, 1979). In some of these species, fighting consists of distinctive phases, for example *Nannacara anomala* whereas in others, for example, *Oreochromis mossambicus* this temporal 'phase' structure is less clear. Nevertheless, many of the behaviour patterns are similar. Fighting begins with contestants raising fins and inflating the branchiostegal membranes below the operculum. Escalation to the next phase is usually defined by the appearance of mouth wrestling. This involves the fish grappling with each other by the mouth and trying throw back the opponent. In the final phase the fish are seen to chase each other nose-to-tail in circles, attempting to bite one another. The fight can end at any point in these phases, usually with the sudden decision by one fish to flee.

T. zillii are well suited to the study of aggression. They will establish territories and spawn readily in captivity. It is therefore a technically straightforward procedure to stage fights between pairs of males in the laboratory aquarium, simply by allowing them to establish territories on either side of a removable partition. Like many other tilapia, they are extremely hardy fish and thus unlikely to present any serious husbandry difficulties. As with other cichlids, they have a complex but distinctive repertoire of aggressive behaviours and colour pattern changes that lend well to the detailed behavioural analysis of fighting. As was pointed out earlier, one slightly unfortunate aspect of this species is that rather little is known regarding aggression and social structure. However, providing this kind of information was one of the aims of the project.

1.4 Aim of this research project

1.4.1 *Philosophy of approach*

I am interested in understanding behavioural mechanisms. Tackling this problem requires linking different levels of study. To start with, it requires an explanation of the functional significance of the behaviour. This then needs to be accounted for in terms of proximate behavioural organisation and this in turn needs to be accounted for in terms of underlying physiology. Thus, I set about designing my experiments in light of what is understood about the functional significance of aggression. Within this functional framework, I have investigated behavioural organisation and possible underlying physiological causes, with the aim of directly relating function to behaviour and behaviour to physiology.

1.4.2 *Specific aims of the project*

I undertook the behavioural studies of this project with the objective of providing new information on aggression in the species and also to demonstrate the functional significance of aggression in *T. zillii*. My first aim was to test game theory predictions regarding the broad features of animal fights, such as the outcome, duration and intensity in relation to relative body size and the value of the contested resource. Relative body size is a variable that is amenable to study because it involves little more than staging fights between individuals of varying sizes. Subjective resource value is more difficult to manipulate experimentally because sometimes it is the case that it is not the absolute value of the resource that is important but the relative value of the resource to a particular individual. For instance, a territory in which to spawn is obviously of greater value to a mature fish than to an immature fish. Such internally-generated variation in resource value may occur in male *T. zillii* where there is large variation in both testis development and size (gonadal state). Thus, it was in relation to relative body size and gonadal state that the present experiments were undertaken.

In addition to testing the broad predictions of game theory, I also undertook a detailed analysis of behaviour with the aim of testing behavioural predictions of game theory regarding the assessment of asymmetries. To understand how short-term behavioural exchanges allow fights to be resolved was an important objective.

Since knowledge of how animals behave in nature is essential to interpreting the functional significance of what they do, and since there is only minimal information available on aggression and social organisation in *T. zillii*, a further aim was to extend

the present project to include a study of aggression and social structure in a semi-natural environment.

The physiological studies aimed to elucidate the behavioural mechanisms underlying aggression in this species. The first study was a simple morphometric analysis of the relationships between internal body variables such as fat reserves and gonadal state. The second was a study of biochemical consequences of energy metabolism in relation to fighting. Finally, a study of sex steroid hormones present in the plasma of the fish was under taken in an attempt to relate gonadal state to aggressive behaviour in these fish. Overall, I have aimed to relate variation in physiology to variation in behaviour and functional context.

1.4.3 *Organisation of the thesis*

Chapters 2 and 3 both describe behavioural studies of fighting and aggression in male *T. zillii*. Chapter 2 reports the methods and results of the core behavioural study of the thesis. This was a series of controlled laboratory experiments that involved the staging of fights between male fish that varied in relative body size and gonadal state. A detailed analysis of the fight structure and behavioural content was undertaken in relation to the predictions of game theory regarding relative body size and assessment. Chapter 3 reports the findings of a semi-natural investigation of social structure, aggression and territoriality of a group of fish in an artificial river. The next three chapters deal with the physiological aspects of the project. Chapter 4 is an investigation of the relationship between external morphology and the internal state of the fish in relation to fighting. Chapter 5 reports a study of the consequences that

fighting imposes for energy metabolism. Chapter 6 reports a study that investigated the associations between a number of sex steroid hormones and gonadal state and the possibility of their role in the regulation of fighting. Finally, Chapter 7 is a general discussion of previous chapters and an attempt to synthesise the various aspects of this research project.

CHAPTER 2

FIGHTING AND ASSESSMENT: THE EFFECTS OF ASYMMETRIES IN BODY WEIGHT AND GONADAL STATE

2.1 Introduction

2.1.1 *Aims and content*

In this chapter, I first review the aspects of game theory that are important to the interpretation of the present research. I then report the main experiment that forms the core of this thesis. The aim of the experiment was twofold; firstly, to account for variation in the outcome, duration and intensity of fights in functional terms and secondly, to demonstrate the proximate behavioural means by which fights are organised and resolved.

2.1.2 *The effect of relative body size and assessment strategies*

It is a regular finding in studies of animal aggression that disputes over resources are won by the larger individual. Examples can be found throughout the animal kingdom, for instance, in teleost fish (Koops & Grant, 1993), in crustaceans (Pavey & Fielder, 1996) and in ungulates (Barrette & Vandal, 1990). Contestants with larger bodies than their opponent are physically stronger and hence able to inflict greater costs on their rival and incur lesser costs themselves. In game theory terms, a size asymmetry leads to differences in 'resource holding potential' or RHP of the contestants (Parker, 1974; Maynard Smith, 1982)

One group of ideas about how the fight is resolved is by the assessment of relative RHP. Parker (1974) first proposed that in an asymmetric fight, an individual benefits from assessing relative RHP because when its RHP is lower, it can withdraw from the fight early on and avoid paying the extra costs of continuing to fight in vain, and when its RHP is greater, it can attack with a high probability of winning. RHP is assessed through visual, audio and contact cues, for example, lateral displaying in cichlid fish (Enquist & Jakobsson, 1986), croaking in toads (Davies & Halliday, 1978) and antler locking in red deer (Clutton Brock *et al.*, 1979). However, there was a problem with Parker's initial model because it assumed the contestants to have perfect information upon which to base their decisions. This means that escalated contests between asymmetric individuals would never happen because the weaker contestant would give up right away.

Hammerstein & Parker (1982) proposed one way out of this dilemma by introducing a stochastic element to the model to accommodate the fact that contestants cannot have perfect information. In the Asymmetric War of Attrition model, resolution is attained by a 'who has more to gain and less to lose by persisting in the fight' rule. The contestant that persists longest wins and persistence time is a function of the ratio of benefits to costs of fighting. A contestant is assigned to play a 'good' role if its benefit to cost ratio is higher than its opponent, that is to say, it has more to gain. The opponent, who has less to gain, plays the 'bad' role. The good role always wins and the bad role always loses. Contestants assess their own role and persist for a greater or lesser duration accordingly. However, because information is not perfect, the

contestants can occasionally mistake their own role for that of their opponent, especially if the difference in cost/benefits between roles is small. Therefore, escalated fights should be expected to occur from time to time.

Enquist & Leimer (1983) proposed an alternative way of modelling error-prone assessment and considered the question of how information on relative RHP is acquired. The key insight of their Sequential Assessment Game is that the information obtained from the performance of a display accumulates in a manner akin to statistical sampling, that is to say, to start with the estimate is very poor but improves with each repetition of the behaviour before reaching an asymptote. The reliability of the information increases with the cost of performing the behaviour; thus fight structure and escalation is modelled as a sequence of behaviours beginning with those that provide the most information for the least cost. If the information contained in the first behaviours is insufficient to determine the asymmetry, then the contestants escalate to the next most cost effective behaviours. Therefore, the fight progresses in a sequence of phases of increasing cost, with fight duration and degree of escalation being a function of how asymmetrical the contestants are; a very accurate estimate is required to detect a very small difference and hence a negative correlation between fight duration and RHP asymmetry is predicted. Thus, the Sequential Assessment Game makes a number of novel predictions, gives a functional account for the process of escalation and finds support from empirical studies in a few species including three cichlid fish (Enquist, et al., 1990; Koops & Grant, 1993; Turner, 1994), a spider (Leimer et al., 1990) and an insect (Englund & Olssen, 1990). It has, therefore, largely superseded alternative models and has become a very influential model itself.

2.1.3 *The effect of subjective resource value*

There are occasions when difference in body size does not predict the outcome of fights and this is usually when there is an asymmetry in the value that contestants place on the disputed resource (the subjective resource value - RV). The more the resource means for the survival and reproduction of the individual, the harder the individual is expected to fight to win or defend the resource. Enquist & Leimer (1987) expanded their model to incorporate the effect of variation in RV. An increase in RV acts to increase the degree of precision of the relative RHP estimate that is accepted as a criterion for giving up. This results in the opponent that places less value on the resource becoming more likely to give up first. As a better estimate requires more time and more costly behaviours, it is predicted that an overall increased RV leads to increased average fight duration and the degree of escalation.

In nature, asymmetries in the subjective value of a disputed resource can arise under a number of circumstances. For example, when bald eagles gather to feed upon post-spawned salmon, newly arrived and unfed individuals will often supplant larger opponents that have fed to satiation (Hansen, 1986). Alternatively, a territory or mate may become extremely valuable at certain times if the opportunity to reproduce is temporally or physiologically constrained. For example, male elephants show individual periodicity in coming into reproductive condition (musthe) which only happens for a few weeks a year (Poole & Moss, 1982). During this period females become extremely valuable for a musthing male, which becomes extraordinarily aggressive toward other males, resulting in it temporarily rising in the dominance

hierarchy. There are a number of studies where the effect of resource value on fighting decisions has been empirically investigated; hermit crabs defending shells of differing qualities (Elwood & Neil, 1992), male spiders fighting over mating access to females (Leimer *et al.*, 1991) and blue footed booby chicks that vary in their food intake needs (Rodriguez-Girones *et al.*, 1996). The results generalise, supporting the predictions concerning fight length, degree of escalation and probability of victory in relation to RV.

2.1.4 *Criticism of the empirical testing of game theory models*

By its treatment of asymmetries in RHP and RV, game theory has provided a conceptual framework that makes robust, generalisable and testable predictions of the broad features of animal fights, such as their length, intensity and outcome. There is, however one main criticism to be made about the empirical testing of the idea that the function of the complex nature of fights is to allow assessment of asymmetries. In my opinion, not enough attention has been paid to contact interaction during fighting. Contact interactions (rather than displays) are where most accurate information is likely to be exchanged during assessment. In the majority of studies contact interactions are recorded as 'mutual occurrences' (for example, mouth wrestling in cichlids, Koops & Grant, 1993; Enquist *et al.*, 1990), but to convincingly demonstrate that the function of such interactions is to allow assessment, it is necessary to tease out the individual components of the interaction and to see if the outcomes for each individual correspond to the asymmetry that is hypothesised to be being assessed. It is possible to uncouple individual contributions in such interactions if a means of assigning individuals to winning and losing individual bouts can be achieved. This is

difficult because of the speed at which fighting happens, but can be tackled if the fight has been recorded on film. By measuring the outcomes for individuals of contact behaviours the assessment hypothesis can be tested in a new way.

2.1.5 Objectives and design of the study

The first objective of this experiment was to investigate the broad predictions game theory makes regarding fight outcome, duration and intensity in relation to asymmetries in body size and resource value. To this end, a series of fights was staged in which I controlled for external territory quality, but varied the degree of asymmetry in body size and gonadal state (assumed to be related to resource value - see section 1.4.2) of the contestants. The second objective was to test the game theory ideas regarding assessment and the resolution of fights. To this end, I video-filmed the fights in order to obtain detailed behavioural data and information on colour pattern changes. The first experiment described was a pilot study that was used to refine the experimental set up for the main experiment that is centre piece of this chapter.

2.2 Pilot experiment

Before I could design a controlled experiment, it was necessary to establish under what circumstances the fish would fight. Assuming that the fish fight over territories, and since the length of time that an occupant has been on its territory is known to influence fighting strategies (for example, Barlow *et al.*, 1986), it was necessary to know how long the fish take to establish a territory and defend it. In addition, it was necessary to have a rough idea of the range of size asymmetries that would be likely to result in fights, since fights are unlikely to start between highly asymmetric opponents. To this end, a series of encounters were staged that varied in both the time the fish had been on their territory and the size asymmetry of the pair. A further aim of this pilot experiment was to construct a mutually exclusive and exhaustive catalogue of behaviours and colour patterns that were be observed during fighting.

2.2.1 Materials and methods

The procedure for staging fights was basically the same as that given for the main experiment (see sections 2.3.1, below). To investigate the length of time it would take for a fish to establish its territory, three groups of pairs were allowed to establish for a period of 24 hours (n = 9 pairs), 48 hours (n = 9 pairs) and 7 days (n = 3 pairs). Weight asymmetry ranged from 2 to 75 % (larger-smaller/smaller).

2.2.3 Results

Out of the nine attempts to stage fights in the 24 hour settling group, only three actually resulted in fights and these were not highly escalated and relatively short (the longest was 220 seconds). Out of nine possible fights in the 48 hours group, five

resulted in escalated fights with durations ranging from 206 to 1214 seconds. Out of

three fights in the 7 days group, three resulted in escalated fights, all of which lasted

for more than 1000 seconds. The maximum weight asymmetry that resulted in a fight

was 51 %. From these first results I decided that a 7 day establishing period and

maximum of 50 % weight difference would be most likely to result in escalated

fighting. The ethogram of aggressive behaviours is presented in Table 2.2.1 (see also

Figure 2.2.1 for a photograph of mouth wrestling) and I have illustrated the colour

patterns in Figure 2.2.2.

Table 2.2.1 Ethogram of behaviours seen during staged fights (continued over)

Behaviour	Description
lateral display	The displaying fish erects its dorsal and anal fins, inflates its branchiostegal membranes and erects its operculae. The fish often swim with exaggerated movements and in parallel with each other.
tail-beat	The opponents align head to tail and beat their tails at each other.
quiver	The fish suddenly makes side-to-side movements with its head pointed downward, followed by a levelling of the body with rapid undulations of the musculature.
zig-zag	The fish rapidly accelerates, brakes, turns direction, accelerates and brakes again.
mouth wrestle	The initiating fish turns to approach its opponent, opens its mouth wide and attempts to make contact. The opponent usually responds by opening its mouth wide. The fish push against each other and whichever fish succeeds in pushing back its opponent is classed as the winner.
Bite	The fish lunges at its opponent, mouth open and snaps the mouth shut upon contact. Inflicting bites and avoiding being bitten results in nose-to-tail chasing.

Table 2.2.1 Ethogram of behaviours seen during staged fights (continued)

Behaviour	Description
mouth lock	The fish approach each other, mouths open and attempt to grip each other by the lips. Once the grip is made, the mouth is shut and the opponents do not attempt to push their opponent. The lock is eventually broken by one fish which folds its fins and makes a rapid side-to-side shake of the head and body.
pause	The fish stop all activity, remaining in close contact with each other. Pausing often follows a mouth-lock.
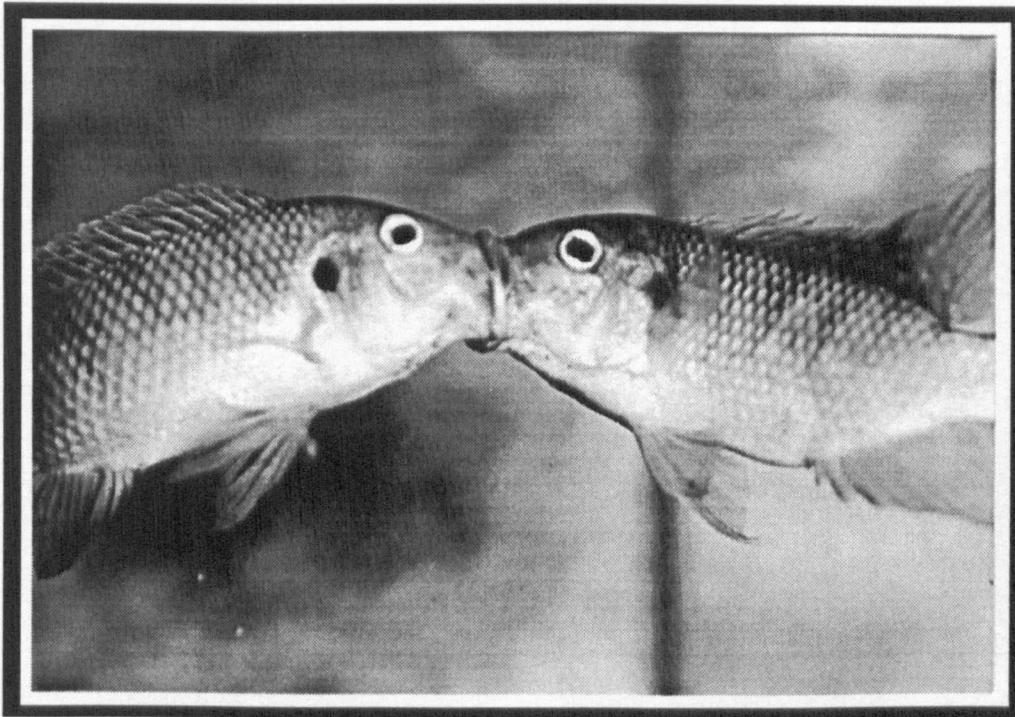
Figure 2.2.1 Photograph taken by the author of mouth wrestling between a pair of male *T. zillii*.
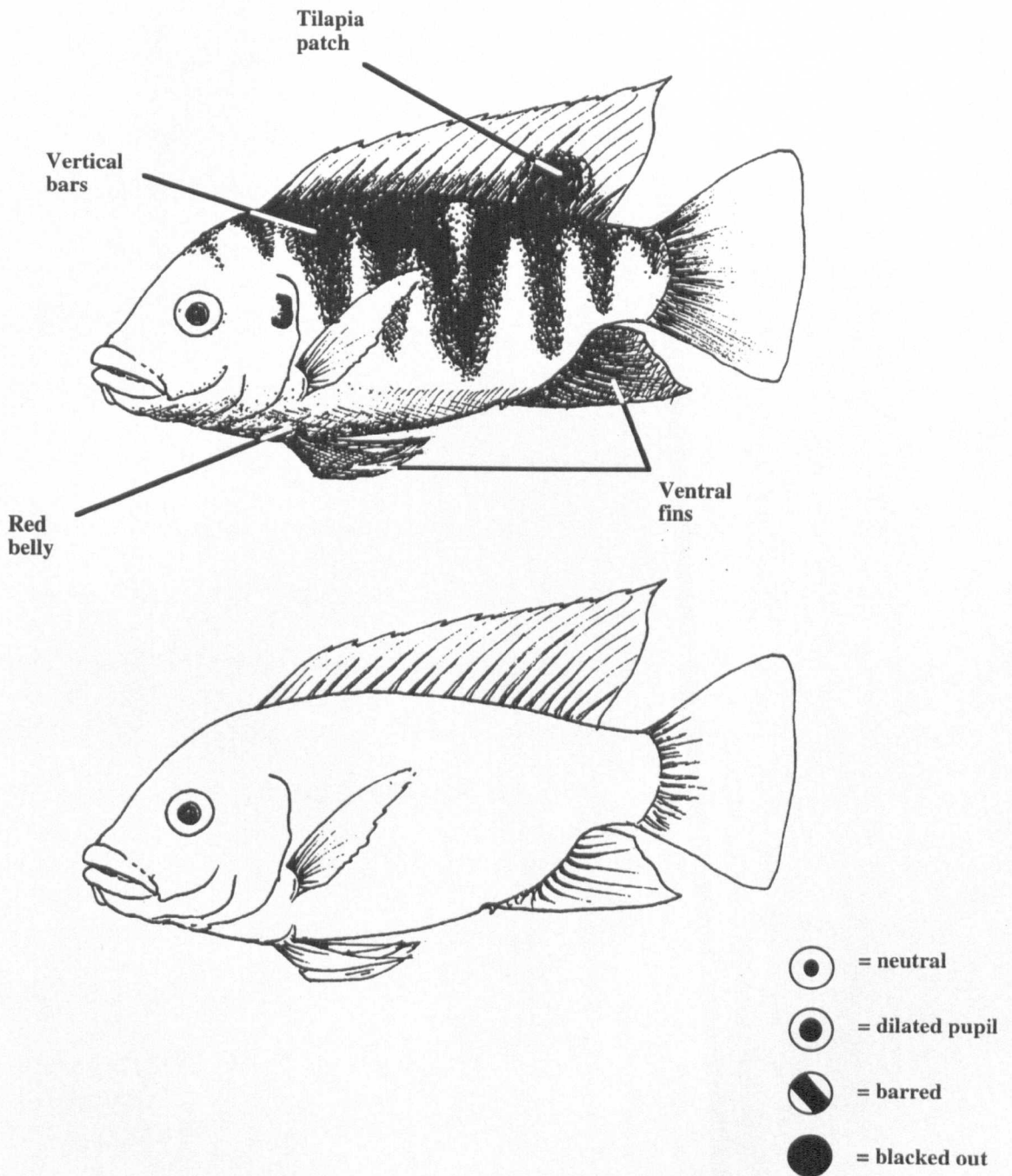
Figure 2.2.2 Illustration of colour patterns expressed by *T. zillii* during fighting (top). Lower illustration shows fish in neutral colouration prior to fighting (scale is approximately 1:1). The different eye patterns are shown in the bottom right corner

2.3 A study of the effects of asymmetries in body size and gonadal state on fighting and assessment in male *T. zillii*

2.3.1 Materials and methods

2.3.1.1 *Fish culture and husbandry*

The original stock of *Tilapia zillii* was obtained from Lake Manzala in Northern Egypt by Dr Brendan McAndrew, University of Stirling. The fish used in all experiments were sexually mature males raised either in the tropical aquarium, Institute of Aquaculture, University of Stirling or by myself in the tropical aquarium at D.E.E.B, University of Glasgow. Prior to experiments the fish were maintained in a large (150 x 60 x 80 cm) glass stock tank. Water temperature was maintained at 27 ± 1°C and water quality was maintained by an external power filter. Light regime was 12:12 light:dark (although, due to technical failure, this was unreliable for part of the duration of the main experiment). Stock feeding was carried out twice a day with commercially available pellets occasionally substituted with blood worm, lettuce or dandelion leaves.

2.3.1.2 *Staging fights*

The fish we used were all from a one year old cohort of sexually mature males raised at the Institute of Aquaculture, University of Stirling. Experiments were carried out under UK Home Office project licence No. PPL 60/01126 (to Prof. Huntingford) and personal licence No. PIL 60/04983 (to myself). Thirty six pairs of fish were selected

on the basis of weight (20 - 140 g) to give a range of size differences varying between 0 - 35 % (heavier - smaller/smaller). Individuals were marked by subcutaneous injection of alcian blue dye (with a pan-jet marker) to aid identification. The pairs were then placed in glass contest tanks (100 x 40 x 30 cm), one fish on either side of a removable opaque partition. To minimise territory variation, each side contained only gravel for nest building and each fish was rationed to eight food pellets per day. The water was aerated and quality maintained by an external power filter. We avoided any owner - intruder asymmetries by allowing each pair to 'own' their territory for 7 days. After this time the partition was raised and the ensuing fight video-filmed. The fish were separated immediately after one fish had lost the fight (which happens suddenly, with a clear behavioural switch, see below) and then killed either by an overdose of benzocaine or by immersion in liquid nitrogen if they were to be used for metabolic study (see Chapter 5). The fish were later weighed and dissected in order to weigh the testis and calculate the gonadosomatic index (GSI), (testes weight as a percentage of total body weight).

2.3.2 Results

2.3.2.1 *Fight structure and behavioural content*

The basic structure of fights between male *T. zillii* was typical of many cichlid fish (see Baerends & Baerends Van Roon, 1951), consisting of three broad phases (see component behaviours in Table 2.2.1). The first was a period of display, in which the fish raised their fins and inflated their opercular membranes. The contestants often swam in parallel with exaggerated movements, beating at each other with their tails. It

was not uncommon for the fish to show courtship activities toward an intruder. This, together with the fact that no physical contact was made, suggests that behaviour in the display phase should not be regarded as escalated fighting. The duration of this phase was very variable (see Table 2.3.1) and all but one fight escalated beyond this stage.

Table 2.3.1 Descriptive statistics of duration (in seconds) of phases in fights.

Phase	Median	Interquartile range	minimum	maximum	n
display	209	100 - 1855	25	10529	35
mouth wrestle	582	325 - 916	116	2182	35
carousel	527	305 - 908	64	1904	34
total escalated	912	690 - 1665	178	2694	35
overall total	1896	1114 - 3704	265	13062	35

Escalated fighting began abruptly with the next phase, termed the 'mouth wrestling' phase (see Table 2.3.1), where contestants engaged in multiple bouts of grappling with open mouths. The first fish to open its mouth was considered to initiate the bout. A fish could attempt to initiate a bout but not actually engage in mouth wrestling, as the opponent may avoid the challenge; conversely, sometimes a bout was initiated simultaneously by both fish. An individual was deemed to win the bout if it succeeded in pushing its opponent backwards. The duration of the mouth wrestling phase was defined as the time from initiation of the first bout, to the time at which 75 % of the total number of bouts of mouth wrestling had been initiated. This cut off was chosen because there is an obvious decrease in the frequency of initiating around this point.

The final phase, termed the 'carouselling' phase, was judged to begin after the 75 % cut off point for the mouth wrestling phase and was terminated with the fleeing of one of the contestants (median duration of this phase is given in Table 2.3.1). Typically, the fish rapidly chased each other nose-to-tail attempting to bite one another. Bites were only scored if they actually landed upon the opponent. Also in this phase there were frequent acts of 'mouth locking', where the fish clamped their mouths on each other's lips (unlike the open mouthed posture of the mouth wrestling) and stayed in this embrace until one fish shook itself free, thereby, 'breaking' the lock. During a mouth lock, the opercular beat rate was observed to increase and, following the lock, the fish often paused and gasped heavily.

Fights ended with one fish suddenly fleeing and making no further aggressive acts toward the opponent. Winning and losing the contest was defined by this criterion, although there was one case where both contestants fled simultaneously. The overall duration of escalated fighting was defined as the time from initiation of the first bout of mouth wrestling, to the time at which the loser flees, minus the duration of any pauses that occurred.

2.3.2.2 Colour pattern changes during fighting

The variable colour patterns exhibited by *T. zillii* in a broad range of social contexts have been described by Hulscher-Emeis (1992). In the present study, a rather crude and limited analysis was undertaken as a first step toward quantifying the relationship between colour patterns and aggression. Only patterns observed to change during

fighting were recorded (tilapia patch, vertical bars, red belly and darkened ventral fins - see Figure 2.2.2). A sample of sixteen fights was chosen for colour pattern analysis. For each minute of time that the fish were engaged in escalated fighting, the expression of a body colour pattern was recorded as on or off. The eye pattern was recorded as being in one of four states (neutral, dilated pupil, barred, or blackened - see Figure 2.2.2). The fight was then split into four periods, namely the first five minutes of escalated fighting (period 1) and then three equal time divisions of the remainder of the fight (periods 2-4).

It was clear that there was a great deal of individual variation between fights in the expression of body colour patterns. In one case, the fight was resolved with both fish still in neutral body colour except for the tilapia patch, whereas in others full colouration was observed in both fish by the end of the fight. The present analysis did not suggest any obvious colour pattern differences between winners or losers at any period in the fight, so the data was pooled to look at how the patterns changed during the fight. Over all fights, Cochran Q tests revealed that all body colour patterns became significantly more likely to be expressed as the fight progressed (notably between periods 1 and 2, see Figure 2.3.1 - $P < 0.05$ for all colour patterns).

The eye pattern was similarly variable between fights and not significantly different between winners and losers, although there was a trend in the data that suggests winners express the barred and blackened pattern sooner than do losers (see Figures 2.3.2a and 2.3.2b). There was quite a clear trend over all fights for eye pattern to change from predominantly neutral and dilated pupil patterns in time period 1 to

being predominantly barred or blacked-out by the later stages of the fight. (Figures 2.3.2a and 2.3.2b).
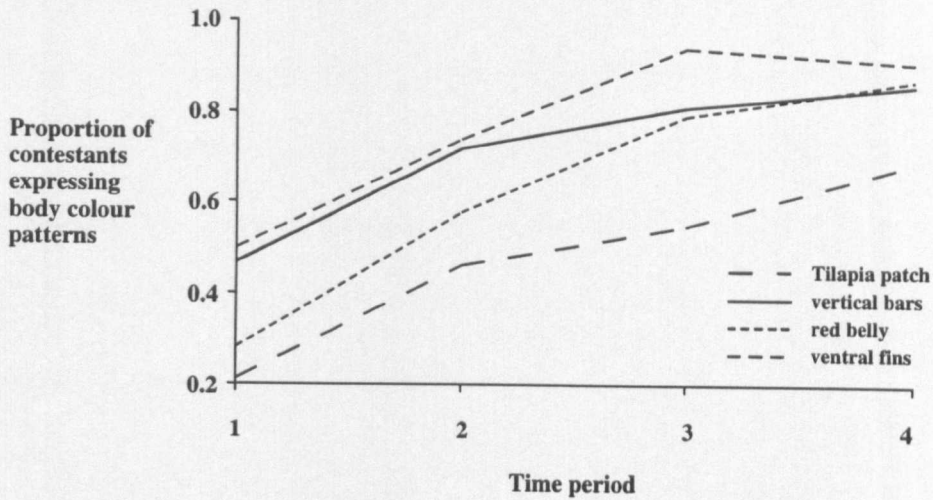
Figure 2.3.1 The proportion of fights in which each colour pattern was expressed at each time period.

a) winners   b) losers
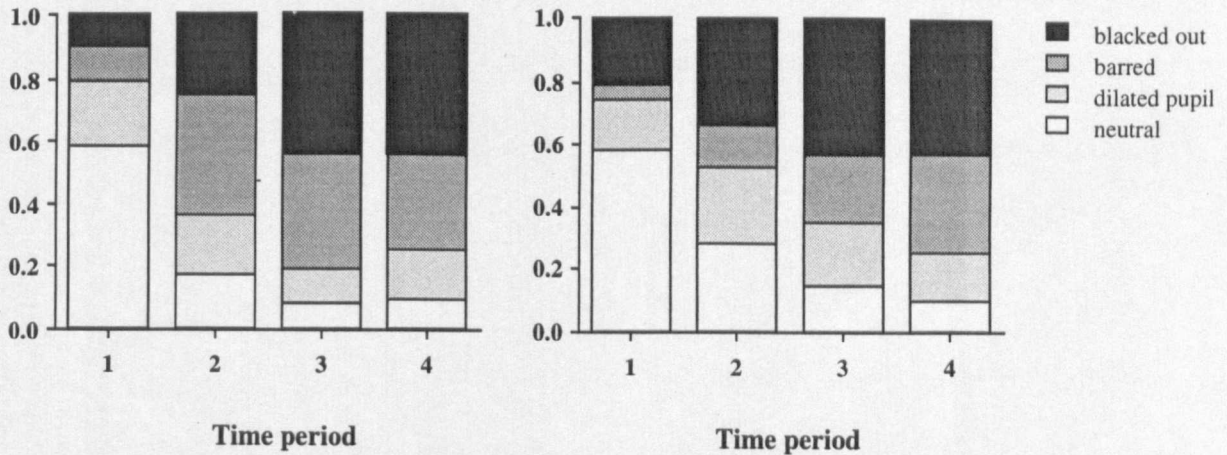
Figure 2.3.2a Proportion of winners adopting each eye pattern at each time period during the fight. Figure 2.3.2b Proportion of losers adopting each eye pattern at each period during the fight.

2.3.2.3 *Asymmetries in body and testis weight and GSI in relation to victory*

Out of the thirty six fights staged, nineteen were won by the larger individual, fourteen by the smaller, one fight remained unresolved, one pair did not fight and one pair were exactly weight-matched. Despite size asymmetries in which the larger fish was up to 35 per cent heavier, and a slight trend for winners to be heavier, median weights of winners and losers, 61.2 and 59.7g, respectively, were not significantly different (Wilcoxon's matched-pairs signed-ranks test, $z = 1.73$, $n = 34$, $P = 0.08$). The most extreme case of a smaller fish winning was where the larger fish was approximately 30 per cent heavier.

GSI ranged from 0.01 percent to 0.61 percent and was not correlated with body weight (Pearson correlation, $r = 0.12$, $P > 0.05$, $n = 72$). Twenty nine fights were won by fish with a greater GSI than their opponent, four by fish with a lesser GSI and in one fight GSI's were equal. Median GSI's of winners and losers were 0.28 % and 0.16 %, respectively, a highly significant difference (Wilcoxon's matched-pairs signed-ranks test, $z = 4.48$, $n = 34$, $P < 0.001$). Similarly, winners had absolutely heavier testis than losers (Wilcoxon's matched-pairs signed-ranks test, $z = 4.44$, $n = 34$, $P < 0.001$).

Condition factor (wt/length ^{3} x 100) did not differ significantly between winners and losers (Wilcoxon's matched-pairs signed-ranks test, $z = 0.35$, $n = 34$, $P = 0.73$) and there was no correlation ($r = - 0.12$, $P > 0.05$) between the condition factor and GSI, indicating that the effect of GSI is not simply a consequence of winning fish being in

better condition. Thus, asymmetry in GSI predicts winning, rather than asymmetry in body weight or condition.

The effects of body weight difference and GSI difference on the probability of victory were further investigated by logistic regression. The dependent variable was the probability of the heavier fish of a pair winning and the independent variables were the difference in weight and the difference in GSI. The difference in weight of a pair of fish $WD_{(a,b)}$, where $_a$ and $_b$ are the larger and smaller fish, respectively, was expressed as:

$$WD_{(a, b)} = \ln (wt_a / wt_b)$$

This has the useful property that $WD_{(a,b)} = -WD_{(b,a)}$ and, therefore, is negative when a smaller fish won, zero when contestants are equal and positive when the larger fish won. The difference in testis weight was expressed in the same way.

The results of the analysis are presented in Table 2.3.2 and show that the probability of the larger fish of the pair winning the fight is strongly related to the difference in GSI. The effect of difference in body weight is weak (not statistically significant) compared to the effect of difference in GSI, but non trivial. The model is best illustrated by a three dimensional probability surface (see Figure 2.3.3) . Out of the 33 fights, the model correctly predicted 12 out of the 14 observed losers and 18 out of 19 observed winners (over 90 % of the observations)..

Table 2.3.2 Results of logistic regression: dependent variable was the probability of the heavier fish of a pair winning and the independent variables were the difference in weight and the difference in GSI.

variables	B	d.f.	significance	R
Testis weight difference	8.69	1	0.017	.28
Body weight difference	22.33	1	0.078	.16
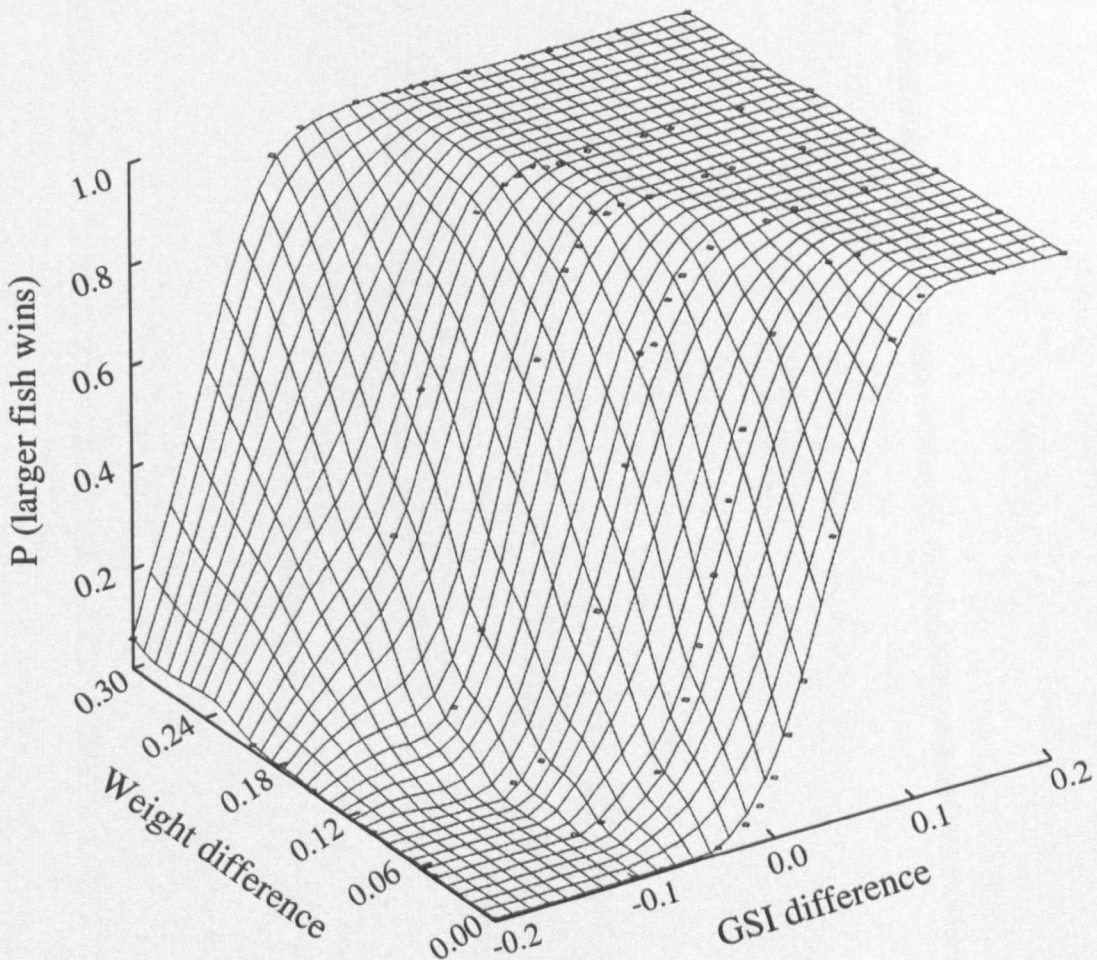
Figure 2.3.3 Relationship between the probability that the heavier fish of a pair wins (y axis), the difference in GSI of the larger fish (z - axis) and difference in body weight of the larger fish (x - axis). The surface illustrates how GSI and body weight combine; difference in GSI is a much stronger effect than difference in body weight. The probability of the heavier fish winning is maximal when the larger fish has a greater relative GSI but very rapidly becomes minimal as the difference in GSI becomes negative. It is only when the weight advantage is very large (> 0.2) that a heavier fish with a smaller GSI is likely to win the contest.

2.3.2.4 *Asymmetries in body weight and testis weight in relation to duration of phases*

Multiple regression was used to determine if the duration of any of the phases was related to the difference in body or testis weight. The results of the analyses are presented in Table 2.3.3 indicating that there was no significant effect of either body weight or testis weight difference on either the duration of the display phase or the duration of the mouth wrestling phase. However, the duration of the carouselling phase was related to both difference in weight and difference in testis weight, in both cases being negatively associated.

Table 2.3.3 Results of multiple regression analyses: dependent variables include duration of phases of the fight and various behavioural measures referred to in the text and independent variables were the weight difference and testis weight difference.

duration of display	$F_{(2,30)} = .07$, Adj. $R^2 = 0.0$, $P < 0.9$		
variables	Beta	$t_{(30)}$	significance
weight difference	-.02	-.12	.90
testis difference	.06	.35	.72

duration of mouthwrestling	$F_{(2,30)} = 1.18$, Adj. $R^2 = 0.01$, $P < 0.31$		
	Beta	$t_{(30)}$	significance
weight difference	-.27	-1.52	.14
testis difference	.002	.01	.98

duration of carouselling	$F_{(2,30)} = 5.13$, Adj. $R^2 = 0.21$, $P < 0.01$		
	Beta	$t_{(30)}$	significance
weight difference	-.41	-2.61	.013
testis difference	-.36	-2.30	.03

Table 2.3.3 continued

proportion of mouthwrestling bouts initiated by larger fish	$F_{(2,30)} = 11.78$, Adj. $R^2 = 0.40$, $P < 0.001$		
	Beta	$t_{(30)}$	significance
weight difference	.66	4.84	.001
testis difference	.08	.61	.68

proportion of mouthwrestling bouts won by larger fish	$F_{(2,30)} = 4.69$, Adj. $R^2 = 0.19$, $P < 0.02$		
	Beta	$t_{(30)}$	significance
weight difference	0.47	2.93	.01
testis difference	0.17	1.06	0.29

proportion of bites inflicted by winner	$F_{(2,25)} = 5.10$, Adj. $R^2 = 0.19$, $P < 0.02$		
	Beta	$t_{(25)}$	significance
weight difference	-.49	-2.81	.01
testis difference	.07	.43	.66

proportion of mouth locks broken by winner	$F_{(2,19)} = .82$, Adj. $R^2 = 0.0$, $P < 0.45$		
	Beta	$t_{(19)}$	significance
weight difference	-.28	1.27	.22
testis difference	.03	.14	.89

2.3.2.4 *Behaviour in the mouth wrestling phase in relation to asymmetries in body weight and testis weight*

Multiple regression was used to determine if there were any effects of difference in body and testis weight on the proportion (arcsine transformed) of mouth wrestling bouts initiated and won by the larger fish. The results of this analysis are presented in Table 2.3.3 and indicate that the difference in body is strongly related to the proportion of bouts of mouth wrestling initiated and won, whereas the difference in testis weight had no significant effects. In other words, the greater the weight asymmetry, the more bouts of mouth wrestling the larger fish initiated and won.

To examine in more detail the relationship between body weight difference and the mouth wrestling behaviour, and to establish if there is a process that may be termed 'assessment', I carried out the following analysis. The sample of thirty three fights that yielded data on mouth wrestling was divided into three equal-sized groups, independent of eventual victory and according to the extent of the percentage weight difference of the pair (0 - 2.5 %, n = 11; 2.5 - 10 %, n = 11 and > 10 %, n = 11). To express the way in which the effect of body weight difference changes as the fight progresses, the total number of mouth wrestling bouts initiated for each fight was divided into quartiles, and the number of bouts initiated and won by the heavier fish in each quartile calculated. For the three groups, at each quartile, the difference between the number of bouts initiated and won by the heavier fish and the lighter fish were analysed using the Wilcoxon's matched-pairs, signed-ranks test. The results of the analysis are presented in Table 2.3.4 and these data are expressed as the proportion initiated and won by the heavier fish in Figures 2.3.5a and 2.3.5b, respectively. Figures 2.3.4a and 2.3.4b show that in the early stages of the fight, both contestants in all groups initiated and won a similar proportion of bouts of mouth wrestling and this is not different from the random expectation (0.5). In the group where contestants are very closely matched (weight difference of 0 - 2.5), this remained the case throughout the fight, indicating that fish that are closely matched for weight are also closely matched in the ability to win at mouth wrestling. However, in the groups with moderate (2.5 - 10 %) and large (> 10 %) weight disparity, as the fight progressed (across quartiles), the heavier fish initiated and won more bouts. Furthermore, as the weight discrepancy increased (across groups), these differences

become greater, for instance, in the > 10 % group, in the last quartile, the larger fish

is winning on average 75 % of the bouts.

Table 2.3.4 Analysis of weight dependent differences in initiating and winning bouts

of mouth wrestling (Wilcoxon's signed-ranks matched pairs test)

GROUP	Initiating			
	Quartile 1	Quartile 2	Quartile 3	Quartile 4
0 - 2.5 %	z = .36 p= .72	z = 1.94 p = .06	z = 1.22 p = .22	z = .21 p = .83
2.5 - 10 %	z = .42 p = .67	z = .91 p = .36	**z = 1.99** **p = .04**	**z = 2.13** **p = 0.03**
> 10 %	z = 1.83 p = .07	z = 1.94 p = .06	**z = 2.49** **p = .01**	**z = 2.83** **p = .005**
	Winning			
0 - 2.5 %	z = .87 p = .39	z = .31 p = .76	z = .40 p = .69	z = .49 p = .62
2.5 - 10 %	z = .25 p = .80	z = 1.70 p = .09	**z = 2.80** **p = .005**	**z = 2.29** **p = 0.02**
> 10 %	**z = 2.49** **p = .01**	**z = 2.65** **p = .008**	z = 1.47 p = .14	**z = 2.80** **p = .005**
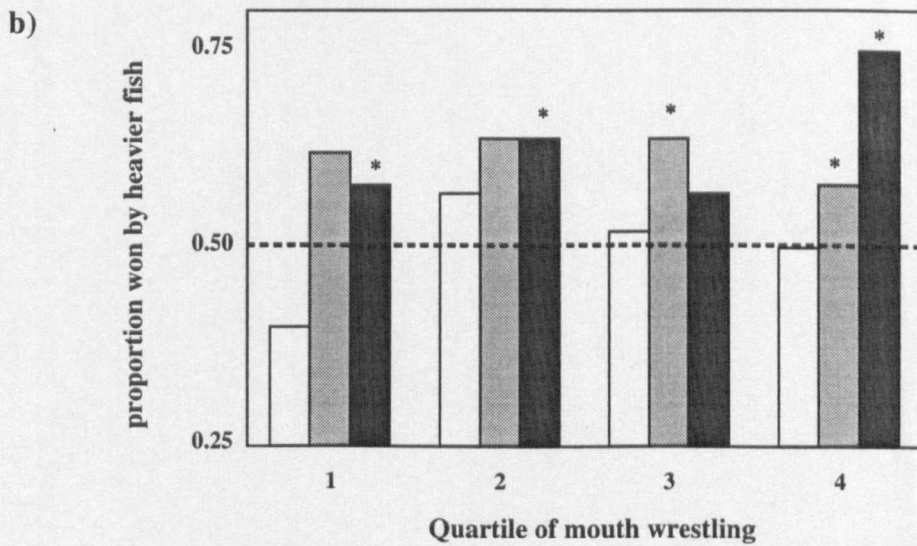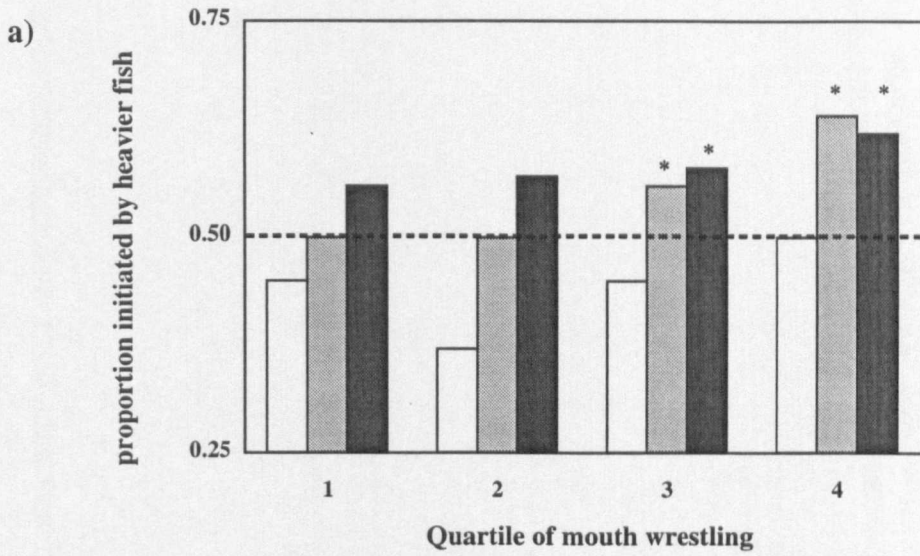
Figure 2.3.4a (top). The proportion of bouts of mouth wrestling initiated by the heavier fish (p) (the lighter fish won 1 - p bouts) for each quartile of the total number of bouts initiated. White bars denote the 0 - 2.5 % weight difference group, hatched bars the 2.5 - 10 % group, and dark bars the > 10 % group. The dashed line is the 0.5 proportion level that would be expected if there was no effect of weight difference. Those bars marked with an * indicate that the heavier fish initiated a significantly greater proportion of bouts (see table 2.3.3). **Figure 2.3.4b** (bottom) The proportion of mouth wrestling bouts won by the heavier fish (format is identical to Fig. 2.3.5a)

2.3.2.5 *Asymmetries in body and testis weight in relation to biting and mouth locking*

Pairs of fish tended to match each other bite-for-bite and, overall, there was no significant differences between winners and losers in the total number of bites inflicted (Wilcoxon's matched-pairs signed-ranks test, $z = 0.21$, $n = 33$, $P = 0.84$). However, upon examination of the proportion of the total count of bites inflicted by the winning fish, a multiple regression revealed a significant effect of the difference in body weight but no significant effect of difference in testis weight (see Table 2.3.3). Figure 2.3.5 shows the negative relationship between the proportion of bites inflicted by the winner and the difference in weight. In other words, winners that are much smaller than their opponents inflict relatively more bites than winners that are larger than their opponents.
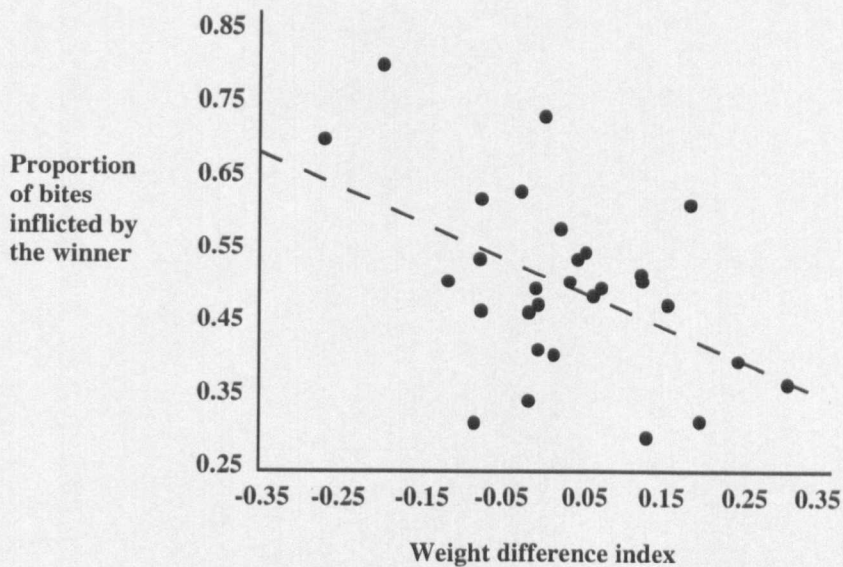
Figure 2.3.5 The proportion of bites inflicted by the winning fish plotted as a function of difference in weight. When the weight difference index is negative, the winner was smaller than its opponent.

The mouth locking behaviour was the only component of fighting in which there were consistent differences between winners and losers. Out of the fights that yielded sufficient mouth locking data (>10 mouth locks), winners broke a significantly lower proportion of mouth locks (median = 0.3) than losers (median = 0.55) (Wilcoxon's matched-pairs signed-ranks test, z = 3.12, n = 29, P < 0.01). Further analysis by multiple regression did not suggest that proportion of mouth locks broken was dependent upon the difference in GSI or weight (see Table 2.3.3).

2.3.3 Discussion

2.3.3.1 *Body size, GSI and the outcome of fights*

Body size (and with it, resource holding potential - RHP) has been shown to be a major factor determining the outcome and structure of animal fights. In the staged fights reported here between male cichlids defending territories, body size evidently does not have the predicted effects. This is in contrast to many studies involving pair-wise contests in fish (Enquist *et al.*, 1990; Ribowski & Franck, 1993 & Turner & Huntingford, 1986). These results suggest that asymmetry in GSI is a better predictor of winning than is asymmetry in body weight.

2.3.3.2 *What is the functional significance of variation in GSI ?*

The present study is not alone in finding an effect of gonadal state on aggressive behaviour in cichlids. Gonadal state predicted dominance in male *Tilapia mariae* (Schwank, 1981), although gonadal state was only measured indirectly by the length of the genital papilla. Similarly, Holder *et al.*, (1991) report that male *Cichlasoma*

citrinellum become increasingly aggressive the closer they get to spawning (they also used the genital papilla as an index of proximity to spawning). Thus, there is clearly some important functional underpinning of gonadal state in the aggressive behaviour of cichlids. In the present study, as we standardised territory quality, this suggests that either GSI relates to the subjective value of a territory or that it reflects stronger, fitter males.

T. zillii breeds all year round in equatorial regions, peaking in intensity during wet months (Siddiqui, 1979). Upon maturation, it is likely that the fish develop endogenous reproductive cycles associated with changes in behaviour and gonadal state. GSI is likely to increase as the fish get closer to spawning, and the closer to spawning, the more valuable the territory becomes to that individual. Thus, the subjective value of the territory may covary with GSI. In addition, it may be that sperm volume is a limiting factor for the capacity to fertilise eggs (as has been shown in some externally fertilising fish, Shapiro & Giraldeau, 1996); thus males that have invested heavily in sperm production may value their territory more because their absolute capacity to fertilise eggs is greater. However, to my knowledge, this aspect of the breeding biology of *Tilapia* sp. has not been researched and it seems unlikely, given that in *T. zillii* clutch size is comparatively small (approximately 500 - 1000 eggs, depending on body size - personal observation) because not all ripe ova are released during a single spawning attempt (Siddiqui, 1979).

Alternatively, GSI may be a consequence, rather than a cause of dominance. Males that are better fighters may be better able to allocate resources to the development of

gonads. In this study, the fish were isolated for seven days prior to fighting, but I cannot be sure that the effects of previous experience and social status are not long lasting. However, there was no evidence that difference in GSI was related to mouth wrestling or biting, which are behaviours one might expect to be related to fighting ability. Thus, it seems more likely that GSI is related to RV. Under this assumption, this study presents an opportunity to evaluate different game theory models of how RHP and RV interact to determine the outcome, duration and behavioural structure of fights.

2.3.3.3 *Is relative body size assessed ?*

It is not obvious whether one should expect body size to be assessed in situations where it does not predict victory. The Sequential Assessment Game that allows for variation in subjective resource value (Enquist and Leimer, 1987) assumes that the assessment of RHP does take place during the fight through a sequence of displays and interactions. The fights reported here did progress in a sequential way, from display through mouth wrestling to carouselling. However, to test the idea that the function of a behavior is to allow assessment of RHP, it needs to be demonstrated that individuals respond to the candidate assessment behavior in accordance with their relative RHP.

While the significance of the display phase is at present obscure, mouth wrestling is clearly a key component of escalated fighting and a likely candidate for an interaction during which the contestants assess body size; it appears to be so in other cichlid fish, for example, Enquist and Jakobssen (1986) and Turner (1994). In the present study, it

is clear that fish heavier than their opponent initiate and win more bouts of mouth wrestling. In addition, our results suggest that assessment of relative size is a dynamic process. If we interpret bout initiation as reflecting motivation to engage in mouth wrestling and winning as an index of relative strength, then these data support Enquist and Leimer's (1983) view that at the start of the fight the contestants have little information on relative RHP, but that this increases as the fight progresses. Relative frequencies of bout initiation are not significantly different from random between contestants in the first quartile of mouth wrestling, but as the fight progresses the difference in relative strength becomes apparent and the smaller fish of a pair initiates less. A reduced motivation to engage in this activity may indicate that information on relative size has been exchanged. These data, therefore, provide evidence that body size is coupled with physical strength and this asymmetry is progressively assessed during the mouth wrestling phase. However, this is evidently not the means by which the fight is resolved, as fish smaller than their opponent often continued to fight in spite of information about its RHP disadvantage and frequently went on to win the fight. The resolution of the fight must come in the final carouselling phase of the fight.

2.3.3.4 *Behaviour in the carouselling phase in relation to relative body size and GSI*

According to the Sequential Assessment Game, the effect of increased RV is to increase the cost of the fight (Enquist and Leimer, 1987). It also predicts that fighting begins with the least costly displays, so we might expect to find an effect of relative GSI in the later, more costly stages of the fight. Consider the two main behaviours in the final carouselling phase, namely biting and mouth locking. Most biting occurs at

this stage and often results in injury (scale loss, skin and fin damage) and provokes retaliation, hence it is likely to be a costly act. Thus, we might expect fish with high GSI's to take greater risks and bite more. Surprisingly, there was no evidence to suggest that difference in GSI was related to biting. However, it is interesting that the proportion of bites inflicted by the winner was highest for severely weight disadvantaged fish, decreased as the opponents became more weight matched and was lowest for weight advantaged winners. This finding suggests that small winners are playing a very high risk strategy. Presumably, when an individual is disadvantaged, a high risk strategy is the only option that offers any chance of winning. Similar high risk strategies are used by subordinate finches when they occasionally beat dominants in disputes over food (Senar, *et al.*, 1992).

The data on the mouth locking behaviour strongly suggest that breaking the mouth lock is related to the likelihood of eventually giving up. However, there was no evidence that this behavior provided information on relative GSI. The significance of the mouth lock is not clear, but it may be a trial in which the contestants assess the relative costs that each opponent is prepared to incur (see section 2.3.3.6).

In summary, winners were not necessarily heavier than their opponents, but they were likely to have a significantly greater GSI. It seems likely that the difference in relative weight was assessed during mouth wrestling, but that the fights were resolved in later stages. The level of risk (in terms of biting) that an eventual winner played depended upon its relative weight; severely weight disadvantaged winners inflicting the highest

proportion of bites on their opponent. Losers broke a significantly greater proportion of mouth locks, but there was no corresponding correlation with relative GSI.

2.3.3.5 *Colour pattern changes during the fight*

Two possible roles of colour patterns in aggressive behaviour in fishes have been suggested. The first is that a change in colour patterns gives general information about motivational state. For example, male bluehead wrasse (*Thalasomma bifasciatum*) rapidly change from an opalescent body colour when courting a female to a green body colour when confronting a rival male (Dawkins & Guilford, 1993). There is also some evidence that colour patterns may give information about specific asymmetries during a fight. For example, Barlow (1963) has shown that in fights between male *Badis badis* (a teleost), the eventual winners adopt a darker body pattern than the losers. In the present study, despite the limited analysis, it was fairly clear that body colour patterns were more likely to be expressed as the fight progressed and that the eye was more likely to be in a barred or blackened state as the fight progressed. However, the analysis did not suggest that there were any differences between winners and losers, although the recording method may have been too crude to detect any subtle changes. Hulscher Emeis (1992) has shown how colour patterns in *T. zillii* change in intensity. The present analysis did not take this aspect into consideration, which may be more important than simply whether the pattern was expressed or not. A more detailed analysis would be needed before conclusions should be drawn about the role of colour pattern changes during fighting and in assessment. Thus, for the present, the only statement that can be made in confidence is that the patterns are more likely to appear as the fight progresses. At the

very least, this suggests, either that they signal an increasing level of aggression between the opponents, or that they are involved in the assessment process in a subtle way. This is an aspect of fighting which clearly deserves further study.

2.3.3.6 *How are the fights resolved ?*

There have been a number of attempts to model fight resolution in relation to both variation in relative RHP and RV. In the Sequential Assessment Game with variation in RV (Enquist & Leimer, 1987), fights are resolved when one contestant gives up because it estimates its RHP to be lower than its opponents. However, because an increase in RV acts to increase the degree of precision of the estimate of relative RHP the contestant accepts before giving up, it is possible for a fish with a greater RHP to lose because it has a lower RV and thus erroneously calculates that it has a lower relative RHP (due to its unwillingness to pay the high costs of getting a precise estimate). The Sequential Assessment Game may be able to account for the effect of RV when the difference in RHP is not too asymmetric or there is a symmetrical increase in RV (for example the spider study of Leimer *et al.*, 1991). The results from the present study are ambiguous in supporting the predictions of the Sequential Assessment Game. On the one hand, our data support the prediction that only the final phase of the fight will be negatively correlated with weight asymmetry (this was also the case for gonad asymmetry in our study). On the other hand, the asymmetries in weight were very large in many cases, and some of the longest fights were those in which fish much smaller than their opponent eventually won. After such a length of time, it seems unlikely that the larger fish gave up because it mistakenly estimated its RHP to be lower than its opponent.

There are at least two other possibilities of how fights could be resolved. One is that relative RHP is assessed, but that because the stakes are so high, the contestants continue regardless and the fight is resolved by the rule 'who has more to gain and less to lose by persisting'. The process of resolution is similar to the Asymmetric War of Attrition model proposed by Hammerstein and Parker (1982) and to Marden and Waage's (1990) 'physical limitation' hypothesis to account for their observation that contests between damselflies are won by the individual that has the highest fat reserves. In the present context, as the fight progresses, costs accumulate and each fish continues until the current cost of fighting outweighs the benefit to be gained from retaining the resource. The first contestant to reach this threshold gives up and the fight is resolved. The level of this threshold increases with increasing RV, thus contestants with higher RV persist longer and are, hence, more likely to win. This idea is expanded and formalised mathematically in Appendix 3.

Alternatively, the contestants may be able to assess each other's cost thresholds, that is how long their opponent is prepared to persist in order to defend the disputed resource. Each contestant continues to fight until one of them assesses that its costs of fighting will exceed the benefits it stands to gain from retaining the resource sooner than it will for the opponent. There are other studies that suggest that contestants can assess relative RV and other asymmetries that are not directly observable, such as energy status and motivational state. Elwood and Neil (1992) present evidence that hermit crabs alter their fighting strategies as they acquire information on each other's shells. Marden and Rollins (1994) have shown that damselflies can assess relative energetic status and settle contests before physical limits are reached and Poole

(1987) reports data for African elephants that suggests that males assess the 'musthe' condition (see section 2.1.3).

2.3.3.7 *Which model best accommodates the data for* T. zillii *?*

Enquist & Leimer's (1987) Sequential Assessment Game may be able to account for the effect of RV when the difference in RHP is not too asymmetric or where there is a symmetrical increase in RV (for example the spider study of Leimer, *et al.* 1991). However, for the present study, where the asymmetry in weight is large in many cases and there is no evidence to suspect that winners are at an advantage in terms of their fighting ability, it seems inappropriate. Furthermore, the longest fights were those in which fish much smaller than their opponent eventually won. After such a length of time, it seems unlikely that the larger fish gave up because it mistakenly estimated its RHP to be lower than its opponent.

In the Asymmetric War of Attrition model (Hammerstein & Parker, 1982), I propose that the carouselling phase is the War of Attrition and that the fish enter this phase with information regarding their relative RHP (which they obtained during mouth wrestling). Each fish has a threshold cost that it is prepared to pay and this is determined by its GSI. The cost of fighting has many components, but two of the most important are likely to be physical injury and exhaustion. The main cause of injuries in fighting in this species is biting and probably the best overall index of how exhausting a fight has been is the duration of escalated fighting. However, there was no evidence for any effects of relative GSI on the proportion of bites received or on

the duration of escalated fighting. Thus, I have no supporting data for this particular model.

If the cost threshold assessment hypothesis is appropriate we should expect assessment to relate to both relative RHP and GSI. We propose that assessment of relative RHP occurs in the earlier stages of the fight (mouth wrestling), just as in the Sequential Assessment Game. However, but because GSI affects how long the contestants intend to persist in the fight, this involves signaling information about motivation. Disclosing information about motivational state and the intention to persist was originally believed to evolutionarily unstable because of its vulnerability to cheating (Maynard Smith, 1982); however, Enquist (1985) has shown that the signaling of intentions can be stable provided the signal honestly reflects the level of intention and to meet this criterion the signal must be costly. It follows that if there are behavioral indicators of GSI and that assessment is occurring, then it should be a feature of the more escalated (costly) stages of the fight. Mouth locking is a feature of the most escalated stage in the fight and breaking the mouth lock is associated with the likelihood of losing. There was not, however, a significant relationship between the proportion of mouth locks broken and relative GSI. Thus, while it seems likely, we cannot conclude that the fish are making available reliable information about their relative cost thresholds. It is interesting that in other detailed studies of fighting, differences between opponents are also only detected in the last stages of fighting, for example, Simpson's (1969) study of fighting in Siamese fighting fish (*Betta splendens*). Similarly, Marden and Rollin's (1994) study on damselflies only found a

significant relationship between energy reserves and fight duration in the most escalated fights.

2.3.3.8 Conclusions

This experiment has revealed that asymmetry in gonadal state has a stronger effect upon fight outcome than does asymmetry in body size (even when this is large). This result is quite surprising but the findings do not contradict game theory, if it is assumed that gonadal state reflects the value of the territory to the individual. However, the study does have implications for the way the resolution of animal fights is usually perceived. In addition to the assessment of RHP, fighting strategies may also serve to obtain information on both how much cost each opponent has paid and how much each opponent is prepared to pay to retain its resource. Signalling intentions or motivation is likely to involve costly signals and thus may only be detected in the later stages of escalated fights.

CHAPTER 3

AGGRESSIVE INTERACTIONS AND SOCIAL ORGANISATION IN A SEMI-NATURAL ENVIRONMENT

3.1 Introduction

The functional analysis of animal behaviour is based on the principle that the animal has evolved under the selection pressures of the natural environment. Where experiments in behavioural ecology are carried out in the laboratory, it is assumed that the conditions of the laboratory are equivalent to a controllable natural environment and that behavioural adaptations can be studied by manipulating certain variables while keeping others constant. However, there is always the risk that observations in the laboratory are spurious artefacts of keeping animals in artificial conditions (Martin & Bateson, 1993). Thus, to justify such an assumption, it is essential to have some information on the behaviour in natural environments with which to compare laboratory results. In addition, information from natural observations and experiments can provide insights that one cannot get from designed experiments alone (Tinbergen, 1958).

There is relatively little information on the social and territorial system of *T. zillii* in the wild. The only published field studies have concentrated on reproductive and spawning behaviour rather than territoriality (Bruton & Gophen, 1991; Siddiqui, 1979). No information is available on the frequency and intensity of aggressive

interactions or on what circumstances provoke aggressive responses. Neither is it known whether *T. zillii* is permanently territorial, or whether during non-breeding phases it is like other *Tilapia sp.* in forming loose social groups. These basic aspects of the natural history would be valuable for the interpretation of the results described in Chapter 2, especially in justifying the functional assumptions made. Furthermore, it would be valuable to know whether the kind of fights observed in the laboratory aquaria are similar to fights seen under more natural circumstances, rather than an artefact of life in captivity. In the scale of the present project, a field study was clearly unfeasible, so use was made of an artificial stream at The University Field Station, Rowardennan.

This chapter describes the social dynamics of a group of male fish allowed to have free range over approximately ten metres of 'river bed' during a one month period. The main aim of the study was to record the occurrence and nature of aggressive interactions and to make observations on the establishment of territories among a group of males under these semi-natural conditions. In addition, I investigated how aggression and social interactions changed when key individuals were removed and females were introduced. The following questions were addressed:

1) What kind of aggressive behaviours are seen ?

2) What kind of social organisation does *T. zillii* exhibit ?

3) What is the functional significance of aggressive interactions ?

The study was undertaken in order to generate a qualitative understanding of the social organisation in *T. zillii* to help in the interpretation of the rest of the project and provide information on which to base a more extensive and quantitative field study. It was necessarily conducted on a small scale and was not designed to provide a definitive quantitative analysis of aggression.

3.2 Materials and methods

3.2.1 *Fish and observational set-up*

Seven sexually mature male and two female fish were selected, weighed and individually marked with a pan-jet ink marker (individual weights of fish are given in Table 3.2.1).

Table 3.2.1 The identity and weight (in grams) of male fish at the start and end of the experiment and percentage weight increase during the period.

Fish I.D	A	B	C	D	E	F	G
weight on day 1	160.2	87.2	70.1	84.8	97.0	74.3	70.4
weight on day 28	-	104.8	87.2	124.3	132.3	82.7	84.4
% weight increase	-	20	25	47	36	11	20

The fish were then transported to an artificial observation stream (flume) at The University Field Station, Rowardennan. Figure 3.2.1 shows the dimensions, and the 'pool and riffle' landscape of the flume. The water in the flume was recirculated, but had a slow trickle of fresh water and overflow drain-pipe. The water temperature was $27 \pm 3°C$ and the light:dark cycle was 12:12. Flow rate varied according to the landscape. Over riffles it was approximately 4 - 8 cm s $^{-1}$, whereas in pools it was 0 - 1 cm s $^{-1}$. The study lasted for twenty eight days. On the first day all males were put into the flume at once and observations (see below) made for five days. On the fifth day, the top ranking fish (see below) was removed and observations continued for a further nine days. Two gravid females were then introduced and observations continued for the remainder of the experiment. At the end of the experiment the males were weighed again and growth rates estimated (see Table 3.2.1).

300 cm

60 cm
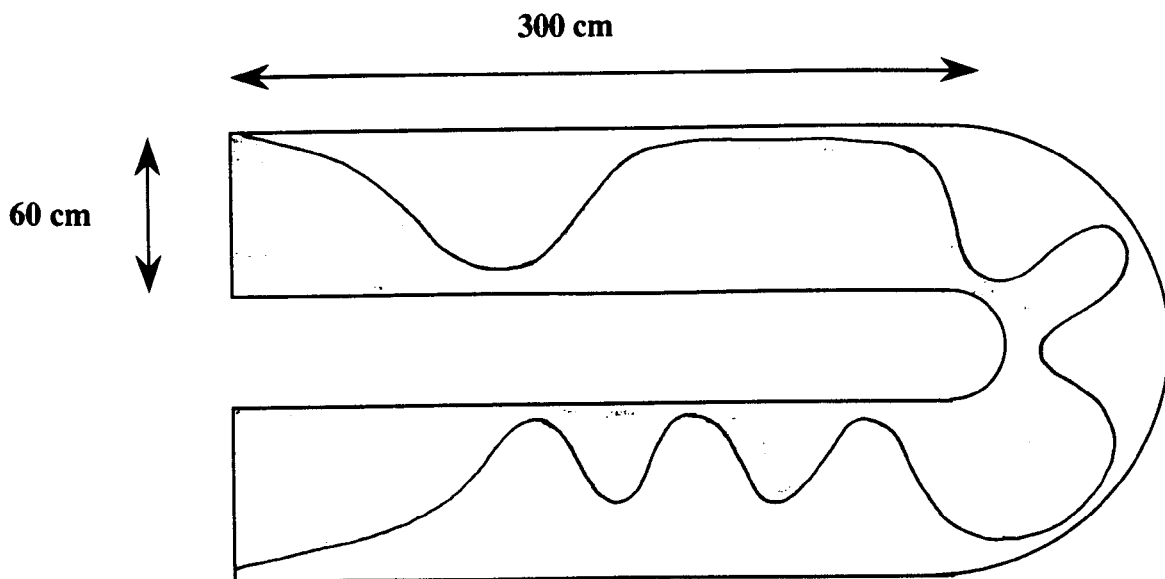
Figure 3.2.1 Diagram of the artificial stream showing pool and riffle landscape (shaded areas are pools). Depth was approximately 35 cm in pools.

3.2.2 *Recording behaviour*

On the first day, observations were made continuously for 1 h and then on and off for the rest of the day. From day 2 onwards, observations were made twice a day (at approximately 09.30 and 15.30) for 30 min., with particular attention being paid to aggressive interactions. Aggressive behaviours (see Chapter 2, Table 2.2.1 for further descriptions of the component behaviours) were classified as follows:

Chases - One fish chases another, which flees immediately, making no attempt to confront the aggressor.

Displays - Both fish raise fins and/or inflate branchiostegal membranes.

Scraps - Both fish perform tail-beating and parallel swimming but neither escalates to mouth wrestling.

Short fights - The fight escalates to mouth wrestling but not to the carouselling phase

Escalated fights - The fight escalates to carouselling and mouth locking.

All the above interactions were terminated with one fish chasing the other or when the interaction was interrupted by another fish.

Social dominance was scored from the outcome of aggressive interactions between individuals. Most interactions were terminated by one fish chasing away the other; the

chaser was assigned 'dominant' to the chased fish and a hierarchy was inferred on the basis of repeated assertion of dominance (Martin & Bateson, 1993). The fish that dominated all others was assigned rank 1 (or the despot), the fish dominant to all but the rank 1 fish was assigned rank 2, and so on.

3.3 Results

3.3.1 *Frequency of aggressive behaviours*

Chasing was the most common aggressive interaction (the median frequency per hour for all fish was 31), followed by displaying (median frequency per hour for all fish was 19). A total of 14 scraps, 8 short fights and 1 escalated fight were observed during the sampling periods over 28 days.

3.3.2 *Dominance structure*

To begin with the males formed a cohesive group and were even observed to be schooling at times. However, this did not last long and was most likely a fright response to being introduced to a novel environment. After a few hours, the group became less cohesive and a dominance hierarchy began to develop through aggressive interactions. The hierarchy is presented in Table 3.2.2. A clear despot had emerged within a few hours and second and third positions in the hierarchy were established by the afternoon of day 1. The lower subordinates did not maintain consistent positions between sampling periods, thus, the hierarchy cannot be strictly classified as linear, although it was usually possible to establish a linear relationship during a single sampling period.

Table 3.2.2 Dominance hierarchy for males fish A - G during the experiment. Rank 1 is assigned to the individual that dominated all other fish, rank 2 to that individual that dominated all other fish except the rank 1 fish and so on. * indicates the point at which the despot was removed and + indicates the point at which the females were introduced.

Fish \ Day	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
A	1	1	1	1	*	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
B	2	2	2	2	2	1	1	1	1	1	1	1	1	1	1	2	4	4	4	2	3	3	3	3	3	3	3	3
C	3	3	3	3	3	2	3	3	4	4	4	4	4	4	5	5	5	5	5	5	4	4	4	4	4	4	4	4
D	4	4	4	5	5	3	2	2	2	2	2	2	2	2	2	3	2	2	3	3	2	2	2	2	2	2	2	2
E	5	5	5	4	4	5	4	4	3	3	3	3	3	3	3	1	1	1	1	1	1	1	1	1	1	1	1	1
F	6	6	6	7	7	4	5	5	5	6	5	5	6	5	4	4	3	3	2	4	5	5	5	5	5	5	5	5
G	7	7	7	6	6	6	6	6	6	5	6	6	5	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6

With reference to Table 3.2.2, one can see that upon removal of the despot on day 5, the second ranked fish became the despot, and the third ranked fish became rank 2. The following day, the rank 2 fish was defeated in a short fight by a lower ranked fish. Further changes in the hierarchy also occurred among the mid and low rank fish. Over the next seven days, the top of the hierarchy stabilised, but again there was instability in the lower half of the table as the two lowest ranked fish alternated between the lowest position.

Following the introduction of the females, there were clear changes in the dominance hierarchy. The fight between the two top ranked fish was presumably an attempt by the second top fish to become the despot. This was not successful, as the despot won the fight. Following this fight, the third ranking male attacked both the despot and the second ranking fish and was successful in both instances resulting in it taking the top position in the hierarchy. Over the next couple of days the hierarchy changed still further. The original despot continued to descend the ranks and a previously low ranking fish secured the number 2 ranked position. The upper region of the hierarchy did not stabilise until around day 21.

3.3.3 Temporal trends in aggressive behaviours

The number of aggressive acts (summed for all fish), for each day (60 minutes of observation) is shown in Figure 3.2.1. In the first 2 days there was a high rate of chasing and displaying, with occasional scraps and one short fight. A single despot was responsible for the majority of chasing and would often interrupt interactions between other fish. Upon removal of the despot, on day 5, there was an increase in aggression, most notably in scrapping and short fighting, between all fish. Following this, the frequency of displays and chases decreased gradually, because low ranking individuals were observed to actively avoid higher ranking individuals.

Following the introduction of two gravid females on day 15, there was a sharp increase in displaying, chasing, and scrapping. Three short fights were observed and the first escalated fight occurred the next day between the two highest ranking individuals. The exact duration of the escalated fight lasted is not known as it was in progress when the observation period started but it continued for 28 observed minutes. Both fish showed signs of injury in the form of lost scales and lip damage. A further two short fights were observed over the next few days but after this sharp rise in aggression, the frequency of aggressive acts began to fall and remained at fairly constant low levels from around day 20 onwards.
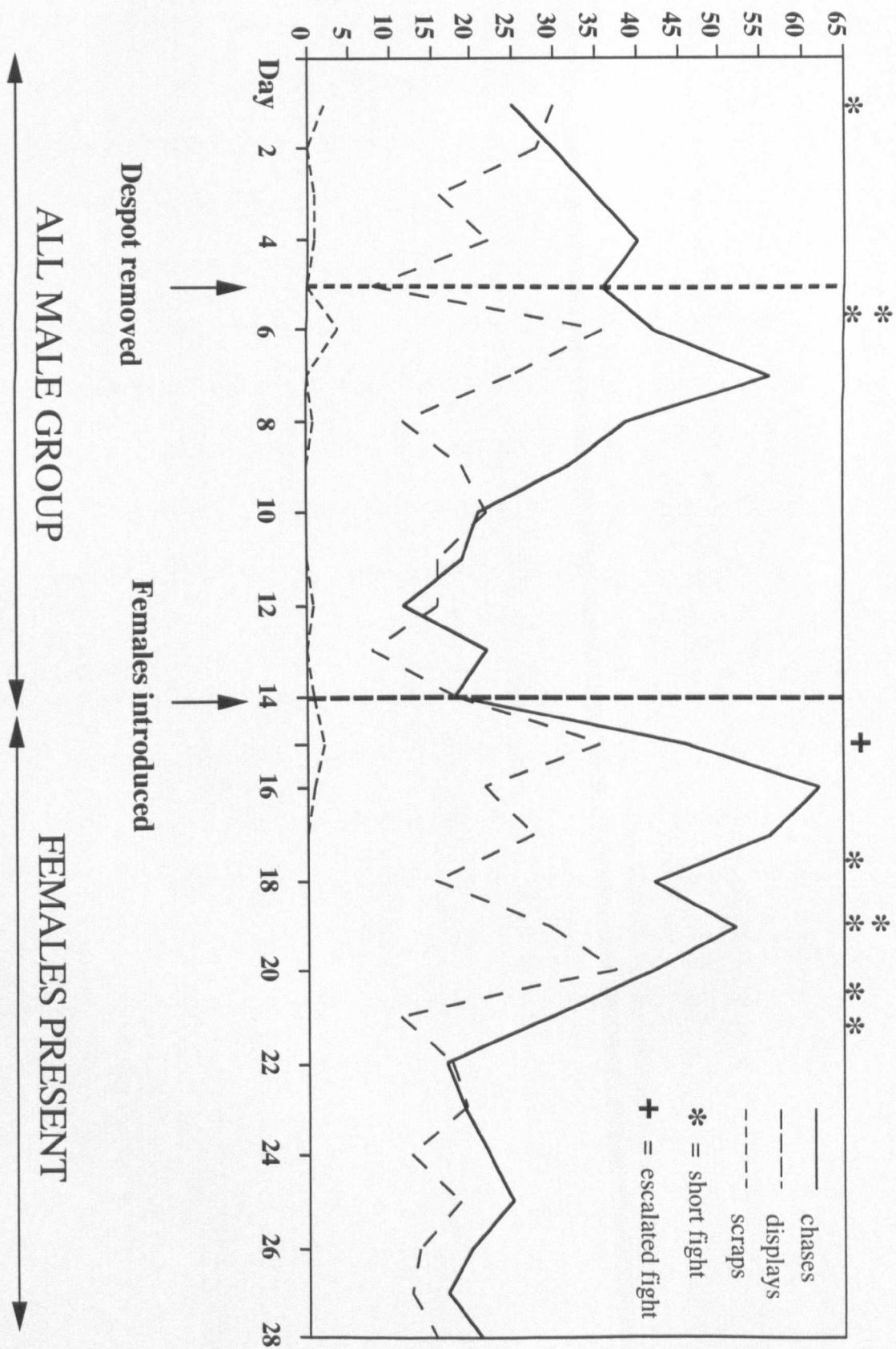
Figure 3.3.1 Frequency of aggressive behavioural acts observed over the period of study

3.3.4 Courtship behaviour and the establishment of territories

The first signs of territory establishment were not observed until five days after the introduction of the females. Most of the males showed courtship behaviours toward the females, such as quivering and digging the gravel with their mouths. The occurrence of courtship behaviours was fairly consistent from the top two ranked males, whereas for low ranked males it was only seen occasionally. First signs of territory establishment, such as the digging of a nest were by a mid ranking male (male A). However, only the two highest ranking individuals (E and D) actually started to dig nests with females, although neither pair spawned. In these cases the females also participated in digging the nest. The territorial pairs frequently left the nest site and it was not obvious if there was a distinctive boundary to the territory. The despot frequently entered the nest area of the rank 2 fish without confrontation.

3.3.5 Body weight in relation to dominance status

Although the heaviest individual became the despot within the first hours, there was no significant correlation between weight and social rank once the hierarchy had established ($R_s = 0.5$, n = 7, P = 0.25). However, by the end of the experiment there was a significant positive correlation between weight and social rank ($R_s = -0.94$, n = 6, P < 0.01). In addition, the top ranked individuals had the highest growth rates.

3.4 Discussion

3.4.1 *Social structure and aggressive behaviours*

This study revealed a number of interesting features of *T. zillii* social behaviour. In the group size I worked with and in the absence of females, males did not establish territories, although there was apparently ample time and space for them do so. The males formed a loose, wide ranging foraging group and no individuals showed any signs of site attachment or site defence. However, aggressive behaviour is clearly a major component of the behavioural repertoire of this species in these conditions. The majority of aggressive interactions were short lived chases and displays; however, eight short fights and one escalated fight were observed. In the presence of females, nest building was observed, but nest owners frequently left the nest and sometimes tolerated the presence of others within the vicinity of the nest; thus the system was not strictly territorial.

Within a few hours of being introduced into the flume, it was clear that a dominance hierarchy was being established. The dominance hierarchy at any one sampling period was linear but was not stable in the long term, particularly during periods of social change. This is similar to the 'linear but unstable social hierarchy' described by Oliveira & Almada (1996) for the mouth brooding tilapia, *Oreochromis mossambicus*.

3.4.2 *What are fights over ?*

Chases and displays occurred at fairly high levels throughout the study and usually did not result in any change in the hierarchy. By definition, chases always involved a higher ranking individual pursuing a lower ranked individual. Thus, chases and probably displays may serve to reinforce dominance relations and maintain an individual's position in the hierarchy. On the other hand, the scraps and short fights sometimes resulted in changes in the dominance hierarchy and were mainly confined to periods when the hierarchy was unstable, for example during the first few days of the experiment, in the days immediately following the removal of the despot and in the days following the introduction of the females. Therefore, the function of scraps and short fights may be in the establishment and challenging of the hierarchy.

The only escalated fight observed was a dispute between the rank 1 and rank 2 fish. Thus, escalated fighting may be reserved for disputes over the top positions in the hierarchy. There was no evidence for the assumption in Chapter 2 that escalated fights occur when territory ownership is disputed, although there was no evidence against this either. It may be the case that top rank positions are equivalent to access to a territory. The fact that there was a dramatic increase in all aggressive interactions among males following the introduction of females suggests that once the opportunity to reproduce arises, there is an added incentive to challenge the established hierarchy or to maintain a high rank.

3.4.3 Benefits of being high in the social hierarchy

The two top ranked males had almost exclusive access to the females and established nest sites. Although in *T. zillii* it is not known whether there is active female choice of males or if top males gain females by excluding other males, clearly being high in the hierarchy pays off in terms of mating opportunities. In addition, the growth rates during the experimental period were highest for the two top ranked fish, which suggests that there may be feeding benefits to be gained from top positions in the hierarchy, as has been demonstrated in this species (Koebele, 1985). Alternatively, it may be that subordinates have lower growth rates as a consequence of the social stress by imposed by dominants, as has been shown in juvenile tilapia, *Oreochromis niloticus*, (Alvarenga & Volpato, 1995) and coho salmon (Ejike & Schreck, 1980).

3.4.4 The consequences of escalated fighting

The circumstances in which the despot lost dominance are a particularly interesting piece of anecdotal evidence for the consequences of becoming involved in an escalated fight. It was immediately following the escalated fight between the despot and the second ranked fish that the despot lost in a short fight to the rank 3 fish. This suggests that the despot was exhausted due to the previous escalated fight and could not fend off the assaults from a fresh attacker. Following its defeat the erstwhile despot fell a further two places down the hierarchy and it was not for a few days that it started to climb again, but it never recovered its previous status. This gives some idea as to how costly engaging in an escalated fight can be.

3.4.5 *Implications of this study for the interpretation of laboratory results*

In this study the escalated fight was very similar in duration and degree of escalation to fights observed in laboratory aquariums. Thus, while escalated fights may be rare in natural circumstances, we do at least know that they happen and that the fights we staged in the laboratory have a natural counterpart.

It was interesting that in the flume, only the top ranked fish established territories, whereas all isolated males in the laboratory studies readily established territories. Presumably this is because in the flume, dominant individuals aggressively prevented territory establishment by the subordinates, whereas in the laboratory, this was not possible. This may explain why the laboratory fights were so escalated; each fish is playing a dominant, territory owner role and thus has much to lose in a fight. Unfortunately, it was not clear from the flume study whether only the top ranked fish were in breeding condition, as subordinates occasionally showed courtship behaviours toward females and all were sexually mature.

While it is likely that during the period of territory establishment the fish invest in the production of gonads, it is still unclear why there should be such large variation in the GSI of the fish used in the laboratory experiment and why relative GSI should predict winning. Clearly, the scale of the present study was inadequate to resolve such issues.

3.4.6 Conclusion

Under the 'semi-natural' conditions of the flume, the social structure of *T. zillii* may be described as a loose group with an unstable dominance hierarchy. The top ranked individuals had better growth rates, almost exclusive access to females and established nest sites. Nest sites were defended but had no clear boundaries that provoked attack, thus, the social system is not simply territorial. Subordinates are prevented from establishing territories by the aggressive actions of dominants. Aggression is clearly a major component of this species' time budget and it is likely that the different classes of aggressive interactions served different functions. Chases and displays appear to serve to maintain dominance relationships, whereas scraps and short fights may be reserved for challenging positions in the hierarchy. Escalated fighting was coincident with the introduction of females and was a result of a dispute over the top rank position, suggesting that it is reserved for situations where the pay-offs of winning are particularly high. As the two top ranked fish also established territories, the possibility that the escalated fight was a territorial dispute cannot be ruled out. Thus, in relation to the laboratory experiments, I have a better idea of why the fights were so escalated, even if I am no closer to resolving the issue of the role of GSI in aggressive behaviour.

CHAPTER 4

A STUDY OF MALE MORPHOLOGY IN RELATION TO FIGHTING AND

DOMINANCE

4.1 Introduction

4.1.1 *Morphometrics, trade-offs and alternative behavioural strategies*

In Chapter 2, the importance of body weight and gonadal state were discussed in

relation to the outcome of staged fights. However, it is important to consider such

variables in relation to other body traits to appreciate the functional morphology of

the animal as a whole (Motta *et al.*, 1995). There are important physiological trade-

offs within the individual between the allocation of resources to the demands of

growth, maintenance and reproduction (Stearns, 1993). Furthermore, when energetic

intake exceeds these demands, the surplus is usually stored as fat in various tissues

around the body for future use when energetic intake fails to meet metabolic demands

(Sheridan, 1994). These four end points of energy partitioning (growth, maintenance,

reproduction and storage) and their interrelations are closely coupled to the life-

history of the animal and thus central to explaining behavioural variation.

The fact that in male *T. zillii*, gonadal state and to a lesser extent body size are

important in determining dominance presents an intriguing problem. Individuals are

most likely to win fights if they are both larger and have a greater GSI than their

opponent, but quite clearly there was a wide range of outcomes, from smaller fish

with larger GSIs winning to larger fish with smaller GSIs doing so. It has been shown in other teleosts, (for example, bluehead wrasse, Warner, 1984), that there is a trade off between growth rate and reproductive activity, which can lead to alternative life history strategies. The bluehead wrasse is an extreme case in which some males are small, invest heavily in gonad production and spawn in groups, while others are larger, defend individual spawning territories and have much smaller testes (Warner & Schulz, 1992). A less extreme case of alternative strategies is described for the cichlid fish *Haplochromis burtoni* (Fernald & Hirata, 1977); some males are territorial and have large gonads, whereas others have small gonads, live in loose groups and await the opportunity to acquire a territory.

Distinct strategies of a similar nature to those shown by *H. burtoni* may also occur in *T. zillii*, which might explain why fish with large gonads fight harder to defend their territory. Alternatively, if the social system is more complex than simply territorial (as is suggested from the findings in Chapter 3), then different combinations of body size and gonadal state may underlie a continuum of behavioural strategies in relation to the social hierarchy as well as territoriality. In either case, it is of interest to explore the relationships between variables such as gonad state, body size and body condition, since these may suggest possible trade-offs which may be interpreted as either consequences or causes of variation in behavioural strategies. Multivariate morphometric analysis is an appropriate technique to use in such investigations.

4.1.2 *Morphometrics, internal physiological dimensions and assessment during fighting*

In a similar vein, but from a different perspective, morphometric analysis can be used to identify different physiological 'dimensions' within the animal. In addition to traits such as weight and length that are clearly related to overall body size, there may be external traits that relate to, and so give information on, nutritional condition, maturity or stage of reproduction. For example, morphological traits have been used to estimate gonad state/stage of reproductive cycle in an cyprinodontid fish (Garcia-Berthou & Mereno-Amich, 1993) and fat condition in Atlantic salmon (Simpson *et al.*, 1992). When individuals fight over resources, asymmetries in such dimensions are often important in the outcome of the fight (see Chapter 1), so it is possible that assessment strategies are directed at acquiring this sort of information. This is likely to be the case in *T. zillii* males, since gonadal state is so important in the outcome of contests (see Chapter 2). Different assessment behaviours often involve emphasis on specific parts of the body, for example, mouth wrestling in cichlids and opercular flaring in Siamese fighting fish (Ma, 1995). Understanding how external morphology relates to internal physiological dimensions may, therefore, shed light on whether certain postures and stances maximise the transmission of such information and, therefore, why certain behaviours are used during fighting and assessment.

4.1.3 *Aims of the study*

The two main aims of this chapter were, first, to investigate the relationships between variation in several internal and external body traits and second, to see whether internal physiological dimensions could be assessed from external morphometrics.

Principle component analysis (PCA) followed by multiple regression was used to explore multivariate relationships among body dimensions.

4.2 Materials and methods

4.2.1 *Morphometrics and internal organs*

The data presented in this chapter were collected from the same fish that provided the data for Chapter 2. From a total of 59 fish, a number of body measurements were taken, using callipers, at the time that the fish were dissected and weighed. In total, nine morphometrics were taken; weight (to the nearest 1/10th gram), length, width at widest point, depth at deepest point of body (see Fig 4.2.1b), mouth width (see Fig 4.2.1a) and genital papilla length (all to the nearest millimetre). In addition, to the removal of the testis, the liver and visceral fat surrounding the intestine were dissected out (see Figure 4.2.2) and weighed to the nearest milligram.

Prior to analysis, the nine variables were log transformed to standardise measures and normalise the data. The PCA with varimax normalised rotation was then performed on the correlation matrix.
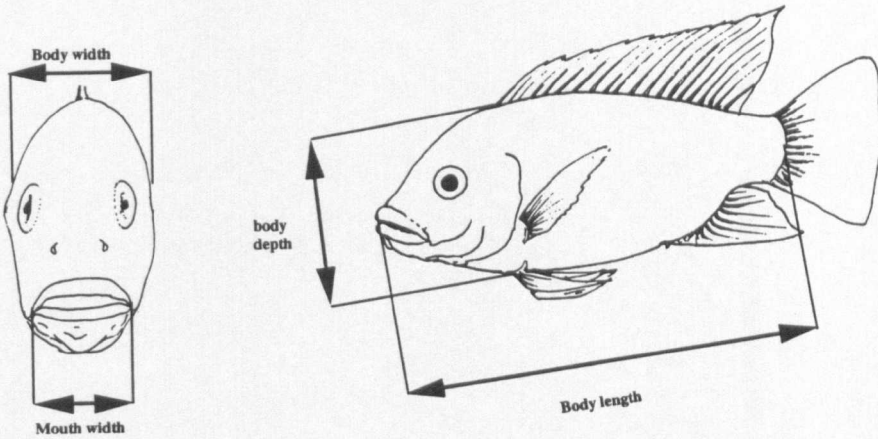
Figure 4.2.1 a and **4.2.1b.** Diagram showing morphometrics taken.
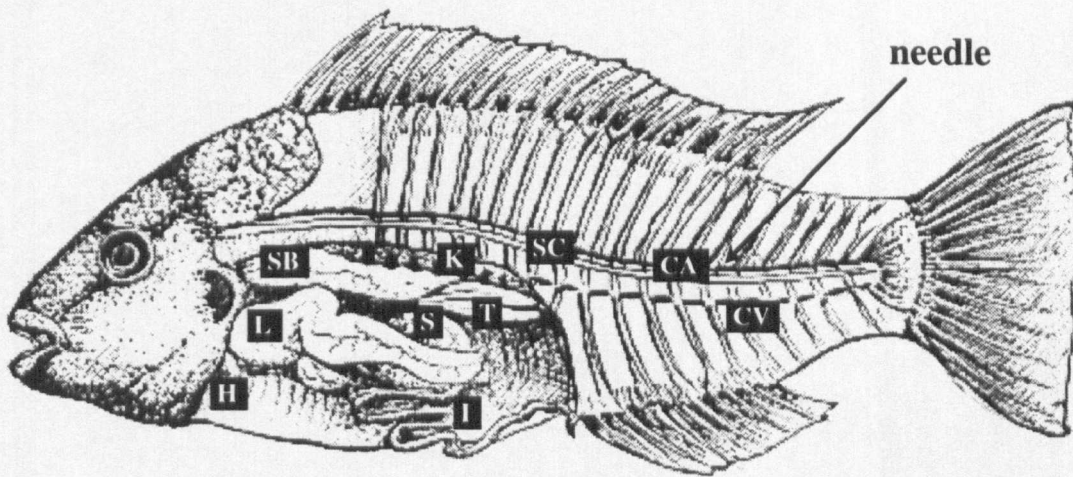
Figure 4.2.2 A male *T. zillii* dissected to show internal organs (SB = swim bladder, L = liver, K = kidney, T = testis, H = heart, S = stomach, I = intestine, SC = spinal; cord). Also shown are blood vessels (CV = caudal vein, CA = caudal artery) and the point from which blood was withdrawn (see Chapter 6).

4.2.2 *Multivariate statistical analysis*

Principle component analysis (PCA) is a data reduction technique that describes major trends in a correlation matrix of the original data set (Manley, 1990). For instance, variables that are highly correlated, such as body weight and length, can be reduced to one component that measures overall size. The first principle component (PC1) to be extracted accounts for the greatest amount of variance in the data. The second principle component (PC2) is uncorrelated to the first and accounts for the greatest amount of variance remaining after the extraction of PC1. Mathematically, the new axes (components) are identified by eigenvectors of the correlation matrix and the variance of the scores of the original variables accounted for by each component is given by the eigenvalue of the matrix. Thus, the eigenvector associated with the largest eigenvalue identifies the largest component, that is to say, PC1. To obtain a clearer discrimination between components, the axes can be rotated to maximise the variances of the component loadings across variables for each component, so that a variable will either load highly or not at all. In theory, components will continue to be extracted until all the variance in the data has been accounted for, so there will be as many components as there were original variables. In practice, as the aim is to reduce the data, a criterion for assigning significance to components is needed. There are no rules for this, although, it is suggested that significance should only be assigned to components that have eigenvalues greater than average, that is to say, if there are 10 original variables, a significant component should account for more than 10 % of the variance. However, biological intuition is justified in interpreting the significance of a component, as PCA is merely an exploratory technique rather than a means of testing hypotheses.

Multiple regression is a technique used to test specific hypotheses, in this case, those relationships exposed by the PCA. Multiple regression enables predictive statements to be made about n number of related variables.

4.3 Results

4.3.1 *Correlations between all morphometrics*

The correlation matrix between all variables is shown in Table 4.3.1. The correlation matrix gives a feel for the relative importance and direction of relationships in the data set. The most obvious are the group of significant correlations between the different measures of body size. However, less obvious but significant correlations were found between the internal variables and external morphometrics; for example, mouth width was positively correlated with testes weight.

Table 4.3.1 Correlation matrix of morphometrics (bold coefficients = $P < 0.05$)

variable	weight	length	depth	width	mouth	genital	liver	testis	fat
weight	-	**.96**	**.96**	**.89**	**.65**	.27	**.5**	-.14	**.3**
length	**.96**	-	**.91**	**.83**	**.62**	.25	**.47**	-.15	**.28**
depth	**.96**	**.91**	-	**.88**	**.69**	.27	**.5**	-.09	.22
width	**.89**	**.83**	**.88**	-	**.76**	.23	**.46**	-.05	.13
mouth	**.65**	**.62**	**.69**	**.76**	-	.37	**.33**	**.38**	-.23
genital	.27	.25	.27	.23	.37	-	**.35**	**.26**	.04
liver	**.5**	.47	.5	**.46**	**.33**	**.35**	-	.14	**.51**
testis	-.14	-.15	-.09	-.05	**.38**	**.26**	.14	-	-.19
fat	**.30**	**.28**	.22	.13	-.23	.04	**.51**	-.19	-

4.3.2 *Principle component analysis on all morphometrics*

Table 4.3.2 shows the results of the PCA. Following Oliviera & Almada (1995), a cut off eigenvalue of 0.6 was chosen for assigning significance to a component. Four principle components were extracted, which together accounted for 92 % of the variance in the original data. Weight, length, depth, width, and to a lesser extent mouth width loaded heavily and positively on PC 1. Testis weight was the only variable that loaded heavily and positively on PC 2. Both liver weight and fat weight loaded heavily and positively on PC 3 . Only genital papilla length loaded heavily and positively on PC 4, which accounted for only a relatively small percentage of the variance. Thus, other than body size (PC 1), the analysis suggested at least two internal physiological dimensions in the data; PC 2 was strongly associated with testes weight and PC 3 was strongly associated with liver and fat weight. Interestingly, the variable loadings for PC2 and PC3 are inversely related to each other, as can be seen in Figure 4.3.1.

Table 4.3.2 Principle component loadings (loadings > 0.6 are denoted in bold)

PC	1	2	3	4
Eigenvalue	4.76	1.62	1.26	.67
variance	52.92 %	17.96 %	14.01 %	7.43 %
weight	**.947**	-.102	.234	.107
length	**.920**	-.118	.214	.101
depth	**.946**	-.034	.182	.106
width	**.944**	.048	.086	.052
mouth	**.790**	.473	-.210	.199
genital	.165	.149	.081	**.969**
liver	.384	.261	**.739**	.224
testis	-.09	**.957**	-.027	.129
fat	.05	-.207	**.923**	-.031
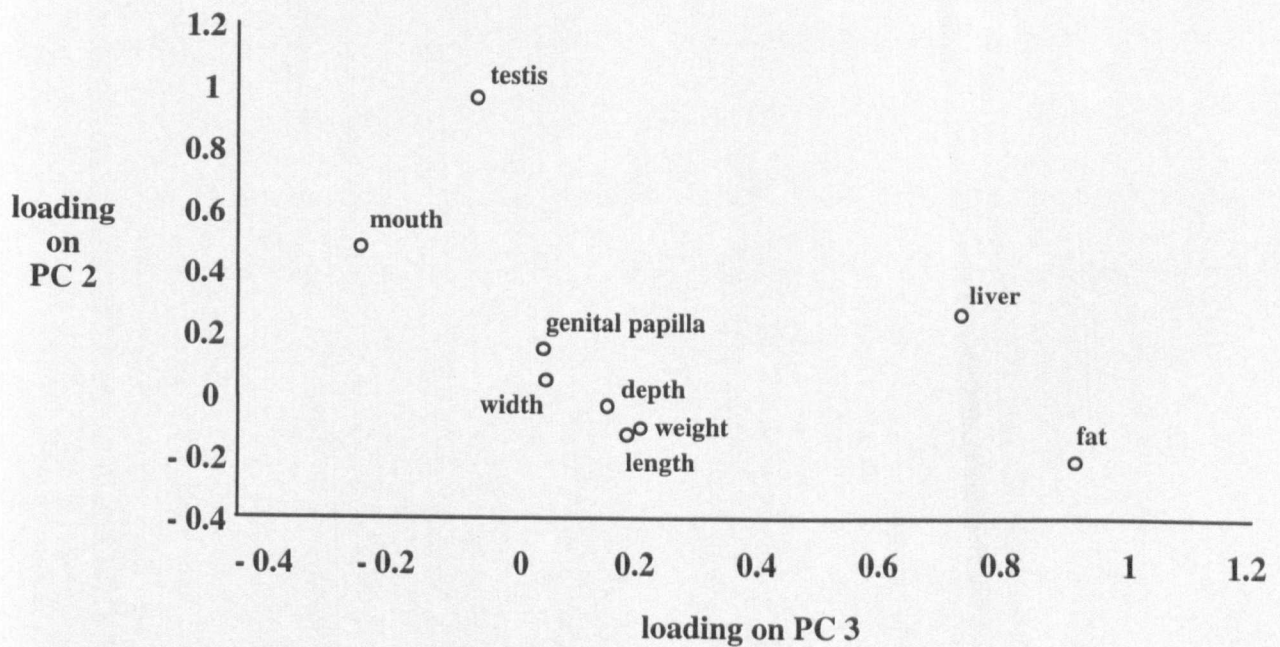
Figure 4.3.1 Plot of principle component loadings of PC 1 and PC 2.

4.3.3 External morphometric predictors of internal variables and genital papilla

Multiple regression was used to test whether any of the external morphometrics could predict the independent physiological dimensions of interest identified by the PCA, namely, the 'gonadal state' dimension (PC 2) and the 'liver and fat' dimension (PC 3). The way in which this was done was to regress the original external morphometric variables either on the single original internal variables or on specified combinations of the original variables suggested by the PCA.

To see if it was possible to predict GSI, a step-wise multiple regression was performed using all external variables as independents and GSI as the dependent variable. The analysis revealed that mouth width and body length and width were significant predictors of GSI that combined to explain 49 % of the variance (see Table 4.3.3). The relationship between mouth width and body width and length were in opposite directions; GSI increased with mouth width but decreased with body length and width. Thus, small fish with large mouths are likely to have a large GSI. As length and width are roughly equivalent to weight, I calculated an index of relative mouth size as mouth width/body weight. This new index was a good predictor of GSI, accounting for almost as much, in terms of per cent variation, as the three variables originally used in the step-wise regression (see Table 4.3.1).

Table 4.3.3 Results of step-wise multiple regressions of GSI, liver weight / body weight (HSI), fat weight / body weight (FSI), fat weight + liver weight / body weight (FHI) on external morphometric variables

GSI $\quad F_{(3, 55)} = 19.38$, Adj. $R^2 = 0.49$, $P < 0.001$

	Beta	R^2 change	P
Body length	-.59	.14	.003
Mouth width	.90	.33	.000
Body width	-.43	.03	.043

GSI $\quad F_{(1, 57)} = 50.9$, Adj. $R^2 = 0.46$, $P < 0.001$

	Beta	R^2 change	P
Mouth/weight	.69	.46	.000

HSI $\quad F_{(1, 57)} = 5.63$, Adj. $R^2 = 0.07$, $P < 0.02$

	Beta	R^2 change	P
Body length	-.30	.07	.02

FSI $\quad F_{(3,55)} = 16.69$, Adj. $R^2 = 0.48$, $P < 0.001$

	Beta	R^2 change	P
Weight	1.27	.12	.06
Mouth width	-.50	.28	.000
Body width	-.67	.06	.01

FHI $\quad F_{(2,56)} = 4.97$, Adj. $R^2 = 0.12$, $P < 0.01$

	Beta	R^2 change	P
Mouth width	-.51	.06	.000
Weight	.38	.08	.02

genital papilla $\quad F_{(1,57)} = 9.21$, Adj. $R^2 = 0.12$, $P < 0.01$

	Beta	R^2 change	P
Mouth width	0.37	0.14	0.004

Separate step-wise regressions were performed for relative fat levels (fat weight/body weight X 100) and relative liver weight (liver weight/body weight X 100) on all external variables. This revealed that weight, mouth width and body width were significant predictors of the relative fat levels that combined to explain 48 % of the variance (see Table 4.3.3). The relationship between mouth width and body weight and width were again in opposite directions, but in contrast to the GSI result, fat decreased with mouth width and increased with weight. Only length was a significant predictor of relative liver weight which only explained 7 % of the variance (see Table 4.3.3). As the PCA suggested that fat levels and liver weight are correlated, once size is allowed for, I calculated a combination index for both fat and liver weight (fat weight + liver weight / body weight) and performed a further step wise multiple regression. Mouth width and body weight were significant predictors and again in opposite directions (see Table 4.3.3).

Step-wise multiple regression revealed that only mouth width was a significant predictor of genital papilla length (see Table 4.3.3). Genital papilla length increased with mouth width.

4.4 Discussion

4.4.1 *Relationships between internal body traits*

Once the variation in overall body size (PC 1) had been accounted for, the PCA identified some interesting relationships suggestive of internal physiological dimensions. Principle component 2 was clearly accounting for variation in testis weight, while PC 3 was accounting for variance that came from the size of the liver and the amount of visceral fat. As testis weight is ubiquitously correlated with reproduction, and both the liver and visceral fat are stores of energy in teleosts (Sherman, 1994), it is intuitive that these represent a dimension of reproductive state and nutritional/energy reserve state, respectively.

It is interesting that these two physiological dimensions appear to be negatively correlated; fish with large gonads tend to have lower fat reserves and smaller livers. One interpretation of this association is that there is a physiological trade-off between the production of gonad and the maintenance of carbohydrate energy reserves. This was not a reflection of differences between mature and immature fish (as is sometimes found, for example in salmonids, Rowe & Thorpe, 1990), because all the fish used in this study were sexually mature and from one cohort (immature fish have virtually no testes). Thus, the trade off would be between how much to invest in gonad once the decision to mature has been taken. While there are many studies that demonstrate a decline of energy reserves associated with egg production, (see review by Wooton, 1990), there are only a handful of studies that have addressed the cost of sperm production. Although individual sperm are tiny, there is increasing evidence

for teleosts suggesting that the volume of sperm required for successful fertilisation is often limiting, especially if fertilisation is external and in an aquatic environment or if sperm competition with other males is prevalent (Nakatsuru & Kramer, 1982; Shapiro & Giraldeau, 1996). The energetic cost of sperm production has received some attention; Diana & Mackay (1979) suggest that the energy reserves of the liver are utilised during the development of testes in Northern pike. Similarly, Chellapa, Huntingford & Strang have evidence to suggest that the development of testes in sticklebacks imposes a significant drain on somatic energy reserves in male three spine sticklebacks, prior to any behavioural consequences of maturation (pers. comm.).

Alternatively, the negative association between large gonads and low energy reserves may be a consequence of the behavioural changes associated with the development of gonad, rather than the production of gonad *per se*. This could work in two ways; either dominant fish have to expend extra energy asserting their dominance through costly displays and fights, whereas subordinate individuals tend to avoid these confrontations and store surplus energy as fat. The other explanation is that there are costs of storing fat and that these depend upon the social status of the individual (Witter & Cuthill, 1993). In European starlings, Witter & Swaddle (1996) have demonstrated that dominant individuals can afford not to store fat as they are likely always to secure enough food, whereas subordinates cannot afford not to store fat as their food supply is unpredictable and they need fat reserves as insurance against failing to meet intake demands.

Unfortunately, the present study was unable to establish whether this negative association between gonad weight and energy reserves is a direct consequence of the production of sperm or whether it results from the consequences adopting 'large gonad' behavioural strategies. However, between the cost of producing a large volume of sperm and the possible consequences for energy partitioning in relation to social dominance, there is likely to be an explanation for the finding that male *T. zillii* with large gonads have low energy reserves.

4.4.2 *Predicting internal traits from external morphometrics*

Body weight and mouth width consistently predicted both the gonadal dimension and the energy reserve dimension. GSI increased with mouth width but decreased with body weight; individuals with a large mouth relative to their body size are likely to have a large GSI. The converse was true for relative fat and liver weight, which decreased with mouth width and increased with body weight. Oliveira & Almada (1995) demonstrated that mouth morphology was sexually dimorphic in the tilapia, *Oreochromis mossambicus*. Thus, it is likely that mouth morphology in *T. zillii* is also a secondary sexual characteristic.

That it was possible to predict GSI is interesting in relation to the findings reported in Chapter 2. On the basis of difference in body size and GSI, it was possible to accurately predict the outcome of a fight. Given that the ratio of mouth width to body weight is an accurate predictor of GSI and that mouth width also predicts body weight, the fish can in theory use their mouths to assess the two variables that will enable them to make a decision about their chances of winning the fight. It cannot be

a coincidence that the majority of behavioural interactions during fighting in this species involve the mouth. Jaw morphology is likely to undergo changes concurrently with gonadal maturation, however, what remains a puzzle is how this finding can be reconciled with the suggestion in Chapter 2 that GSI varies cyclically, increasing as the fish get closer to spawning. It may be that mouth morphology changes with GSI in a similar way that the jaw and head morphology of male Atlantic and Pacific salmon changes rapidly as the fish get closer to spawning (Tchernavin, 1938). If this was the case then mouth morphology should regress (as it does in salmon) during non-reproductive periods (when the GSI is small). Clearly, the present study could not resolve this issue; however, it should be possible to do so if a means of non-destructively measuring GSI could be found. Ultrasound has been used by fish biologists to measure gonad weight (Mattson, 1991) and may be a good means of doing this.

4.4.3 Conclusion

This study has shown that variation in GSI and body size have consequences for the physiology of the animal in relation to energy partitioning. The causal and functional reasons for the relationship between body reserves, gonadal state and dominance could not be demonstrated in the present work, but the information provided by this study has set the stage for further work that could experimentally tease apart these effects. In addition, this study demonstrates that the morphology of the mouth in combination with body size can be used to predict GSI. This suggests that it is a male secondary sexual characteristic and thus, may explain why so much of the fighting behaviour of this species involves the mouth.

CHAPTER 5

PROXIMATE COSTS OF ESCALATED FIGHTING: ENERGY

METABOLISM AND INJURIES

5.1 Introduction

5.1.1 *Proximate causes of strategic decision-making*

In Chapter 2, the fighting strategies of male *T. zillii* were discussed within the frame-
work of game theory. Game theory approaches to animal aggression examine the
functional significance of different patterns of fighting through an analysis of their
consequences for the reproductive success of the individual (Maynard Smith, 1982).
The positive consequences, or 'benefits' of fighting, include gaining access to or
defending limited resources, and the negative consequences, or 'costs' of fighting,
may include the increased risk of predation and the time and energy expended
(Huntingford & Turner, 1987). Whatever behaviour this approach identifies as
optimal must be realised by a proximate mechanism that ensures the appropriate
behavioural option is executed in any given set of circumstances. These behavioural
mechanisms are little understood and are the focus of this chapter.

An important concept in this chapter is the behavioural decision rule and the idea of
causal factors (McFarland & Houston, 1981). As was discussed in Chapter 1, causal
factors are variables resulting from the animal's perception of the external
environment and variables relevant to the animal's internal environment. These

combine to form a motivational state variable, which provides the animal with information relevant for making functional decisions. Decision rules are specific responses to a given level of a motivational state variable. Elwood & Neil (1992) extended this approach to the context of fighting where the immediate cost associated with fighting is the relevant motivational state variable for making decisions on whether the contestant should continue, escalate or flee from the fight. Here I build upon Elwood & Neil's ideas in an attempt to uncover the mechanisms underlying the making of strategic decisions during fighting in *T. zillii*.

The causal factors underlying the proximate cost of fighting may be defined as all those variables that have detrimental consequences for the individual. Perhaps the most obvious causal factor of the cost of fighting is physical injury; however, there have been few attempts to quantify its effects. In addition to injury, further adverse consequences associated with fighting relate to energy metabolism. It has been shown in fish (Alvarenga & Volpato, 1995; Metcalfe *et al.*, 1995) and reptiles (Wilson *et al.*, 1990) that variation in aggressive behaviour and social rank of individuals are related to metabolic rate. More specifically, there is evidence that the metabolic consequences of fighting, such as energy depletion and the accumulation of metabolites such as lactate, represent a significant cost to the animal concerned (Haller, 1995). Clearly, the role of energy metabolism is an important component in the control mechanisms of animal aggression.

As described in Chapter 2, fights between male *T. zillii* in this study were often extraordinarily long and fierce and injuries occurred as a result of biting, most often

in the form of scale loss. Scales take time to regenerate and if the numbers lost are great, the damaged skin can become infected with fungus (personal observation). Escalated fighting appears to be energetically costly, and in the longer fights the fish are often observed to pause and hyperventilate for periods during and after fighting. For these reasons, therefore, *T. zillii* was thought to be a good species to investigate the role of physical damage and energy metabolism in the choice of behaviour during fighting.

5.1.2 *Carbohydrate metabolism and the muscle system of fish*

Fish, in general, are well suited to studies of energy metabolism because of their relatively simple muscular system. The mytomal musculature consists of two functionally and anatomically distinct muscle types (Bone, *et al.*, 1995). The red muscle system, or the *m. lateralis superficialis*, is a superficial layer of muscle comprising an estimated ten per cent of the myotomal block in *T. zillii*. It is found beneath the skin and particularly along the flanks beneath the lateral line. It is aerobic and used for low intensity, sustainable swimming. The white muscle system, or *m lateralis profundus*, comprises the bulk of the mytomal block. It is anaerobic and used for high intensity activities such as burst swimming. Escalated fighting is likely to involve predominately the white muscle system.

Muscle glycogen reserves are the primary source of catabolisable carbohydrate for immediate activity requirements. The other main store of glycogen is the liver but there is conflicting evidence as to whether these reserves can be mobilised fast enough to meet immediate activity requirements (Haller, 1991). Use of the white

muscle system results in anaerobic glycolysis and the production of lactate ions and protons (Johnstone & Goldspink, 1973). Although anaerobic glycolysis can fuel rapid and powerful responses, it is not sustainable because muscle glycogen reserves are rapidly depleted and it can take hours to convert lactate back into glycogen (Gleeson, 1996). In addition, anaerobic respiration is associated with a decrease in blood pH that disrupts fluid and electrolyte balance (Wood, 1991). Thus, anaerobic activity quickly depletes energy reserves and is associated with considerable detrimental effects on the physiological state of the animal. For these reasons, lactate is likely to be a good index of metabolic cost where activity is anaerobic and has been used by physiological ecologists to estimate the energetic consequences of behaviours such as territorial defence in lizards (Pough & Andrews, 1985).

5.1.3 *Aims of experiment*

The aim of the experiment was to investigate the possible proximate costs of fighting in male *T. zillii*, with reference to physical injury and metabolic changes. I measured three metabolic parameters: the concentrations of total sugars and lactate in the white muscle, and the concentration of total sugars in the liver. Injury was scored as the number of scales lost by the end of the fight. These variables are related to the behavioural content, duration and outcome of the fight.

5.2 Materials and Methods

5.2.1 *Staging fights and sampling procedure*

Fights were staged as described in Chapter 2, but immediately after dominance was established, the fish were electro-stunned with 180 volts of AC current for 10 seconds delivered from a specially constructed electro-anaesthetiser (L. Ross, University of Stirling, pers. comm.). Control fish were treated indentically but stunned as soon as the partition was raised (before they had time to fight). The electro-anaesthetiser rapidly immobilises the fish and induces anaesthesia within 5 - 10 seconds. The anaesthetised fish were then immersed in liquid nitrogen (-196°C). This is a very fast means of killing fish and one that rapidly arrests metabolic responses. The fish were then stored at - 70°C. The fish were then partially thawed and samples of white and red muscle were removed from an area below the dorsal fin along the lateral line. The liver was also removed and then all samples were stored at -70°C until the experiment was complete at which point they were freeze dried for 24 hours.

5.2.2 *Quantifying behaviour and injury*

The criteria for defining the duration of escalated fighting, the outcome of the fight and the weight difference and behavioural measures are as described in Chapter 2. *T. zillii* has large scales and it is relatively easy to score the number of scales lost as a result of being bitten. For analysis I used the following as a measure of relative scale loss; number of scales lost by the loser - number lost by the winner. To this value was added 10 (to make all numbers positive).

5.2.3 *Biochemical analyses*

All reagents were supplied by the Sigma chemical company (Poole, Dorset). Tissue samples were prepared for analysis using the perchloric acid (PCA) extraction method (Gäde *et al.*, 1978). This involved adding 150 mg of powdered sample to 500 μl of chilled 0.3M PCA in an eppendorf. This was then mixed thoroughly with an ultrasonic cell disrupter. Following centrifuging for 10 minutes at 13,000 rpm, the supernatent was decanted off and stored on ice. A further 500 μl of PCA was added to the pellet, ultrasonicated again and centrifuged as before. The resultant supernatent was added to the volume obtained previously. This was then neutralised by adding 2M potassium bicarbonate. Again the solution was centrifuged as before and the supernatent decanted. This was then the working sample which was stored at - 20 °C until used.

L-lactate concentrations were determined by an enzymatic assay based on Gutmann & Wahlefeld (1974). The lactate is oxidised to pyruvate in a reaction catalysed by lactic dehydrogenase.. This involved adding the 50 μl of the sample solution to 1000 μl of glycine-hydrazine buffer, 50 μl of 40mM NAD + and 5 μl lactic dehydrogenase. The mixture was vortexed and incubated in a water bath at 37°C for 2 hours. The concentration of pyruvate was determined spectrophotometerically at 340 nm and calibrated using L-lactate standards.

Total hexose and pentose sugars were determined by the anthrone method of Carrol *et al.* (1956). This involves acid hydrolysis of glycosidic bonds to yield glucosyl units; this is dehydrated to furfural, which in turn, reacts with anthrone. The concentration

of glycosyl units was determined spectrophotometerically at 620 nm and calibrated by using D-glucose standards. All samples were processed simultaneously for each assay. White muscle lactate and total hexose and pentose sugars concentrations were determined for all pairs of fish and 16 control fish, but total hexose and pentose concentrations for the liver were only be determined for 18 fought pairs and 10 controls. Due to difficulties of removing the thin layer of red muscle from the white muscle, red muscle lactate and total hexose and pentose sugars concentrations were only determined for 11 fought pairs and six controls. Metabolite concentrations of muscle were expressed in μmoles g^{-1}dry tissue.

5.3 Results

5.3.1 *Biting and scale loss in relation to weight asymmetry, fight duration and outcome.*

From Chapter 2 I already knew that in terms of the total number of bites inflicted, winners and losers did not differ significantly and that there was a negative correlation between the proportion of bites inflicted by the winner and the difference in weight. In terms of injuries, losers lost significantly more scales by the end of the fight (median number of scales lost were 4 and 7 , for winners and losers, respectively -Wilcoxon's signed ranks matched pairs: $z = 2.06$, $P = 0.03$, $n = 23$). There was also a negative trend between difference in scale loss and the difference the difference in body size (see Figure 1, $R_s = - 0.37$, $n = 23$, $P = 0.08$) suggesting that smaller winners inflict the greatest injury. Thus it appears that winners were more effective at inflicting damage on their opponent, especially if when smaller than their opponent.

5.3.2 *Metabolic consequences: glucose depletion*

Inspection of Table 5.3.1 shows that control fish had significantly higher concentrations of white muscle total sugars than either winners or losers (t - test: $t = -2.81$, d.f. = 33, $P < 0.01$ and $t = -3.49$, d.f. = 32, $P < 0.01$, respectively for winners and losers). Thus, participation in a fight does deplete white muscle energy reserves, although winners and losers did not differ significantly in this respect (paired t - test: $t = -1.15$, n = 28, $P = 0.26$). There was a significant negative correlation between the concentration of total sugars in the white muscle and the duration of escalated fighting for winners, but this trend was not significant for losers (see Figure 5.3.1).

Table 5.3.1 Tissue metabolites in winners, losers and unfought controls. All metabolites are expressed as the mean (± standard error) in μm per g $^{-1}$ of dry tissue.

Tissue	Metabolite	Winners	Losers	Controls
white muscle	sugars	4.24 ± 1.76 (n = 29)	3.94 ± 1.98 (n = 29)	5.5 ± 1.72 (n = 16)
	lactate	21.36 ± 6.12 (n = 29)	23.14 ± 6.23 (n = 29)	17.96 ± 6.24 (n = 16)
red muscle	sugars	6.15 ± 0.47 (n = 11)	7.84 ± 0.85 (n = 10)	12.01 ± 1.93 (n = 6)
	lactate	19.53 ± 1.61 (n = 11)	20.57 ±1.45 (n = 10)	14.86 ± 1.85 (n = 6)
liver	sugars	34.96 ± 11.41 (n = 18)	27.8 ± 11.43 (n = 18)	37.47 ± 8.94 (n = 10)
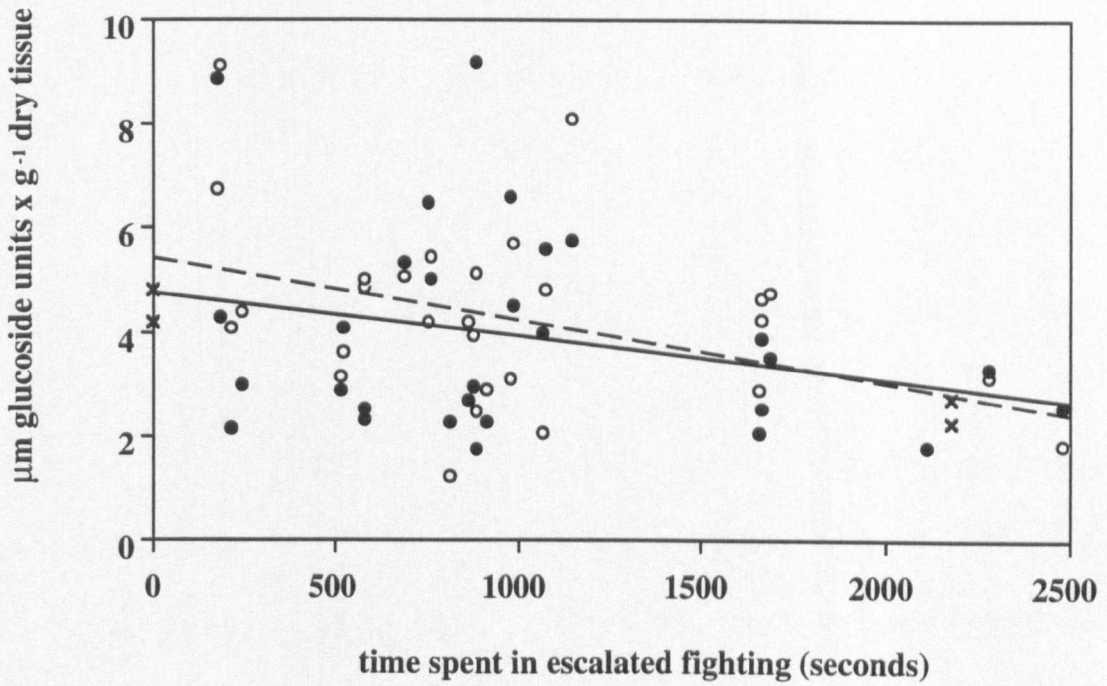
Figure 5.3.1 Concentration of glucoside units in the white muscle (expressed as μm per gram of dry tissue) as a function of time spent in escalated fighting. Winners are represented as unfilled circles (correlation = - 0.43, P < 0.05 - dashed line), losers as filled circles (correlation = - 0.26, P > 0.05 - solid line). Unresolved fights are represented as crosses (one that never escalated i.e had zero time, and one where both contestants gave up).

Table 5.3.1 shows that for the red muscle, total sugars concentrations were approximately twice those found in the white muscle (paired t-test: t = 8.56, n = 21, P < 0.001). Control fish had higher concentrations of total sugars in the red muscle than either winners or losers (t - test: t = - 3.82 , d.f = 15, P < 0.01; t = - 2.26, d.f = 14, P < 0.05, respectively for winners and losers). Although winners tended to have lower total sugars concentrations than losers, this difference was not significant (paired t - test: t = -2.14, n = 10, P = 0.06). Thus, the red muscle was affected in a similar way to the white muscle, though absolute concentrations of total sugars were higher in the first place.

For the liver, Table 5.3.1 shows that controls had significantly higher concentrations of total sugars than losers (t-test: t = - 2.31, d.f. = 26, P < 0.05) but not winners (t - test: t = - 0.6, d.f. = 26, P = 0.55). There was a trend for winners to have higher concentrations than losers, but this was not significant (paired t - test: t = 1.78, n = 18, P = 0.09). No correlations were detected between liver total sugars and the duration of escalated fighting in winners (r = -.06, P > 0.05) or losers (r = 0.22, P > .05).

Thus it appears that during escalated fighting glycogen and sugar reserves are depleted from the muscle equally for both contestants but liver reserves are only depleted to a significant extent in losers.

5.3.3 *Metabolic consequences: L-lactate accumulation*

For the white muscle Table 5.3.1 shows that control fish had significantly lower concentrations of lactate than winners and losers (t - test between winners and controls: t = -2.42, d.f. = 43, P < 0.05 and between losers and controls: t = 4.16, d.f. = 43, P < 0.001) and that losers had significantly higher concentrations of lactate than winners (paired t - test: t = -3.06, n = 29, P < 0.01). Lactate levels increased significantly with fight duration for both winners and losers (see Figure 5.3.2).

Figure 5.3.2 Concentration of lactate in the white muscle (expressed as μm of lactate per gram of dry tissue) as a function of time spent in escalated fighting. Winners are represented as unfilled circles (correlation = 0.38, P < 0.05 - dashed line), losers as filled circles (correlation = 0.38, P < 0.05 - solid line). Unresolved fights are represented as crosses (one that never escalated i.e had zero time, and one where both contestants gave up).
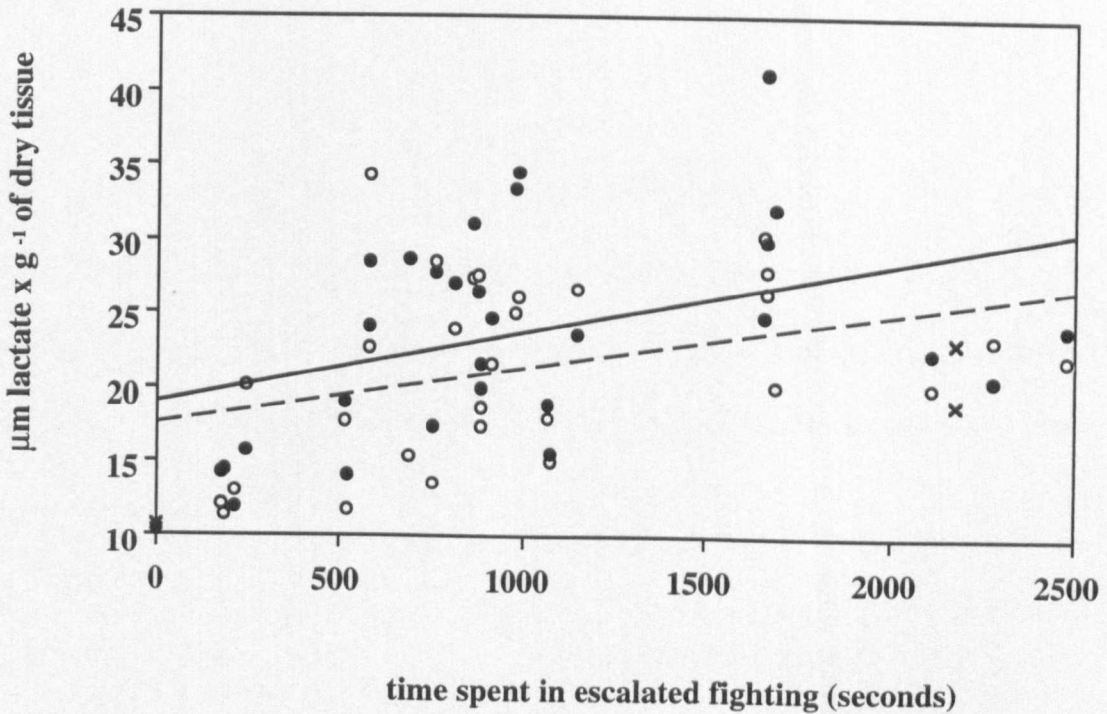
A similar pattern was found in the red muscle (Table 5.3.1), with controls having significantly lower lactate concentrations than losers (t - test: t = - 2.46, P < 0.05) but not winners (t - test: t = - 1.8, P = 0.09). Winners and losers did not differ significantly (paired t-test: t = -2.14, n = 10, p = 0.06) and there were no correlations between the concentration of lactate in the red muscle and the duration of escalated fighting. Furthermore, there was no significant difference between the lactate concentrations in the white and red muscle (paired t - test: t = - 19, n = 20, p = 0.8).

5.4 Discussion

5.4.1 *Proximate costs of fighting*

The present study is consistent with the idea that fighting incurs significant metabolic costs (Haller, 1995), as well as physical injury and that these costs increase with fight duration and can differ between winners and losers. Such costs need to be quantified in order to test the hypothesis that strategic decisions are based on estimation of these proximate levels.

5.4.2 *Injuries during fighting in relation to winning and losing*

It is difficult to measure the cost of external injury directly, because there are many aspects of skin damage; for example, inflammation, infection, ionoregulatory disruption and localised cell death. However, measuring scale loss at the end of a fight may give a crude idea. In addition, measuring the number of bites inflicted may give an idea of aggression or 'intent' to injure. Biting and scale loss were evident in

all fights, but most severe in those fights won by the smaller contestant. Winners appeared to be able to bite more effectively in terms of removing scales, especially if they were smaller. This suggests that winners are inflicting greater costs on their opponents than they are receiving themselves and that smaller winners inflict the greatest relative cost.

5.4.3 *Differences between the red and white muscle system*

In general, the effects of fighting on the metabolic state of the white muscle system were greater than for the red muscle. In the red muscle, there was a lower accumulation of lactate and the concentrations of sugars was absolutely higher. This is not surprising given that the red muscle is well supplied with blood for aerobic respiration. These results are congruent with the idea that the white muscle system is utilised during intensive activity and the red muscle during sustainable swimming (Goldspink & Johnstone, 1973). For this reason, further discussion is based upon the results of the white muscle analysis.

5.4.4 *The effect of escalated fighting on metabolic parameters*

Significant metabolic consequences of escalated fighting occurred in the white muscle and may be attributed to the anaerobic respiration of the glycogen and sugar reserves. The total sugar concentrations in fought fish were approximately two thirds of those of control fish, suggesting that fighting does deplete local energy reserves. However, there was a surprising amount of scatter in the data which may reflect considerable variation in glycogen and sugar reserves prior to fighting. The glycogen reserves of the liver were depleted as a result of fighting (although only significantly in losers)

and there was a trend for controls to have higher reserves than losers. Similarly, Chellapa & Huntingford (1989) found that in territorial fights between male three spine sticklebacks (*Gasterosteus aculeatus*) losers had lower liver glycogen than winners. However, there is some disagreement about whether the liver provides short term energetic support for muscles in fish, as it does in mammals (Haller, 1991). It is possible that liver glycogen is mobilised as a stress response (see review by Pickering, 1980), rather than in the energetic support of muscles used for fighting, which may explain the lower levels sometimes found in losers.

As fighting appears to result in the depletion of muscle glycogen, it is not surprising that lactate concentrations were significantly lower in the control fish than in fought fish. Furthermore, the concentration of lactate increased significantly with the time spent engaged in fighting. What is not clear, however, is whether the magnitude of the difference in concentration of muscle lactate between control fish and that of fought fish, while significant was not great and it may be questioned as to whether this is a biologically meaningful finding. No data are available for effects of exhaustive exercise on muscle biochemistry in *T. zillii*. However, data from other species suggest that the concentrations of lactate we observed are typical of fish subjected to vigorous exercise. The mean amount of white muscle lactate for fought fish in my study (22 μm g^{-1}) which was slightly lower than measurements (27.02 μm g^{-1}) made on Rainbow trout immediately after being chased for 5 minutes (Milligan & Girard, 1993). However, the lactate data for *T. zillii* control fish was much higher (17.69 μm g^{-1}) than resting rainbow trout (5.07 μm g^{-1}) used in the Milligan & Girard's study. Thus, I tentatively suggest that in terms of its consequences for

accumulation of lactate in white muscle tissue, fighting is equivalent to vigorous activity. That fighting in *T. zillii* is energetically costly but not totally exhausting is consistent with what has been found in wide variety of animals; crabs (Thorpe *et al.*, 1995), lizards (Wilson *et al.*, 1990) and damselflies (Marden & Rollins, 1994).

5.4.5 *Differences between winners and losers*

The only significant biochemical differences we detected between winners and losers were in the concentration of lactate in the white muscle, with concentrations tending to be higher in losers. Extensive anaerobic respiration is associated with a decrease in intracellular and blood pH (Wood, 1991). This is a significant problem for fish because this disrupts acid-base balance, causing problems with osmotic, ionic and fluid volume homeostases. As the process of recycling lactate back to glycogen is slow, lactate production effectively represents an end for the capacity for immediate energy production. It follows that losers are in a worse energetic state than winners and may suffer a more severe internal acidosis. Thus, fighting is metabolically costly for both parties, but especially so for losers.

While the anaerobic metabolism of glycogen reserves is a significant component of the energetic consequences of fighting and exercise, it is worth bearing in mind that there are also other components of exercise-induced energy metabolism that have important physiological consequences, such as the depletion of creatine phosphate, ATP, amino acids and lipids (Haller, 1991; Wood, 1991). These may well play a role in strategic decision-making.

5.4.6 Proximate costs as cues for strategic decision-making

A central idea in the functional analysis of decision-making is the 'decision-rule', which dictates the behavioural options that an optimally designed individual should take in any given set of circumstances (McFarland & Houston, 1981). The decision-rules in fighting can be thought of as a response to a given level of relative cost. Overt behavioural changes, such as escalating to a specific level or giving-up the fight, are made when the level of relative cost reaches the threshold level needed to express the appropriate behaviour.

Do the data from the present study support such a picture of the causal basis of decision-making ? Losers did tend to incur higher costs than winners, both in terms of physical injuries and adverse metabolic consequences. What is especially interesting is that large losers tended to be the most severely affected by biting and consequent scale loss. Thus, it does seem likely that high costs relative to your opponent are associated with the decision to give up. It is possible that losers use their estimate of high relative cost level to make the decision that they would eventually lose the fight and thus would do better by giving up sooner rather than later.

How information is obtained regarding relative costs is a matter for speculation. It is possible that the fish can monitor the number of scales they bite off their opponent and the number of bites they have inflicted. Alternatively, there is an intriguing possibility that the fish may be able to assess relative metabolic states. In Chapter 2, the only differences that were detected between winners and losers were in a behaviour termed 'mouth locking', in which the contestants clamp shut their mouths

on one another's lips. They stay in this embrace until one of them suddenly shakes itself free. The mouth lock is likely to restrict ventilation of the gills and thus may impair oxygen uptake. Throughout the fight the fish are accumulating an oxygen debt as a consequence of anaerobic metabolism. Thus, it may be that the fish with the greater oxygen debt is forced to break the lock sooner and, thereby, gives away information on its level of accumulated physiological cost. Marden & Rollins (1994) came to a similar conclusion about territorial fighting in damselflies, where victory can be predicted by relative fat reserves. It appears that the damselflies can assess relative fat reserves through complex and lengthy flight displays.

A further problem concerns the manner in which individual fish integrate and combine different causal factors (injury, lactate accumulation, depleted sugars and any other adverse consequences) to obtain an estimate of overall relative cost. A possible candidate for the physiological basis of such a mechanism is the catecholamine hormone system that includes the 'fight or flight' hormone adrenaline. There is evidence in teleosts suggesting that sub-optimal metabolic states such as decreased blood pH, increased CO_2 blood tension and increased lactate levels result in the release of catecholamines (Aota et al. 1991; Randall & Perry 1992). In addition, physiological responses to injury, such as inflammation, and psychological effects can stimulate catecholamine release (Brown 1994). I have some preliminary data (see Appendix 1) that suggests, surprisingly, that winners have very much higher plasma levels of the catecholamine hormone, adrenaline, than losers. The situation is clearly complex and would likely require analysis at the neural level. However, the

relationship of the catecholamines with behaviour and fighting in animals may be promising avenue for future research.

5.4.7 Conclusion

The data from this study regarding the infliction of injury and consequences of anaerobic energy metabolism suggest that fighting is costly for both winners and losers, but that this is especially marked for losers. Thus, it seems possible that losers are basing their decisions to give up on such costs, although the exact mechanism by which this is achieved is still unclear.

CHAPTER 6

SEX STEROID HORMONES IN <u>T. ZILLII</u> AND THEIR ASSOCIATIONS

WITH GONADAL STATE, AGGRESSION AND SOCIAL DOMINANCE

6.1 Introduction

6.1.1 *The endocrine system and behaviour*

The nervous system, the endocrine system and the immune system are the body's

main communication systems. All three respond to the internal state of the body and

the external environment and integrate this information. The brain is the predominant

processor of this information and enables the animal to respond physiologically and/or

behaviourally to a wide range of environmental circumstances. Although there is a

blurring between the mechanisms of action of the endocrine system and the nervous

system, one can broadly define a hormone as a molecule secreted by specialised cells

(endocrine glands) into the blood where it is transported to distant cells that have

specific receptors for that hormone (Brown, 1994). Hormone release occurs when the

endocrine cell is activated by neural or hormonal stimulation. The interaction of the

hormone with the target cell receptor is complex but ultimately leads to an activation

of genes that regulate protein synthesis in the cell. The overall effect of the hormone is

to alter cellular function in such a way that the cell has an modified sensitivity to a

particular stimulus that causes a physiological and/or behavioural change. Behavioural

endocrinology is the study of the bi-directional interaction between hormones and

behaviour (Nelson, 1995).

6.1.2 Sex steroids and reproductive behaviour

Steroids are hormones that are synthesised in the adrenal glands and the gonads. There is a chain of hormonal events that lead to the release of sex steroids (see Handelsman, 1995, for review). At the top of the chain are the gonadotropic releasing hormones, which are produced in the hypothalamus in response to external and internal sensory stimulation. The gonadotropic releasing hormones activate the production of the gonadotropins in the pituitary, which in turn activate the production of the sex steroids in the testis or ovary. This mechanism of action is termed the hypothalmus-pituitary-gonadal axis. There are three types of sex steroid, the C_{21} steroids or progestogens, the C_{19} steroids or androgens and the C_{18} steroids or estrogens (see Figure 6.1.1). The progestogens are the precursors of all other steroids, but have also been implicated in having specific behavioural effects themselves. The androgens have many different roles in the stimulation of male physiology and in the maintenance of the male phenotype and are produced in the interstitial cells of the testis. The estrogens have many different roles in the generation and maintenance of female characteristics and physiology. They are produced mainly in the ovaries by an enzymatic conversion of androgens (a process called aromatisation).

During evolution, the sex steroids involved in the physiological control of gamete maturation have been co-opted to synchronise the social behaviours that ensure there is successful transferring of mature gametes to the opposite sex. Intraspecific aggression is a major component of reproductive behaviour and there is much evidence in mammals and birds to suggest that aggression is mediated by sex steroids (Schlinger & Callard, 1990). Many studies have demonstrated an association between

Progesterone

Testosterone

Oestradiol

17,20β-Dihydroxy-4-pregnen-3-one

11β-Hydroxytestosterone

11-Ketotestosterone
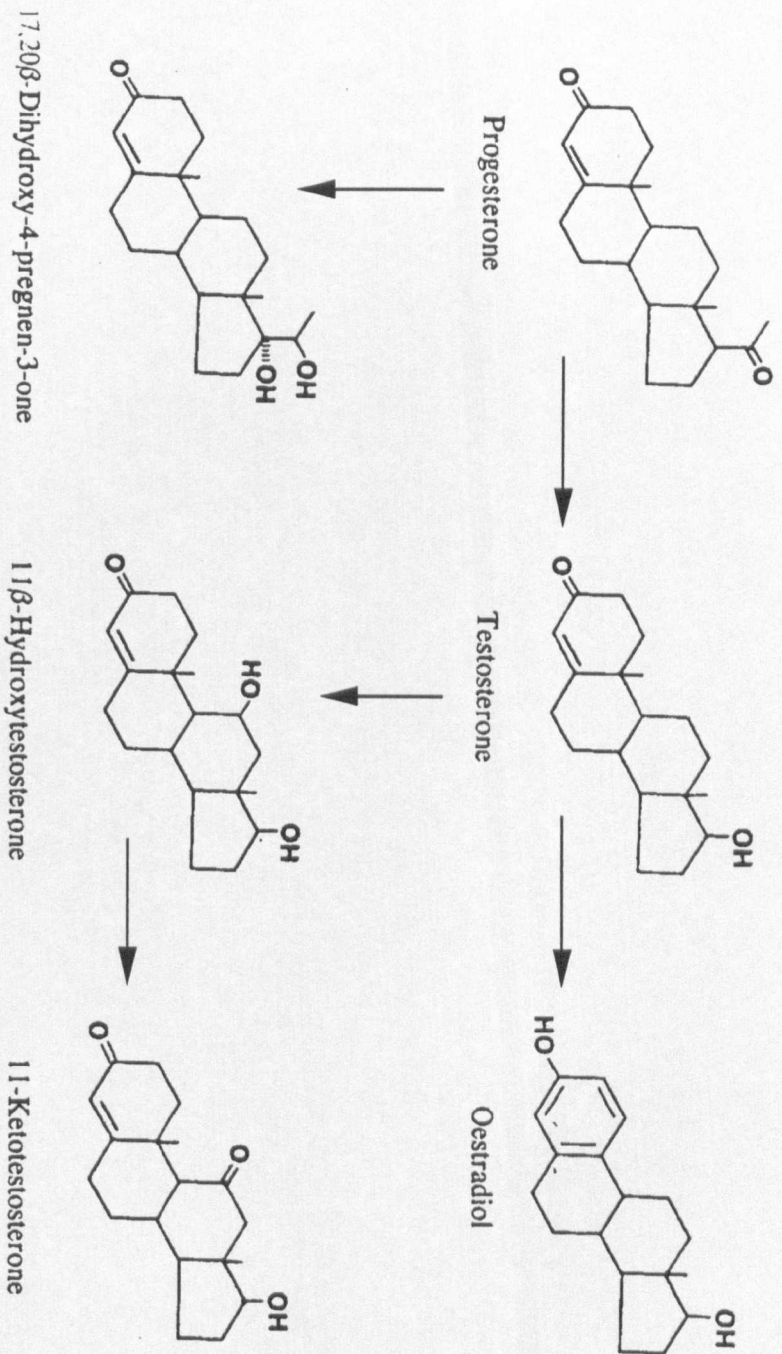
Figure 6.1.1 Sex steroids found in teleosts and conversion pathways between them (arrows)

increased plasma concentrations of testosterone and the appearance of territorial behaviour and increased aggression, for example, in lizards (Greenberg & Crews, 1990), birds (Wingfield *et al.*, 1987) and rodents (Gaines *et al.*, 1985). As more information has become available, the subtleties and complexities of androgen action have been teased apart and our understanding of the association between androgens and male aggression has become more refined. It is now realised that androgens have adverse side effects such as reduced immunocompetance and parasite resistance (Saino *et al.*, 1995). Thus, it is necessary to analyse the functional consequences of androgen levels in relation to these costs. Wingfield *et al.*'s, (1990) Challenge Hypothesis proposed that androgens levels are regulated in relation to aggressive challenges and thus are only high when necessary. This may explain why there is a correlation with aggression only during periods of repeated interactions between males and why there is a surge of plasma androgens that occurs during and after a fight (Wingfield & Wada, 1990).

6.1.3 *Sex steroids in teleost fish*

While the basic hypothalamus-pituitary-gonadal mechanism is similar in teleosts and other vertebrates, the gonadal steroids and their functions differ considerably. In addition to testosterone (T), the teleost testis produces an 11-oxygenated steroid, 11-ketotestosterone (11kT). In species studied to date, 11kT is predominant to, and has greater androgenic potency than T, suggesting that it is the major androgen in male teleosts (Borg, 1994). Other androgens produced by the testis include 11β-hydroxyandrostenedione, 11-ketoandrostenedione and 5α-dihydrotestosterone,

although they are all less effective than 11kT or T in stimulating male sexual characters (Borg et al., 1993). The estrogen, 17β-estradiol (E_2) is also a major teleost sex steroid, although in species studied to date (for example, Arctic charr, *Salvelinus alpinus*), the evidence suggests a functional role in the control of female maturation and reproduction (Mayer et al., 1992). Not much is known about the role of E_2 in males, although in black porgy (*Acanthopagrus schlegeli*) it has been shown to suppress testicular development and stimulate sex reversal (Chang et al., 1995). A further sex steroid specific to teleosts is the progestogen, 17α,20β-Dihydroxy-4-pregnen-3-one (17,20-P) that has been shown to have an important role in the final stages of testicular maturation (see review by Kime, 1993) and has been implicated in the control of spawning behaviour of salmonids (Mayer et al., 1994). The relationships between some of these steroids is shown in Figure 6.1.1. In nearly all teleosts studied to date there is close association between these steroids and the expression of reproductive behaviour (see reviews by Borg & Mayer 1995; Pankhurst, 1995).

6.1.4 *Sex steroids, aggression and social dominance in teleosts*

The evidence for a relationship between androgens and male aggressive behaviour in teleosts is less clear than it is for mammals and birds. In a tilapia (*Oreochromis mossambicus*), Oliveira et al. (1996) found that in social groups, the dominant territorial males had significantly higher urine androgen levels than did subordinates. Perhaps the best evidence for such an association comes from an elegant field study by Cardwell & Liley (1991), who found that dominant, territorial Stoplight parrotfish (*Sparisoma viridae*) had higher levels of plasma T and 11kT than subordinate males

and that these high levels were a consequence of repeated aggressive interactions. On the other hand, for two salmonid species, rainbow trout (*Oncorhyncus mykiss*) (Liley & Kroon, 1995) and kokanee salmon (*Oncorrhyncus nerka*) (Liley *et al.*, 1993) no significant differences in plasma androgen levels were found between dominants and subordinates.

6.1.5 *Aims of the present study*

In Chapter 2 it was shown that in pair-wise fights, GSI is a better predictor of dominance than is body size. While the functional reasons for this finding have yet to be demonstrated, it seems likely that GSI is related in some way to maturation, proximity to spawning and hence aggression (see section 2.4.3 in Chapter 2). T, 11kT and 17,20-P have all been implicated in the physiological regulation of testis maturation (Borg, 1994) and as such are the likely candidate components in the mechanistic link between gonadal state and behavioural output. In an attempt to understand the mechanism by which GSI is influencing behaviour, the present study was undertaken to look at plasma concentrations of these gonadal steroids in relation to GSI and success in pair-wise fights.

6.1.6 *Technique for measuring steroids*

The most commonly used technique for measuring steroids is radioimmunoassay (RIA). The logic behind the RIA is the principle of competitive binding between an antibody and an antigen (Barnard *et al.*, 1995; Nelson, 1995). Antibodies can be specifically raised to bind only to the steroid of interest. The binding affinity of the antibody is not affected if the steroid is labelled with a measurable radioactive isotope.

This means that in a solution composed of radiolabelled steroid and unlabelled steroid, there will be competition for the binding sites of the antibody. This will equilibrate at concentrations that are proportional to the original amounts of each present in solution. Thus, by measuring how much radiolabelled steroid remains unbound in solution, it is possible to work out the amount of unlabelled steroid. The technique is very sensitive and can be used to measure picogram (10^{-12} gram) quantities of hormone.

6.2 Materials & Methods

6.2.1 *Fish and staging of fights*

The fights staged in this experiment form a sub-set of the sample of fights reported in Chapter 2; thus the methodology is identical with the exception of blood sampling. This study generated individual blood samples from twenty four staged fights and ten unfought control fish. Blood was also obtained from additional control fish for the purposed of validating the RIA for *T. zillii* (see section 6.2.4). In addition, for interest and comparison, five female fish were also sampled.

6.2.2 *Blood sampling and plasma extraction*

Following the establishment of dominance and subsequent electro-stunning, the fish were placed in strong bezocaine (200 mg per litre) for 60 seconds to ensure long term anaesthesia and a blood sample withdrawn from the caudal aorta (refer back to Figure 4.3) using a 1 ml syringe with a 25 gauge needle primed with anti-coagulant heparin sodium solution (supplier: ICN chemicals). It was usually possible to obtain between

200 and 500 µl of blood which was immediately centrifuged for 6 minutes at 13,000 rpm. The plasma was then decanted and stored at -70 °C. Following blood sampling the fish were killed by immersion in liquid nitrogen. All sample preparations and RIAs were performed in the laboratory of Drs Bertil Borg and Ian Mayer, Department of Zoology, University of Stockholm (with financial support provided by the NERC). During the period of storage, the samples were accidentally thawed for a period of approximately 18 hours, however, it was demonstrated that this had no detectable effects on steroid concentrations - see Appendix 2.

6.2.2 *Preparation of plasma samples and reagents*

Prior to RIA, individual plasma samples (200-300 µl) were mixed with distilled water containing NaN_3 in the ratio of 1:2, and heat-treated for 1 hour at 80°C. Following centrifuging (30 minutes at 13,000 rpm) the supernatant was decanted and the sample stored at 3°C prior to assaying. Radiolabelled (radioactive tritium - ^3H) T and E_2 were supplied by Amersham, International, UK. Tritiated 11kT and 17,20P were synthesised by Dr I. Mayer, University of Stockholm. Pure T and E_2 were supplied by Sigma Chemicals and pure 11kT and 17,20P were synthesised by Dr I. Mayer. Antiserum for all steroids was raised by Dr. R. Schulz at the University of Leiden, Netherlands.

6.2.4 *Validation of radioimmunoassay of steroids in* T. zillii *plasma*

Ideally, the antibody should only react with the steroid under study, but in practice it is often the case that many substances may compete with the labelled steroid for binding. This 'cross-reactivity' is particularly a problem with steroids that have an

similar molecular structure as the steroid under study. The cross reactivities of the RIA's used had been previously determined for T, 11kT and E_2 (Schulz, 1985), and 17,20P (Mayer *et al.*, 1990a). However, when working on a new species, there is always a risk that the antibody reacts differently in the plasma of the new species. The RIAs were validated for use with *T. zillii* by taking a sample of untreated plasma and removing all steroids and proteins by adding an excess of a chilled dextran-coated charcoal (DCC - see section 6.2.5 below). 1000 µl of the stripped plasma was then 'spiked' with known concentrations (10 ng) of T, 11kT, E_2 and 17,20P. The spiked sample was then treated as in section 6.2.3 and the RIAs run for each steroid according to section 6.2.5 (below). Provided the RIA accords with the known amount of steroid in the spiked sample, the RIA can be assumed to be accurate.

6.2.5 *Radioimmunoassay of plasma steroids*

The original procedures for these RIA's are published in Schulz (1985) (T, 11kT and E_2) and Mayer *et al.* (1990a) (17,20P). The following reagents used in the preparation of standards are the same for samples; 1 l of stock RIA buffer was made up which comprised the following reagents;

NaH2PO4 + H2O - 3.87g

Na2HPO4 + 2H20 - 10.67g

NaN3   - 0.05g

NaCl   - 9.00g

Gelatine   - 1.00g

The gelatine was dissolved in 200 ml hot distilled water. The other reagents were dissolved in 700 ml distilled water, added to the gelatine solution and the pH adjusted to 7.0.

Activated dextran coated charcoal was prepared by mixing 10 parts charcoal to 1 part dextran in 100 ml of RIA buffer. The mixture was stirred for 1 h on ice prior to use.

The stock solutions of steroids were stored in ethanol in the concentration 100ng/50 μl. The stock solutions of radiolabelled steroids were stored in ethanol and prior to RIA were diluted in RIA buffer to give a reading of approximately 30,000 disintigrations per minute/50 μl (see below). The stock solution of antiserum was diluted 1:10 with RIA buffer and then further diluted (in RIA buffer) according to the optimal 50 % binding affinity for the particular RIA (1:7 for T, 1:70 for 11kT, 1:7 for E_2 and 1:35 for 17,20P).

A set of standard curves were constructed with which to obtain calibrations for the spiked validation sample and the real samples. A set of standards were prepared from the stock solution of pure steroids. The stock solution was diluted with RIA buffer to 2000 pg/50μl. The other standards (1000, 500, 250, 125, 62.5, 31.25, 15.625 and 7.8125 pg per 50 μl) were prepared by 1 + 1 dilution with RIA buffer. For standard curve assays, duplicate 10 X 75 mm borosilicate vials contained 50 μl standard. To these was added 50 μl of radiolabelled steroid, 50 μl RIA buffer and 200 μl antiserum. The total volume in each vial was always equal to 350μl. The procedure for assaying

samples is identical with the exception that the volume of sample added should be varied according to the absolute concentration of steroid in the sample. This is because the standard curve is sigmoid and most accurate in the linear section where the percentage bound is between approximately 25 - 75. For T and 11kT 50 μl was optimal, whereas for E_2 and 17,20P 100 μl was needed (in which case no RIA buffer was added). In addition to the standards and/or samples, the following controls were run simultaneously and in duplicate;

Total bound: 50 μl 3 H steroid, 100 μl RIA buffer + 200 μl antiserum

Non specific bound: 50 μl 3 H steroid + 300 μl RIA buffer

Total radioactivity count: 50 μl 3 H steroid (no charcoal treatment)

The vials were then vortexed to mix the contents and incubated over night at 4°C. The following day, 300 μl chilled DCC was added to each vial and incubated for 5 minutes to remove all unbound steroids. The vials were then centrifuged at 4°C at 400 rpm to remove the charcoal (and unbound steroids). The supernatent (containing the bound steroid) was then decanted into scintillation vials containing 4 ml of scintillation fluid (Optiphase "HiSafe" II, LKB Wallac, Finland). These were then vortexed and the disintigrations per minute counted for 5 min. in a liquid scintillation beta-counter (1214 Rackbeta, LKB Wallac, Finland) connected to a computer. For each RIA all samples were processed together. Final concentrations were calculated as the mean of the two duplicate samples and expressed as nanograms of steroid per ml of plasma.

6.3 Technical results

6.3.1 *Validation of radioimmunoassays for* <u>T. zillii</u>.

All steroid RIAs indicated accurate measurement (+- 1.5 ng per ml plasma) of the

concentration of steroids in *T. zillii* plasma (values for the validation tests for T, 11kT,

E_2 and 17,20P are presented in Table 6.3.1 The sensitivity of all RIAs was 0.4 ng

steroid per ml plasma.

Table 6.3.1 Mean values for spiked samples (ng steroid per ml plasma)

RIA	T	11kT	E_2	17,20P
Mean concentration of steroid measured in spiked sample	9.6 ng	11.48 ng	9.35 ng	10.4 ng

6.4 Biological results

6.4.1 *GSI and body weight in relation to outcome of fights*

Just to reiterate, the fights from which the blood samples were collected are a sub-set of the fights reported in Chapter 2. Within this subset GSI was still a significant predictor of winning, whereas body weight was not.

6.4.2 *Plasma concentrations of testosterone*

Table 6.3.2 shows the mean concentrations of plasma T in winners, losers and controls. Two pairs of fish were excluded from the analysis because the T concentrations of the losers were below the limit of detection (0.4 ng per ml) and both fish had extraordinarily low GSIs. A paired t-test showed that there were no significant differences between winners and losers (t = - 0.53, n = 21, P = 0.6). Control fish were not significantly different from either winners (t-test: t = 0.44, d.f = 31, P = 0.66) or losers (t-test: t = 0.47, d.f = 29, P = 0.64). Figure 6.4.1 shows plasma T concentrations are not significantly correlated with GSI (r = - 0.18, n = 56, P = 0.63). There were no significant correlations between plasma T concentrations of either winners or losers and the duration of time spent in escalated fighting (r = - 0.3, n = 23, P > 0.05 and r = - 0.15, n = 21, P > 0.05, for winners and losers respectively).

Table 6.3.2 Mean (± standard error) of plasma concentrations of steroids in male winners, losers and controls, and females. ND = not accurately detectable.

Steroid	T	11kT	E_2	17,20P
Winners (n = 21)	4.56 (± 0.64)	10.77 (± 1.72)	ND	ND
Losers (n = 21)	4.55 (± 0.63)	11.94 (± 1.24)	ND	ND
Controls (n = 11)	4.51 (± 0.56)	12.07 (± 2.92)	ND	ND
Females (n = 5)	23.64 (± 4.51)	2.45 (± 0.64)	6.43 (± 2.16)	ND
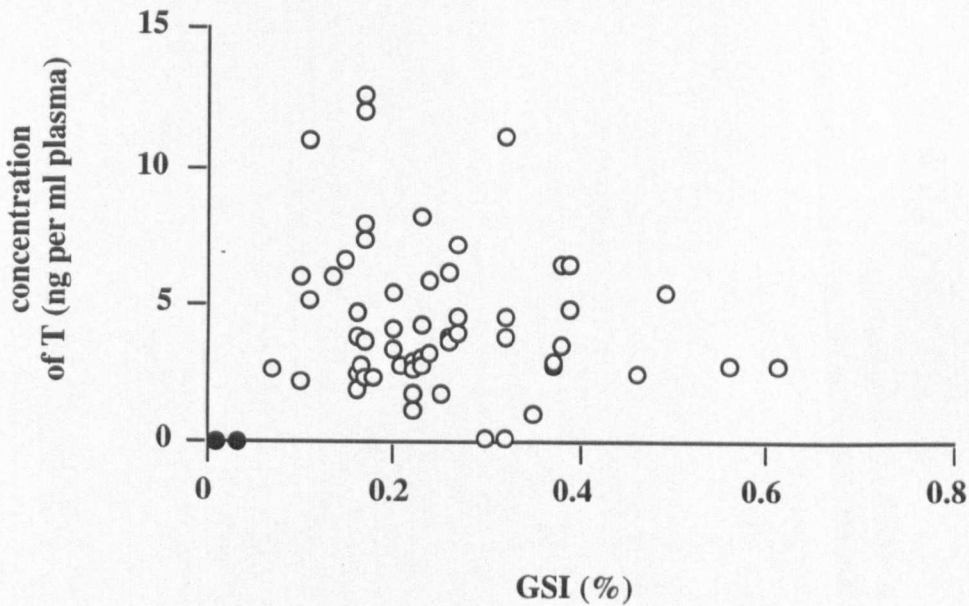
Figure 6.4.1 Concentration of plasma T plotted against GSI (filled circles represent those fish excluded from the analysis)

6.4.3 *Concentrations of plasma 11kT*

The same two pairs of fish were excluded from the T data set were also excluded from this analysis because 11kT concentrations were below the limits of detection. Table 6.3.2 shows mean concentrations of plasma 11kT in winners, losers and controls. Plasma 11 kT concentrations were on average twice those of T. A paired t-test showed that there were no significant differences between winners and losers in plasma 11kT concentrations (- 0.71, n = 21, P = 0.48). Control fish were not significantly different from either winners (t-test: t = - 0.39, n = 31, P = 0.7) or losers (t-test: t = - 0.03, n = 29, P = 0.97). There were no significant correlations between plasma 11kT concentrations of either winners or losers and the duration of time spent in escalated fighting (r = - 0.1, n = 23, P > 0.05 and r = - 0. 18, n = 21, P > 0.05, for winners and losers respectively). Figure 6.4.4 illustrates a significant negative correlation between 11 kT concentrations and GSI (r = - 0.34, n = 56, p < 0.05). There was a significant positive correlation between 11 kT and T concentrations (r = 0.56, p < 0.01, n = 56).
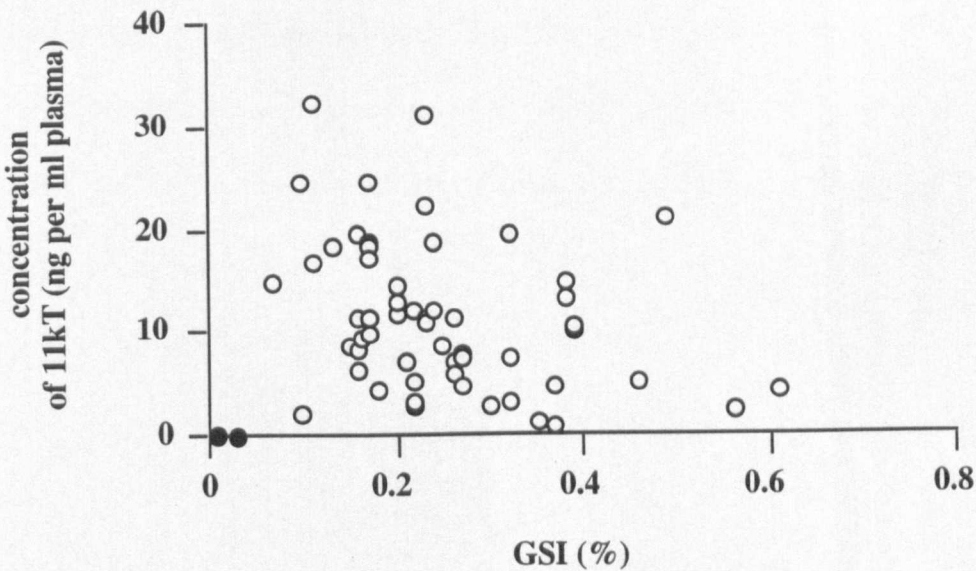
Figure 6.4.2 Concentration of plasma 11kT plotted against GSI (filled circles = those fish excluded from the analysis).

6.4.4 *Concentrations of plasma 17,20 P*

The maximum concentration of plasma 17,20-P detected was 1.18 ng per ml and many of the samples were below the limits of detection of the RIA. Thus, the data set cannot be treated as reliable. Since there was no reason to suspect a technical problem, it may be concluded that concentrations of 17,20P are very low in male *T. zillii* in this study.

6.4.5 *Concentrations of plasma E_2*

The RIA for E_2 was only carried out on six fought pairs and 3 controls, because E_2 is a female sex steroid and the concentrations were expected to be low. The maximum concentration of plasma was 0.57 ng per ml. Thus, as with 17,20 P it is only possible to conclude that E_2 concentrations are very low in males.

6.4.6 *Concentrations of plasma T, 11kT, 17,20P and E_2 in females*

Five female fish were blood sampled and assayed with the male samples for comparison and general interest. The mean concentrations of T, 11kT and E_2 are shown in Table 6.3.2 (concentrations of 17,20P were below the detection limits of the RIA). Plasma T was approximately 5 times that of males, 11kT half that of males and plasma E_2 6 times that of males.

6.5 Discussion

6.5.1 *Sex steroids in male* T. zillii

While the gonadal endocrinology of female *T. zillii* has been studied (K. Coward & C. Randall, University of Stirling, pers. comm.), there appears to be no data on the gonadal steroids and their relative quantities in males. The validation procedure for the use of the RIAs with *T. zillii* were successful and T, 11kT, 17,20P and E_2 were all detected in plasma of males. However, only T and 11kT concentrations were present in quantities sufficient to be measured reliably. Plasma concentrations of 11kT were approximately twice as high as T concentrations in *T. zillii* males, which is in agreement with what has been found in most other teleosts studied (Borg, 1994). These data support the general view that that 11kT is the major androgen in mature male teleosts. The absolute plasma concentrations detected were in agreement with what is found in other species, for example, Mayer *et al.*, (1990b) measured plasma 11kT and T in wild male sticklebacks and found 11kT to vary from approximately 2 - 3 ng during the non breeding season up to. 20 - 30 ng per ml during breeding and T to remain constantly around 2-3 ng per ml throughout the season.

6.5.2 *Sex steroids as a possible mechanism linking relative GSI to victory*

As with Chapter 2, GSI was still a very reliable predictor of the outcome the fights staged in this study. This suggested that GSI influences how valuable the territory is to the fish. In functional terms GSI may reflect proximity to and capacity for reproduction, in that, a fish with a high GSI would have a greater vested interest in maintaining a territory. Fish with high GSIs are assumed to have a greater motivation

in fighting, that is to say, are more aggressive. In other teleosts, for example three spined sticklebacks (*Gasterosteus aculeatus*), androgen changes associated with reproductive cycles are associated with territorial and aggressive behaviour (see review by Borg & Mayer, 1995). That androgens are causal to increased aggression is supported by a castration experiment on a cichlid (*Haplochromis burtoni*); castrated males had drastically reduced plasma concentrations of T and 11kT and reduced aggression but oddly, not social dominance (Francis *et al.*, 1992). Furthermore, the Challenge Hypothesis, that is to say, the idea that the experience of aggressive interactions has a positive feedback effect on androgen secretion, has been supported by both a laboratory study of cichlid fish (Oliveira, *et al.*, 1996) and a field study of parrot fish (Cardwell & Liley, 1991). It should, however, be noted that at least two studies, both on salmonids, have failed to detect an association between androgen levels and social dominance (Liley *et al.*, 1993 and Liley & Kroon, 1995)

Thus, the evidence is good for androgen-mediated aggression for teleosts other than salmonids and for these reasons, it was thought that a similar mechanism may underlie the persistence in fighting (and high probability of winning) observed in *T. zillii* males with high relative GSIs. The sex steroids T, 11kT and 17,20P have all been implicated in the maturation of the testis (Borg, 1994) and are likely candidates as components in the mechanism linking gonadal state to behavioural output.

6.5.3 *Sex steroids in relation to outcome and duration of fights*

The results of this study do not support the view that plasma concentrations of T or 11kT are associated with dominance or with particularly aggressive and persistent

fighting strategies. The observation that unfought control fish had similar levels of T to fought fish and the fact that there were no correlations between T and 11kT and the duration of fighting did not suggest that fighting results in surges of T, as has been reported for birds (Wingfield & Wada, 1989) and as was inferred by Cardwell & Liley (1991) for Stoplight parrotfish. Overall, there was no evidence in *T. zillii* that the gonadal steroids play a simple role in mediating short-term behavioural responses.

It is possible that absolute concentrations of androgens are less important than relative changes in androgen concentration. To identify such an effect, it would have been necessary to take blood samples prior to staging the fight and this has problems of its own, such that the sampling process itself causes stress. One way of overcoming the stress problem would be to take urine sample, as was done by Oliveira *et al.* (1996). However as neither the relationship between urinary concentrations of steroids and plasma nor the time course of changes in plasma to changes in urine is known, this approach also has problems. Ideally, one would have to some kind of micro-cannulation of an artery of the fish, from which samples could be withdrawn by remote control.

6.5.4 *Relationships between the sex steroids and gonadal state*

To date, the function of T in teleosts is not clear. In fact, whether it can be regarded an androgen in the sense that it 'generates the male phenotype' is debatable as in many teleosts (see Borg, 1994 for review) females have much higher plasma T concentrations than males. This was certainly the case in *T. zillii*, even though female sample sizes were small. T is clearly produced by the testis in *T. zillii*, as was

indicated by the non-detectable concentrations found in the two fish with extraordinarily small GSIs, but no clear relationship was found between concentrations of plasma T and GSI.

The fact that 11 kT was also undetectable in the two fish that had the very low GSIs suggests that this too is a testicular product. More importantly, the negative correlation found between GSI and 11kT concentrations is interesting with regard to what is already known about the endocrinology of sperm production in other teleosts. In the Atlantic salmon *(Salmo salar)*, plasma 11kT levels peak during the early stages of spermatogenesis and then decline during later stages of sperm maturation (Mayer *et al.*, 1990). Sikkel (1993) similarly reported a decline in levels of plasma 11kT of the Garibaldi *(Hypsopops rebicundus)* as males get closer to spawning. If 11kT has a role in the early stimulation of spermatogenesis and if a high GSI is indicative of a large reserve of mature sperm, then one would expect 11kT concentrations to be correspondingly low in fish with high GSIs. There can be little benefit in producing more sperm than is necessary, thus spermatogenesis can be relaxed and the sperm reserve maintained until such time that a ripe female is present.

The final maturation and subsequent release of sperm (spermiation) can then be initiated. 17,20-P has been implicated in this process in rainbow trout (Olsen & Liley, 1993). Olsen & Liley's findings suggest that there are pheromones in the female urine that stimulate the production of 17,20-P which in turn initiates spermiation. Plasma levels of 17,20-P were very low or undetectable in all males. In light of these recent findings, the observed low levels of this hormone in males may be due to the fact that

they had had no stimulatory exposure to ripe females. However, the possibility that the gonads of *T. zillii* produce a further maturation-inducing steroid other than 17,20-P cannot be excluded.

The question still remains as to the nature of the mechanism that links GSI with winning. It is possible that 11kT has an slight inhibitory effect on aggression, but it is more likely that if aggression behaviour is mediated by sex steroids, then it is by steroids other than those measured in this study. Alternatively, the problem is perhaps best framed as GSI being a consequence, rather than a cause of dominance, and that the environmental circumstances and social experiences leading to a large GSI have led to the development of a neuronal organisation that predisposes these males to fight vigorously and win contests. There is some good evidence that social dominance regulates GSI via gonadotropic-releasing hormone containing neurones in the hypothalamus of the cichlid, *H. burtoni* (Francis *et al.*, 1993).

6.5.5 Conclusions

It seems likely that the role of the androgen hormones measured in this experiment is in the physiological regulation of testicular maturation and spermatogenesis. There was no evidence that these androgens play a direct role in the mediation of aggressive responses in the short term. The possibility that subtle effects may have been masked because pre-fight concentrations of steroids were not measured cannot be ruled out. However, given that the fish endocrine system differs radically from the mammalian system, it is perhaps timely to reassess the putative role of androgens in aggression in fish.

CHAPTER 7

GENERAL DISCUSSION

7.1 Synopsis of aims, approach and questions

The overall aim of this project was to examine the functional significance and proximate causation of fighting in *Tilapia zillii*. To this end, I planned experiments with three objectives in mind:

1) To examine the behavioural ecology of fighting and to test the broad predictions of game theory regarding fight outcome, duration and intensity.

2) To obtain detailed behavioural records of fighting in order to examine behavioural organisation and the means by which fights are resolved.

3) To investigate physiological aspects of fighting.

Once these had been accomplished, the remaining challenge was to try to show how functional and causal analyses of behaviour are mutually beneficial for understanding animal behaviour as a whole. In this chapter I review the main results and conclusions of the previous chapters. I then offer my personal opinion on how I think the different levels and approaches taken in the study interrelate and why I think this integration between disciplines is so important to the study of animal behaviour. If, by the end of this chapter, it can be seen that causal approaches can improve our functional understanding of animal behaviour and vice versa, then my goal has been attained.

Tinbergen (1972) summed up this perspective when, upon describing the persuit of ethology, he wrote "One finds oneself turning all the time from asking 'what is its use?' to 'how is it done ?'".

7.2 What are male *T. zillii* fighting over?

While this question is probably the most fundamental of all, it is the one that I can answer with least certainty. This is mainly because so little information is available on *T. zillii* in the wild. At the start of the project, it was clear that males showed fairly typical territorial behaviour in the laboratory aquaria; they dug nests and would aggressively confront and fight any intruding males. However, in the flume study reported in Chapter 3, it transpired that the social system of *T. zillii* was more complicated than simply territorial. The fish were much more socially interactive, forming loose groups and establishing a dominance hierarchy. The expression of territorial behaviour appeared to be conditional on social status, as only the two top ranked males were seen to dig nests and consistently court females. The escalated fight observed in the flume study was between the two highest ranked fish, but as both had built nests, it was not clear whether this was a territorial dispute, a challenge for the top position, or a dispute over both. Thus, it appears that the immediate reasons for fighting between male *T. zillii* depends upon the social and physical environment. However, whatever the environment-specific reasons for aggression, be it over social position or territory ownership, it probably ultimately boils down to the same thing, namely, mating opportunities. Obviously, a field study of the social organisation of *T. zillii* is needed to properly understand the behavioural ecology of aggression in this species.

7.3 Functional insights into fighting in male *T. zillii*

Chapter 2 showed that, as far as the outcome of fights goes, there were two important variables, namely difference in GSI and difference in body size (RHP). Difference in GSI had a much stronger effect than did difference body size, at least in pairs that differed in weight by no more than approximately 30 %. Although this finding was initially unexpected, game theory could account for it, if, as seems likely, GSI actually reflects resource value (RV). Assuming this to be the case and that there is simultaneous variation in RHP and RV, then predicting duration and intensity of fights becomes much more complicated. Enquist & Leimer's (1987) Sequential Assessment model is sufficiently sophisticated to handle RHP and RV simultaneously. However, in this model decisions are depicted as being made on the basis of the accuracy of the estimate of relative RHP which is clearly inappropriate for the present study, in which fish 30 % smaller than their opponent are able to secure victory (see Chapter 2 for further expansion of this argument). It was certainly the case that no simple relationships existed between either GSI or body size difference and fight duration, although upon more detailed analysis some subtle and complex patterns did start to emerge

Analysis of the mouth wrestling phase showed that the fish were assessing body size. This suggested that information on relative body size is important in making strategic decisions, but at a gross behavioural level its effect is masked by the effect of GSI asymmetry. The two main behaviours in the highly escalated carouselling phase were biting (and consequent nose-to-tail chasing) and mouth locking. It appeared that smaller fish respond to information about relative RHP by biting more, the greater

their size disadvantage. This was most marked if the smaller fish went on to win the fight. This suggests that when a smaller fish chooses to continue a fight, it compensates for its disadvantage by increasing its intent to inflict cost. The magnitude of this biting response does not, however, appear to be related to GSI difference.

The functional significance of mouth locking is harder to pin down. Breaking the mouth lock was not related to asymmetry in body size or GSI, but losers consistently broke more mouth locks than winners, suggesting that breaking the mouth lock is related to the likelihood of giving up. This behaviour is clearly related to resolving the fight, but just how it relates to the determinants of fight outcome still remains obscure. It seems asymmetries in body size and GSI, in combination, influence behaviour in such complex ways that it is very difficult to work out the individual contribution of each variable. If one had a large enough pool of fish, one could experimentally tease these effects apart by carefully matching pairs for size and also GSI (which could be done using the morphometric predictors presented in Chapter 4).

7.4 What are the behavioural mechanisms underlying decision-making during fighting ?

The behavioural mechanisms that underlie the effect of asymmetries in body size and GSI are likely to have important differences but also share important characteristics. An asymmetry in body size probably exerts its effects by conferring an immediate advantage or disadvantage in terms of the ability to inflict and withstand cost. Thus, contestants may use information on relative body size as a mechanism to weigh up

their strategic options. On the other hand, an asymmetry such as GSI probably exerts its effects by influencing the threshold level of cost that each contestant is prepared to pay to retain its resource (rather than conferring any immediate (dis)advantage in terms of the ability to inflict cost). Thus, contestants may use information on current cost levels in relation to cost thresholds set by GSI to weigh up their strategic options.

While the mechanistic bases of the effects of RHP and RV may be different, both must be part of one overall decision-making mechanism as both operate in relation to the same variable, namely the proximate cost of fighting. As RHP affects the rate at which costs accrue and RV affects the amount of cost that will be tolerated before giving up, a contestant should ideally decide to give up when its estimate of its current level of cost relative to that of its opponent means that it will reach its threshold level of cost before its opponent does. Thus, it must be able to estimate its own current level of cost, its own threshold cost and the same two variables for its opponent. In other words, a very complicated assessment mechanism must be in operation.

What evidence is there that such mechanisms underlie behavioural decisions in the fights investigated in this study? As mentioned earlier, and contrary to what is normally found, smaller fish adjust their biting upward in relation to body size difference. Therefore, it does seem likely that information on body size asymmetry underlies the level of risk that individuals adopt in a particular fight. In relation to mouth locking, it may be also be the case that engaging in the activity is itself costly, but there are at least two other possible roles for mouth locking; on the one hand it

may provide information on the current cost that each fish has incurred; this would be the case if, for instance, the mouth lock impairs oxygen uptake from the gills and forces the opponent with the greater oxygen to break sooner. Alternatively, mouth locking could give away information on the eventual costs that each contestant is prepared to pay; this would be the case if, for instance, the degree of persistence in any one bout of mouth locking is proportional to how motivated the individual is to retain its resource. In sum, it appears that the mechanisms underlying fight resolution may be of two kinds. Firstly, there is the infliction of cost realised by behaviours such as biting and chasing; this depends upon relative RHP. Secondly, there may also be assessment of the relative levels of cost that have been inflicted, realised by mouth locking; this depends on RV.

7.5 What are the physiological bases of decision-making mechanisms?

If the above idea of a 'two component' mechanism of strategic decision-making is correct, then we should expect to find two corresponding physiological components of the mechanism. On the one hand, we should see a set of physiological parameters tracking the proximate cost of fighting. On the other we should also see a physiological basis for the differential activation of strategic decisions based on the setting of threshold levels of cost.

Possible physiological bases for the proximate level of cost were revealed in Chapter 5. As discussed there, there are significant detrimental consequences of anaerobic energy metabolism associated with fighting, such as internal acidosis, oxygen debt and energy depletion. Furthermore, the data suggested that winners and losers

differed with respect to at least some of these, for example in terms of the accumulation of lactate. In addition, the infliction of injuries also represents a proximate cost of fighting, although the physiological basis of injury is a much more complicated issue. Nevertheless, the biochemical analyses did suggest that differential accruement of costs during fighting has a physiological basis, which may be used by the contestants as cues for making strategic decisions.

In terms of the physiological basis for the variation in the threshold-setting rules of decision-making, it seemed logical to look for some association with GSI. The gonadal steroids were the obvious candidates, because of the various physiological roles they play in gonad maturation and because of their association with aggression in many animal species. Such a mechanism would have been supported if, for example, it could have been demonstrated GSI was positively correlated with levels of circulating androgens. One could then hypothesise that androgens act as a cue to the setting of the threshold level of cost that should be incurred before giving up the fight. However, this idea was not supported by the finding presented in Chapter 6 which suggested that plasma concentrations of two main androgens in this species were not related to variation in persistence and aggression in fighting. Thus, if there is such a mechanism linking GSI to fight outcome, its physiological basis remains obscure.

7.6 Physiology, behaviour and evolution

In the research presented in this thesis, it has been necessary to cross back and forth between physiology, behavioural organisation and functional outcome to eventually

make some sense of what was a complicated and unusual example of animal behaviour. A level of analysis that was particularly fruitful was the detailed study of behavioural interaction. Not only did this illuminate the functional significance of body size and GSI, but it was also the starting point for the development of many of the ideas for the physiological studies. This shows the importance of the behavioural level of analysis. Without the insights gained from this aspect of the analysis, the gap between physiology and functional endpoint might well have been too big to bridge. With it, however, the three levels of analysis (functional, behavioural and physiological) relate and mutually reinforce one another in an illuminating way.

7.7 Conclusion: bridges and levels

At the start of this Chapter I quoted Tinbergen because he so often stressed the cause/function duality of the nature of ethological investigation. Few would disagree with this perspective. However, no matter how philosophically satisfying this perspective is, practically and intellectually, bridging levels of study is very difficult. Thus, some may question whether, given that causal and functional studies work well on their own, the benefits of building the bridge justifies the effort needed to do so. My opinion is that levels and bridges are one and the same; that is to say, the causal level is one end of a bridge and the functional level the other. Thus, convergence between disciplines is an inevitable consequence of the advancement of the science and should be welcomed rather than shied away from.

REFERENCES

Aota, S., Holmgren, K. D., Gallaugher, P. & Randall, D. J. 1990 A possible role for catecholamines in the ventilatory responses associated with internal acidosis or external hypoxia in rainbow trout *Oncorhynchus mykiss*. *J. exp. Biol.* **151**, 57 - 70.

Alvarenga, C. M. D. & Volpato, G. L. 1995 Agonistic profile and metabolism in alevins of the Nile Tilapia. *Physiol. Behav.* **57**, 75 - 80.

Archer, J. 1988 *The Behavioural Biology of Aggression*. Cambridge: Cambridge University Press.

Archer, J. & Huntingford, F. A. 1993 Game theory models and escalation of animal fights, In. *The Dynamics of Aggression: Biological and social processes in dyads and groups* (eds. M. Potegal & J. F. Knutson), p.p 3 - 31. Hove, U.K.: Lawrence Erlbaum Ass.

Baerends, G. P. 1975 An evaluation of the conflict hypothesis as an explanatory principle for the evolution of displays. In: *Function and Evolution of behaviour*. (Eds. G. P. Baerends, C Beer, & A. Manning) p.p 187 - 227. Oxford: Clarendon Press.

Baerends, G.P. 1993 Behavioural and the evolution of behavioural strategies. In: *Behavioural Ecology of Fishes* (eds. F. A. Huntingford & P. Torrecelli) p.p. 301 -316. Chur, Switzerland: Harwood Academic.

Baerends, G. P. & Baerends - Van Roon, J. M. 1950 An introduction to the study of the ethology of cichlid fishes. *Behav. Suppl.* **1**, 1 - 251.

Barlow, G. W. 1963 Ethology of the Asian teleost *Badis badis*. II. Motivation and the signal value of the colour patterns. *Anim. Behav.* **11**, 97 - 105.

Barlow, G. W., Rogers, W. & Fraley, N. 1986 Do Midas cichlids win through prowess or daring? It depends. *Behav. Ecol. Sociobiol.* **19**, 1 - 8.

Barnard, G., Read, G. F. & Collins, W. P. 1995 Immunoassay. In: *Steroid analysis*. (Eds. H. L. J. Makin, D. B. Gower & D. W. Kirk), pp. 185 - 216. London: Blackie Academic & Professional.

Barrette, C. & Vandal, D. 1990 Sparring, relative antler size and assessment in male caribou. *Behav. Ecol. Sociobiol.* **26**, 383 - 387.

Bone, Q., Marshall, N. B. & Blaxter, J. H. S. 1995 *Biology of Fishes*, 2nd edition. London: Blackie Academic & Professional.

Borg, B. 1994 Androgens in teleost fishes. *Comp. Biochem. Physiol.* **109 C**, 219 - 245.

Borg, B. & Mayer, I. 1995 Androgens and behaviour in the three-spined stickleback. *Behaviour* **132**, 1025 - 1035.

Borg, B., Antonopoulou, E., Andersson, E., Carlberg, T & Mayer, I. 1993 Effectiveness of several androgens in stimulating kidney hypertrophy, a secondary sexual character, in castrated male three-spined sticklebacks, *Gasterosteus aculeatus. Can. J. Zool.* **71**, 2327 - 2329.

Brown, D. & Rothery, P. 1993 *Models in Biology: Mathematics, Statistics and Computing.* New York: Wiley.

Brown, R. E. 1994 *An Introduction to Neuroendocrinology.* Cambridge: Cambridge University Press.

Bruton, M. N. & Gophen, M. 1992 The effect of environmental factors on the nesting and courtship behaviour of *Tilapia zillii* in Lake Kinneret (Israel). *Hydrobiol.* **239**, 171 - 178.

Cardwell, J. R. & Liley, N. R. 1991 Androgen control of social status in males of a wild population of Stoplight parrotfish, *Sparisoma viride* (Scaridae). *Horm. Behav.* **25**, 1 - 18.

Carroll, N. V., Longely, R. W. & Roe, J. D. 1956 The determination of glycogen in liver and muscle by use of anthrone reagent. *J. biol. Chem.* **220**, 583 - 593.

Chang, C-F., Lau, E-L. & Lin, B-Y. 1995 Estradiol-17β suppresses testicular development and stimulates sex reversal in protandrous black porgy, *Acanthopagrus schlegeli. Fish Physiol. Biochem.* **14**, 481 - 488.

Chellapa, S. & Huntingford, F. A. 1989 Depletion of energy reserves during reproductive aggression in male three-spined stickleback, *Gasterosteous aculeatus* L. *J. Fish Biol.* **35**, 315 -316.

Chellappa, B., Huntingford, F. A. & Strang, R. C. (in prep) Energy reserves and reproductive investment in male three spine sticklebacks (*Gasterosteous aculeatus*). MS.

Clutton Brock, T. H. & Albon, S. D., Gibson, R. M. & Guinness, F. E. 1979 The logical stag: adaptive aspects of fighting in red deer ((*Cervus elephus* L.). *Anim. Behav.* **27**, 211 - 225.

Cosmides, L. & Tooby, J. 1987 From evolution to behavior: Evolutionary psychology as the missing link. In, *The Latest On The Best: Essays on Evolution and Optimality* (ed. J. Dupré), p.p 278 - 306. Cambridge, Mass.: MIT Press.

Darwin, C. R. 1859 *On the Origin of Species*. London: John Murray.

Davies, N. B. (1991) Studying behavioural adaptations. In: *The Tinbergen Legacy* (eds. M. S. Dawkins, T. R. Halliday & R. Dawkins), p.p 18 -30. London: Chapman & Hall.

Davies, N. B. & Halliday, T. R. 1978 Deep croaks and fighting assessment in toads, *Bufo bufo*. *Nature, Lond.* **274**, 683 - 685.

Dawkins, M. S. & Guilford, T. 1993 Colour pattern in relation to sexual and aggressive behaviour in the Bluehead wrasse *Thalassoma bifasciatum*. *Behav. Proc.* **30**, 245 - 252.

Diana, J. S. & Mackay, W. C. 1979 Timing and magnitude of energy deposition and loss in the body, liver, and gonads of northern pike (*Esox lucius*). *J. Fish. Res. Board Can.* **36**, 481 - 487.

Edwards, D. H. 1995 The neural mechanisms of social dominance in crustaceans. In: *Nervous Systems and Behaviour: Proceedings of the 4th International Congress of Neuroethology*. (Eds. M. Burrows, T. Matheson, P. L. Newland & H. Schuppe), p.p. 3. Stuttgart: Georg Theim Verlag.

Ejike, C. & Schreck, C. B. 1980 Stress and social hierarchy rank in Coho salmon. *Trans. Am. Fish. Soc.* **109**, 423 -426.

Elwood, R. W. & Neil, S. J. 1992 *Assessment and Decisions - A Study of Information Gathering by Hermit Crabs*. London: Chapman & Hall.

Englund, G. & Olssen, T. I. 1990 Fighting and assessment in the net spinning caddis larva *Arctopsyche ladogensis*: a test of the sequential assessment game. *Anim. Behav.* **39**, 55 - 62.

Enquist, M. 1985 Communication during aggressive interactions with particular reference to variation in choice of behaviour. *Anim. Behav.* **33**, 1152 - 1161.

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

Enquist, M. & Leimer, O. 1983 Evolution of fighting behaviour: decision rules and assessment of relative strength. *J. theor. Biol.* **102**, 387 - 410.

Enquist, M. & Leimer, O. 1987 Evolution of fighting behaviour: the effect of variation in resource value. *J. theor. Biol.* **127**, 187 - 205.

Enquist, M., Leimer, O., Lunjberg, T., Mallner, Y. & Segerdahl, N. 1990 A test of the sequential assessment game: fighting in the cichlid fish *Nannacara anomala. Anim. Behav.* **40**, 1 -14.

Fernald, R. D. & Hirata, N. R. 1977 Field study of *Haplochromis burtoni*: quantitative behavioural observations. *Anim. Behav.* **25**, 964 - 975.

Francis, R.C., Jacobson, B., Wingfield, J. C. & Fernald, R. D. 1992 Castration lowers aggression but not social dominance in male *Haplochromis burtoni* (Cichlidae). *Ethology* **90**, 247 - 255.

Francis, R. C., Soma, K. & Fernald, R. D. 1993 Social regulation of the brain-pituitary-gonadal axis. *Proc. Nat. Acad. Sci. USA.* **90**, 7794 - 7798.

Fryer,G. & Iles, T. D. 1972 *The Cichlid Fishes of the African Great Lakes: Their Biology and Evolution.* Edinburgh: Oliver & Boyd.

Gäde, G. E., Weeda, E. & Gabbott, P.A. 1978 Changes in the level of octopine during the escape response of the scallop, *Pecten maximus* (L.). *J. comp. Physiol.* **124 B**, 121 - 127.

Gaines, M. S., Fugate, C. L., Johnson, M. L., Johnson, D. C., Hisey, J. R., & Quadagno, D. M. 1985 Manipulation of aggressive behavior in male prairie voles (*Microtus ochragaster*) implanted with testosterone in silastic tubing. *Can. J. Zool.* **63**, 2525 - 2528.

Garcia-Berthou, E. & Moreno-Amich, R. 1993 Multivariate analysis of covariance in morphometric studies of the reproductive cycle. *Can. J. Fish. Aquat. Sci..* **50**, 1394 -1399.

Gleeson, T. T. 1996 Post exercise lactate metabolism: A comparative review of sites, pathways, and regulation. *Annu. Rev. Physiol.* **58**, 565 -581.

Grafen, A. 1987 The logic of divisively asymmetric contests: respect for ownership and the desperado effect. *Anim. Behav.* **35**, 462 - 467.

Grant, J. W. A. 1993 Whether or not to defend? The influence of resource distribution. *Mar. Behav. Physiol.* **23**, 137 - 153.

Greenberg, N. & Crews, D. 1990 Endocrine and behavioural responses to aggression and social dominance in the Green Anole lizard, *Anolis Carolinensis. Gen. Comp. Endocrin.* **77**, 246 - 255.

Gutmann, I. & Wahlefeld, A. W. 1974 L-(+)-lactate determination with lactate dehydrogenase and NAD+. In: *Methods of enzymatic analysis, 2nd Edition* (Ed. H. U. Bergmeyer), pp. 1464 - 1468. New York: Academic Press.

Haller, J. 1991 Biochemical cost of a fight in fed and fasted *Betta splendens. Physiol. Behav.* **49**, 79 - 82.

Haller, J. 1995 Biochemical background for an analysis of cost - benefit interrelations in aggression. *Neurosci. Biobehav. Rev.* **19**, 599 - 604.

Hammerstein, P. & Parker, G. A. 1982 The asymmetric war of attrition. *J. theor. Biol.* **96**, 647 - 682.

Handelsman, D. J. 1995 Testosterone and other androgens: physiology, pharmacology and therapeutic use. In: *Endocrinology* 3rd Edn. (Ed. L. J. De Groot) p.p 2351 - 2378. London: Saunders.

Hansen, A. J. 1986 Fighting behavior in bald eagles: a test of game theory. *Ecology* **67**, 787 - 797.

Harvey, P. H. & Bradbury, J. W. 1991 Sexual selection. In: *Behavioural Ecology: An Evolutionary Approach* 3rd Edn. (Eds. J. R. Krebs & N. B. Davies), p.p 203 - 233. Oxford: Blackwell Scientific Publishing.

Holder, J. L., Barlow, G. W. & Francis, R. C. 1991 Differences in aggressiveness in the Midas cichlid fish (*Cichlasoma citrinellum*) in relation to sex, reproductive state and the individual. *Ethology*, **88**, 297 - 306.

Huber, R. 1995 Serotonin controls decision making aspects of fighting in crayfish. . In: *Nervous Systems and Behaviour: Proceedings of the 4th International Congress of Neuroethology* (Eds. M. Burrows, T. Matheson, P. L. Newland & H. Schuppe). p. 168. Stuttgart: Georg Theim Verlag.

Hulscher-Emeis, T. M. 1992 The variable colour patterns of *Tilapia zillii* (Cichlidae): Integrating ethology, chromatophore regulation and the physiology of stress. *Neth. J. Zool.* **42**, 525 - 560.

Huntingford, F. A. 1991 War and peace revisited. In: *The Tinbergen Legacy* (eds. M. S. Dawkins, T. R. Halliday & R. Dawkins) p.p 40 -59. London: Chapman & Hall.

Huntingford, F. A. 1993 Behavioural mechanisms in evolutionary perspective. *T.R.E.E.* **8**, 81 - 83.

Huntingford, F. A. & Turner, A. K. 1987 *Animal Conflict.* London: Chapman & Hall.

Huntingford, F. A., Taylor, A. C., Smith, I. P. & Thorpe, K. E. 1995 Behavioural and physiological studies of aggression in swimming crabs. *J. Exp. Mar. Biol. Ecol.* **193**, 21 - 39.

Jakobsson, S., Radesäter, T. & Jarvi, T. 1979 On the fighting behaviour of *Nannacara anomala* (Cichlidae, Pisces). *Z. Tierpsychol.* **49**, 210 - 220.

Johnstone, I. A. & Goldspink, G. 1973 A study of the swimmimg performance of the Crucian carp *Carassius carassius* (L.) in relation to the effects of exercise and recovery on biochemical changes in the myotomal muscles and liver. *J. Fish Biol.* **5**, 249 - 260.

Keenleyside, M. H. A. (ed) 1993 *Cichlid Fishes: Behaviour, ecology and evolution*. London: Chapman & Hall.

Kime, D. E. 1993 'Classical' and 'non-classical' reproductive steroids in fish. *Rev. Fish Biol. Fish.* **3**, 160 - 180.

Koops, M. A. & Grant, J. W. A. 1993 Weight asymmetry and sequential assessment in convict cichlid contests. *Can. J. Zool.* **71**, 475 - 479.

Koebele, B. P. 1985 Growth and the hierarchy effect: an experimental assessment of three proposed mechanisms; activity differences, disproportional food acquisition, physiological stress. *Env. Biol. Fish.* **12**, 181 - 188.

Krebs, J. R. & Davies N. B. 1991 (eds) *Behavioural Ecology: An Evolutionary Approach*. 3rd Ed. Oxford: Blackwell Scientific.

Leimer, O., Austad, S. & Enquist, M. 1991 A test of the sequential assessment game: fighting in the bowl and doily spider *Frontinella pyramitela. Evolution* **45**, 862 - 874.

Liley, N. R. & Kroon, F. J. 1995 Male dominance, plasma hormone concentrations, and availability of milt in male rainbow trout (*Oncorhynchus mykiss*). *Can. J. Zool.* **73**, 826 - 836.

Liley, N. R., Olsen, H. K., Foote, C. J. & Van Der Kraak, G. J. 1993 Endocrine changes associated with spawning behavior in male Kokanee salmon (*Oncorhynchus nerka*) and the effects of anosmia. *Horm. Behav.*, **27**, 470 - 487.

Lowe-McConnell, R. H. 1959 Breeding behaviour patterns and ecological differences between *Tilapia* species and their significance for evolution within the genus *Tilapia* (Pisces: Cichlidae). *Proc. Zool. Soc. Lond.* **132**, 1 - 30.

Ma, P.M. 1995 On the agonistic display of the Siamese fighting fish. I. The frontal display apparatus. *Brain. Behav. Evol.* **45**, 301 - 313.

Manley, B. F. 1990 Multivariate statistics: A primer. London: Chapman & Hall.

Marden, J. H. & Rollins, R. A. 1994 Assessment of energy reserves by damselflies engaged in aerial contests for mating territories. *Anim. Behav.* **48**, 1023 - 1030.

Marden, J. H. & Waage, J. K. 1990 Escalated damselfly territorial contests are energetic wars of attrition. *Anim. Behav.* **39**, 954 -959.

Martin, P. & Bateson, P. 1993 *Measuring Behaviour: An Introductory Guide.* Cambridge, Cambridge University Press.

Mattson, N. S. 1991 A new method to determine sex and gonad size in live fishes by using ultrasonagraphy. *J. Fish Biol.* **39**, 673 - 677.

Mayer, I. Berglund, I., Rydevik, M., Borg, B. & Schulz, R. 1990a Plasma levels of five androgens and 17α-hydroxy-20β-dihydroprogesterone in immature and mature Baltic salmon (*Salmo salar*) parr, and the effects of castration and androgen replacement in mature parr. *Can. J. Zool.* **68**, 263 - 267.

Mayer, I., Borg, B. & Schulz, R. 1990b Seasonal changes in and effect of castration/replacement on the plasma levels of five androgens in the male three-spined stickleback, *Gasterosteous aculeatus* L. *Gen. Comp. Endocrinol.* **79**, 23 - 30.

Mayer, I., Liley, N. R. & Borg, B. 1994 Stimulation of spawning behavior in castrated rainbow trout (*Oncorhynchus mykiss*) by 17α-20β-Dihydroxy-4-pregnen-3-one, but not by 11-ketoandrostenedione. *Horm. Behav.* **28**, 181 - 190.

Mayer, I., Schmitz, M., Borg, B. & Schulz, R. 1992 Seasonal endocrine changes in male and female Arctic charr (*Salvelinus alpinus*). I. Plasma levels of three androgens, 17α-hydroxy-20β-dihydroprogesterone, and 17β-estradiol. *Can. J. Zool.* **70**, 37 - 42.

Maynard smith, J. 1982 *Evolution and The Theory of Games.* Cambridge: Cambridge University Press.

Maynard Smith, J. & Price, G. R. 1973 The logic of animal conflict. *Nature, Lond.* **246**, 15 - 18.

Maynard Smith, J. & Reichert, S.E. 1984 A conflicting-tendency model of spider agonistic behaviour: hybrid pure line comparisons. *Anim. Behav.*, **32**, 564 - 578.

McFarland, D. & Houston, A. I. 1981 *Quantitative ethology.* London, Pitman Publishing.

Metcalfe, N. B., Taylor, A. C. & Thorpe, J. E. 1995 Metabolic rate, social status and life history strategies in Atlantic salmon. *Anim. Behav.* **49**, 431 - 436.

Milligan, C. L. & Girard, S. S. 1993 Lactate metabolism in rainbow trout. *J. exp. Biol.* **180**, 175 - 193.

Millinski, M. & Bakker, T. C. M. 1990 Female sticklebacks use male coloration in mate choice and hence avoid parasitised males. *Nature, Lond.* **344**, 330 - 333.

Møller, A. P. & Saino, N. 1994 Parasites, immunology of hosts, and sexual selection. *J. Parasitol.* **80**, 850 - 858.

Motta, P. J., Norton, S. F. & Luczkovich, J. J. 1995 Perspectives on the ecomorphology of bony fishes. *Envir. Biol. Fish.* **44**, 11 - 20.

Nakatsuru, K. & Kramer, D. 1982 Is sperm cheap ? Limited male fertility and female choice in the lemon tetra (Pisces, Characidae). *Science* **216**, 753 - 755.

Nelson, R. 1995 *An Introduction to Behavioral Endocrinology.* Sunderland, Mass.: Sinauer Associates.

Oliveira, R. F. & Almada, V. C. 1995 Sexual dimorphism and allometry of external morphology in *Oreochromis mossambicus. J. Fish Biol.* **46**, 1055 - 1064.

Oliveira, R. F & Almada, V. C. 1996 On the (In)stability of dominance hierarchies in the cichlid fish, *Oreochromis mossambicus. Agg. Behav.* **22**, 37 - 45.

Oliveira, R. F., Almada, V. C. & Canario, A. V. M. 1996 Social modulation of sex steroid concentrations in the urine of male cichlid fish *Oreochromis mossabicus. Horm. Behav.* **30**, 2 - 12.

Olsen, K. H. & Liley, N. R. 1993 The significance of olfaction and social cues in milt availability, sexual hormone status, and spawning behaviour of male rainbow trout (*Oncorhynchus mykiss*). *Gen. Comp. Endocrin.* **89**, 107 - 118.

Pankhurst, N. W. 1995 Hormones and reproductive behaviour in male damselfish. *Bull. Mar. Sci.* **57**, 569 - 581

Parker, G. A. 1974 Assessment and the evolution of fighting behaviour. *J. theor. Biol.* **47**, 223 - 243.

Parker, G. A. & Maynard Smith, J. 1990 Optimality theory in evolutionary biology. *Nature, Lond.* **348**, 27 - 33.

Pavey, C. R. & Fielder, D. R. 1996 The influence of size differential on agonistic behaviour in the freshwater crayfish, *Cherax cuspidatus* (Decapoda: Parastacidae). *J. Zool., Lond.* **238**, 445 - 457.

Philippart, J-Cl. & Ruwet, J-Cl. (1982) Ecology and distribution of Tilapias.. In: *The Biology and Culture of Tilapias* (eds. R. S. V. Pullin & R.H. Lowe-McConnell), p.p 15 - 59. Manilla, Philippines: ICLARM.

Pickering, A. D. 1981 *Stress and Fish* (ed. by A.D. Pickering) London: Academic Press.

Poole, J. H. 1987 Announcing intent: the aggressive state of musthe in African elephants. *Anim. Behav.* **37**, 140 - 152.

Poole, J. H. & Moss, C. J. 1981 Musthe in the African elephant, *Loxodonta africana*. *Nature, Lond.* **292**, 830-831.

Pough, F. H. & Andrews, R. M. 1985 Use of anaerobic metabolism by free-ranging lizards. *Physiol. Zool.* **58**, 205 - 213.

Randall, D. J. & Perry, S. F. 1992 Catecholamines. In: *Fish Physiology Vol. XIIB* (eds W. S. Hoar, D. J. Randall & A.P. Farrell). San Diego: Academic Press: 255 - 300.

Ribowski, A. & Franck, D. 1993 Demonstration of strength and concealment of weakness in escalating fights of male Swordtails (*Xiphophorus helleri*). *Ethology* **93**, 265 - 274.

Rodriguez-Girones, M. A., Drummond, H. & Kacelnik, A. 1996 Effect of food deprivation on dominance status in blue-footed booby (*Sula neboxii*) broods. *Behav. Ecol.* **7**, 82 - 88.

Rowe, D. K. & Thorpe, J. 1990 Differences in growth between maturing and non-maturing male Atlantic salmon, *Salmo salar.*, L. *J. Fish Biol.* **36**, 643 - 658.

Saino, N., Møller, A. P. & Bolzern, A. M. 1995 Testosterone effects the immune system and parasite infestations in the barn swallow (*Hirundo rustica*): an experimental test of the immunocompetance hypothesis. *Behav. Ecol.* **6**, 397 - 403.

Schlinger, B. A. & Callard, G. A. 1990 Aggressive behaviour in birds: An experimental model for studies of brain-steroid interactions. *Comp. Biochem. Physiol.* **97A**, 307 - 316.

Schwank, E. 1980 The effect of size and hormonal state on the establishment of dominance in young males of *Tilapia mariae* (Pisces: Cichlidae). *Behav. Proc.* **5**, 45 - 53.

Schulz, R. 1985 Measurement of five androgens in the blood of immature and maturing male rainbow trout, *Salmo gairdneri* (Richardson). *Steriods* **46**, 717 - 726.

Senar J.C., Camerino M. & Metcalfe N.B., 1992 Fighting as a subordinate in finch flocks: escalation is effective but risky. *Anim. Behav.* **43**, 862 - 864.

Shapiro, D. Y. & Giraldeau, L. C. 1996 Mating tactics in external fertilisers when sperm is limited. *Behav. Ecol.* **7**, 19 - 23.

Sherman, M. A. 1994 Regulation of lipid metabolism in poikilothermic vertebrates. *Comp. Biochem. Physiol.* **107 B**, 495 - 508.

Siddiqui, A. Q. 1979 Reproductive Biology of *Tilapia zillii* (Gervais) in Lake Naivasha, Kenya. *Env. Biol. Fish.* **4**, 257 - 262.

Sikkel, P. C. 1993 Changes in plasma androgen levels associated with changes in male reproductive behaviour in a brood cycling marine fish. *Gen. Comp. Endocrin.* **89**, 229 - 237.

Simpson, A. L, Metcalfe, N. B & Thorpe, J. E. 1992 A simple non-destructive biometric method for estimating fat levels in Atlantic salmon, *Salmo salar* L., parr. *Aquacult. Fish. Manage.* **23**, 23 -29.

Stearns, S. C. 1992 *The Evolution of Life Histories.* Oxford, Oxford University Press.

Tchernavin, V. 1938 Changes in the salmon skull. *Trans. Zool. Soc. Lond.* **24**, 103 - 172.

Thorpe, K. E., Taylor, A. C. & Huntingford, F. A. 1995 How costly is fighting ? Physiological effects of sustained exercise and fighting in swimming crabs, *Necora puber* (L.) (Brachyura, Portunidae). *Anim. Behav.* **50**, 1657 - 1666.

Tinbergen, N. 1951 *The Study of Instinct.* Oxford: Oxford University Press.

Tinbergen, N. 1958 *Curious Naturalists.* New York: Basic Books.

Tinbergen, N. 1964 On aims and methods of ethology. *Z. Tierpsychol.* **20**, 410 - 433

Tinbergen, N. 1972 *The Animal in its World: Explorations of an Ethologist.* London: George Allen & Unwin.

Trewavas, E. 1982 Tilapias: taxonomy and speciation. In: *The Biology and Culture of Tilapias* (eds. R. S. V. Pullin & R.H. Lowe-McConnell). p.p 3 - 13. Manilla, Philippines: ICLARM.

Trewavas, E. 1983 Tilapine Fishes of the Genera Sarotherodon, Oreochromis and Danakilia. London: British Museum (Natural History).

Turner, G.F. 1994 The fighting tactics of male mouthbrooding cichlids: the effects of size and residency. *Anim. Behav.* **47**, 655 - 662.

Turner, G. F. & Huntingford, F.A. 1986 A problem for game theory analysis: assessment and intention in male mouthbrooder contests. *Anim. Behav.* **34**, 961 - 970.

Turner, G. F. & Robinson, R. (in prep.) Reproductive biology of Tilapias. MS.

Tyrrell, T. 1993 The use of hierarchies for action selection. In: *Animals to Animats 2: Proceedings of the Second International Conference on Simulation and Adaptive Behavior.* (Eds. J. A. Meyer, H. L. Roitblat & S. W. Wilson), p.p 138 - 147. Cambridge, Massachusetts: MIT press.

Warner, R. R. 1984 Deferred reproduction as a response to sexual selection in a coral reef fish: a test of the life historical consequences. *Evolution* **38**, 148 - 162.

Warner, R. R. & Schulz, E. T. 1992 Sexual selection and male characteristics in the Bluehead wrasse, *Thalassoma bifasciatum*: Mating site acquistion, mating site defence and female choice. *Evolution* **46**, 1421 - 1442.

Wilson, M. A., Gatten, R. E. & Greenberg, N. 1990 Glycolysis in *Anolis carolinensis* during agonistic encounters. *Physiol. Behav.* **48**, 139 - 142.

Winberg, S. & Nilsson, G. E. 1993 Roles of brain monoamine neurotransmitters in agonistic behaviour and stress reactions, with particular reference to fish. *Comp. Biochem. Physiol.* **106C**, 597 - 614.

Wingfield, J. C., Ball, Dufty, A. M., Jr. G. F., Hegner, R. E. & Ramenofsky, M. 1987 Testosterone and aggression in birds. *Amer. Sci.* **75**, 602 - 608.

Wingfield, J. C., Hegner, R. E., Dufty, A. M. Jr. & Ball, G. F. 1990 The "challenge hypothesis": Theoretical implications for patterns of testosterone secretion, mating systems and breeding strategies. *Am. Nat.* **136**, 829 - 846.

Wingfield, J. C. & Wada, M. 1989 Changes in plasma levels of testosterone during male-male interactions in the son sparrow, *Melospiza melodia*: time course and specificity of response. *J. Comp. Physiol.* **A 166**, 189 - 194.

Wingfield, J. C., Whaling, C. S. & Marler, P. 1994 Communication in vertebrate aggression. In: *The Physiology of Reproduction*, 2nd Ed. (Eds. E. Knobil & J. D Neill), p.p 303 - 342. New York: Raven Press.

Witter, M. S & Cuthill, I. 1993 The ecological costs of avian fat storage. *Phil. Trans. R. Soc. Lond. B.* **256**, 73 - 90.

Witter, M. S. & Swaddle, J. P. 1996 Dominance, competition, and energetic reserves in the European starling, *Sturnus vulgaris. Behav. Ecol.* **6**, 343 - 348.

Wood, C. M. 1991 Acid-base and ion balance, metabolism, and their interactions, after exhaustive exercise in fish. *J. exp. Biol.* **160**, 285 - 308.

Wooton, R. J. 1990 *Ecology of Teleost Fishes* London: Chapman & Hall.

Yeh, S-R., Fricke, R. A. & Edwards, D. H. 1996 The effect of social experience on serotonergic modulation of the escape circuit of cray fish. *Science* **217**, 366 - 369.

A PRELIMINARY INVESTIGATION OF THE CATECHOLAMINE HORMONES, ADRENALINE AND NORADRENALINE IN THE PLASMA OF T. ZILLII IN RELATION TO FIGHTING.

Introduction

The catecholamine hormone system is known to be an important component of the physiological stress response of animals (Randall & Perry, 1992) and has been reported to be activated in aggressive contexts (Haller, 1995). As discussed in Chapter 5, the catecholamines may play a role in the integration of qualitatively different costs associated with fighting. Thus, this preliminary study was undertaken to establish if two catecholamines, adrenaline and noradrenaline could be measured in the plasma of *T. zillii.*

Materials & methods

Two fights were staged between male *T. zillii* (as in Chapter 2). After the fight had been resolved the fish were blood sampled and plasma extracted (as in Chapter 6). One pair of control fish (as in Chapter 2) were also blood sampled. The samples were then processed by Dr D. Watson at Strathclyde University to determine concentrations of the catecholamine hormones adrenaline and noradrenaline. This was done by gas-chromatography mass-spectrometry.

Results

The results of the analysis are presented in Table a1.1 below. Winners had plasma adrenaline levels approximately 100 times that of losers and approximately 2-3 times higher than controls. Winners also had higher plasma noradrenaline levels than losers, although this result is much less striking than for adrenaline.

Table a1.1 Concentration of the catecholamines adrenaline and noradrenaline in the plasma of winners, losers and control *T. zillii* (ND = non-detectable).

Sample	winner/loser	Adrenaline (ng per ml)	Noradrenaline (ng per ml)
Fight 1	winner	128.78	2.65
	loser	2.34	ND
Fight 2	winner	346.4	13.94
	loser	2.08	ND
Control	A	37.01	1.58
	B	52.12	0.57

Conclusions

Clearly, one cannot draw much from such a small sample size, but the differences between winners, and controls is so marked that it seems likely that fighting is having a major effect upon the release of these hormones. What is particularly striking is that losers had such low levels of adrenaline compared with winners and (to a lesser extent) controls. It is difficult to even speculate what this actually means in terms of physiology and function but at the very least it suggests something important and interesting is happening and that this should be investigated further.

APPENDIX 2

AN EXPERIMENT TO DETERMINE IF THERE IS SIGNIFICANT BREAKDOWN OF PLASMA SEX STEROIDS AS A CONSEQUENCE OF THAWING AT ROOM TEMPERATURE.

Introduction

During the work reported in Chapter 6 there was an accidental thawing of the deep-frozen plasma for a period of between 12 and 18 hours. I thought that there may have been some biological breakdown of steroids and that this could give spurious results. Thus, I planned an experiment that would show whether this was the case or not. This involved obtaining plasma from three replicate fish and assigning the plasma from each fish to one of three treatments: controls (continuous deep frozen at - 70°C) and experimentals (thawed and left at room temperature (20°C) for 12 or 24 hours).

Materials and methods

Three large (> 150g) male *T. zillii* were anaesthetised with benzocaine and approximately 1ml of blood was withdrawn (see Chapter 6). Following plasma extraction (see Chapter 6), each sample was divided into three eppendorfs which were either permanently frozen, frozen and then allowed to thaw for 12 hours and frozen and allowed to thaw for 24 hours. Following treatment the samples were all frozen at - 70°C until RIA (see Chapter 6).

Results

The samples were assayed for testosterone (T) and 11-ketotestosterone (11kT). Table a2.1 shows that there were no detectable differences between the treatments for either steroid.

Table a2.1 Concentrations (ng per ml plasma) of T and 11kT in frozen and thawed samples.

Sample (fish)	1			2			3		
time	0	12	24	0	12	24	0	12	24
T	7.2	7.24	6.64	3.83	4.31	3.63	5.93	6.29	6.47
11kT	36.2	35.1	35.8	10.55	11.31	9.78	14.95	15.14	16.52

Conclusion

This study strongly suggests that the accidental thawing of the plasma samples in Chapter 6 would not have caused any significant breakdown of the steroids measured.

APPENDIX 3

A THEORETICAL STUDY OF FIGHT RESOLUTION

A3.1 Theoretical modelling in biology

Modelling is a way of formally expressing ideas about how the world works (Brown & Rothery, 1993). Different people see the role of mathematical modelling in biology differently. Some biologists, who are able to think and communicate very clearly in words, can test their ideas without having to invoke any mathematics. However, for others mathematical modelling can be a way of clarifying ideas in order that they can be tested scientifically. In this chapter I aim to show how mathematical modelling firstly clarified and then allowed me to test an idea that I had about how fights between the fish were resolved. That the model's predictions were not supported by empirical data allowed me to reject the idea, thereby progressing my understanding of the mechanisms by which fights are resolved.

A3.2 Natural selection and modelling optimality of behavioural adaptations

As Darwin (1859) and the many who have followed him argue, adaptation is a pervasive feature of organisms, although not the only force that has shaped life on Earth. Therefore, studying adaptation is one way of understanding life on Earth. The concept of adaptation by natural selection rests upon the premise that individuals maximise their reproductive success (or ultimate fitness). This axiom lends itself well to mathematical analysis of the biological traits in question. A trait is assumed to have both costs and benefits in terms of ultimate fitness (usually equated with lifetime

reproduction) and that natural selection will act to minimise costs, maximise benefits or obtain the best compromise between the two. This approach to the analysis of adaptation has been termed 'optimality' theory (Parker & Maynard Smith, 1990). As was shown in Chapter 2, much effort has been put into the modelling of fighting in animals. Game theory is a special class of optimality models that takes into account frequency dependent effects.

In the fights that were reported in Chapter 2, none of the published game theory models of fighting could fully account for the observations. It was, therefore, a priority to try and explain the observations. What follows is an account of how initial ideas were gradually formalised and expressed mathematically as a model in an attempt to account for the observed data.

A3.3 Characterising the problem of how fights are resolved

In Chapter 2 three main conclusions were reached about fighting in *T. zillii*. First, the relative GSI and body size of a pair of fish could be used to predict the outcome of the fight. Second, body size is assessed during the mouth wrestling phase, but is evidently not the means by which the fight is resolved. Third, resolution is attained during the carouselling phase, where the fish chase, bite and engage in mouth locking. The fact that the fish were prepared to escalate to a dangerous level of fighting even when they were size disadvantaged suggested that the resolution of the fight must be related to how much cost each individual is prepared to pay to retain its territory. Furthermore, as it seemed that relative GSI caused the fish to fight longer, so it was

suggested that the cost an individual was prepared to pay to retain its territory was positively correlated to its GSI.

I was interested in examining how GSI affects behaviour during fights. There are two possible scenarios assuming that the fight is resolved during the carouselling phase; firstly, difference in GSI is assessed during the carouselling phase, much as differences in body size are assessed (c.f Enquist & Liemer, 1983), and the fight is resolved when one fish estimates its GSI to be less than that of its opponent. Secondly, it may be impossible to assess this asymmetry (because GSI is not directly observable), so the carouselling phase is a 'War of Attrition' (c.f Hammerstien & Parker, 1982; Maynard Smith, 1982) in which the fish fight as hard as they can, inflict as much cost as they can, and persist until one of them reaches its threshold level of cost that it is prepared to pay. As fish with larger GSI have a higher threshold they are more likely to win. This second possibility is the simpler of the two and the one that I and Prof. Marc Mangel, University of California at Santa Cruz, endeavoured to model. As a basis for modelling, what happens during a fight was represented as a flow diagram with a series of decision points (see Figure 7.3.1).
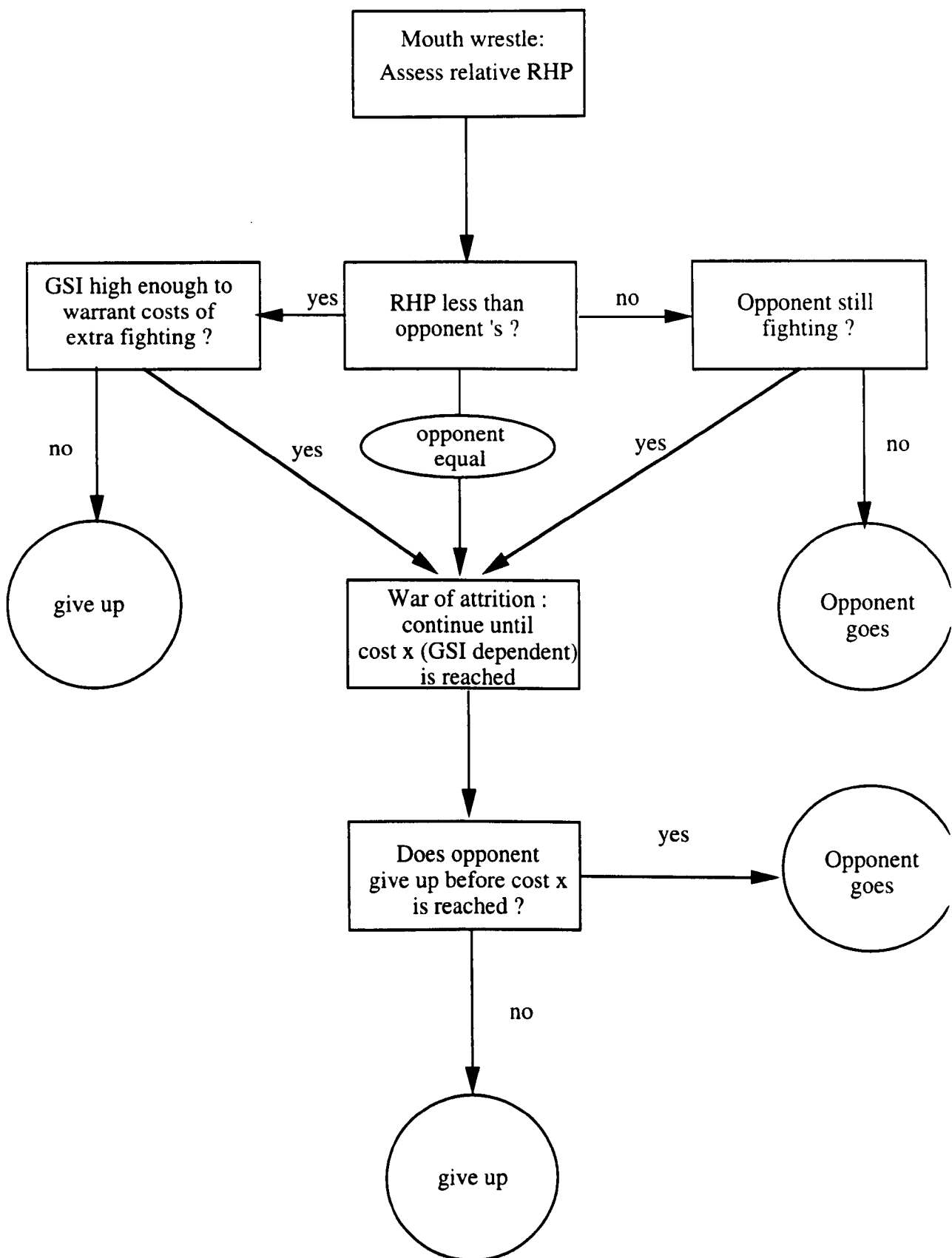
Figure A3.3.1 Flow diagram representation of how fights may be resolved by a GSI-dependent persistence rule.

A3.4 Formalising ideas and constructing the model

As we have no way of measuring the overall cost of fighting (see Chapter 5), we needed some measurable correlate of this function. For this we chose the duration of fighting because, whatever the exact nature of these costs, they must be an increasing function of the length of the fight (for example, in Chapter 5 it was shown that lactate production was positively correlated to fight duration). Our aim was to develop a model that would make predictions regarding fight outcome and the duration of the carouselling phase on the basis of asymmetries in body size and GSI. Thus, we proposed that each fish has good information on its size asymmetry (obtained from mouth wrestling) and that on the basis of this it can estimate the further time it will take it to win the fight (represented as T). T takes into account difference in body size by assuming that a fish larger than its opponent should expect to win the fight sooner due to its advantage. Mathematically, this is handled as follows (where t is simply the actual time since the process begins): for a pair of fish (i and j) that are equal in body size $T_i\,(t) = T_j\,(t)$ which we represent as $T_0\,(i,j)$. Then for each increment in asymmetry in body weight there is a proportional increase the elevation between $T_i(t)$ and $T_j(t)$ (see solid lines in Figure 7.4.1 where fish i is larger than fish j). Thus

$$T_i\,(t) = T_0\,(i,j) + k(B_j - B_i) - t$$

where

$T_0\,(i,j)$ = constant (the time it takes to win when fish have equal body weights)

$(B_i - B_j)$ = difference in body size

k = constant

t = time elapsed

Next, we consider that each fish has a maximum time for which it is prepared to fight for and after which it gives up because of physiological costs. Mathematically, this time-dependent physiological state variable, is represented as P (see dashed line in Figure 7.4.1). P is necessarily steeper than T, that is to say, it must 'run' faster than real time. This is handled by multiplying t by the term α (B_j) that is defined as being greater than 1, (although how much greater than 1 depends on body size). P also takes into account GSI, the effect of which is defined as being additive. Effectively this means that, a fish with a GSI above (or below) the average for the population will have an increased (or decreased) P. P is also affected (again additively) by absolute body size which was included because larger fish are likely to have a larger physiological capacity and inflict and sustain absolutely more damage. Thus;

$$P(i)t = cB_i + c1G_i - \alpha(B_j)t$$

where:

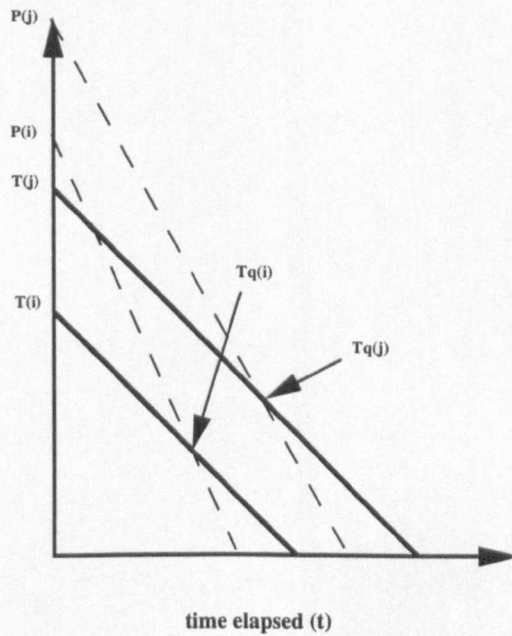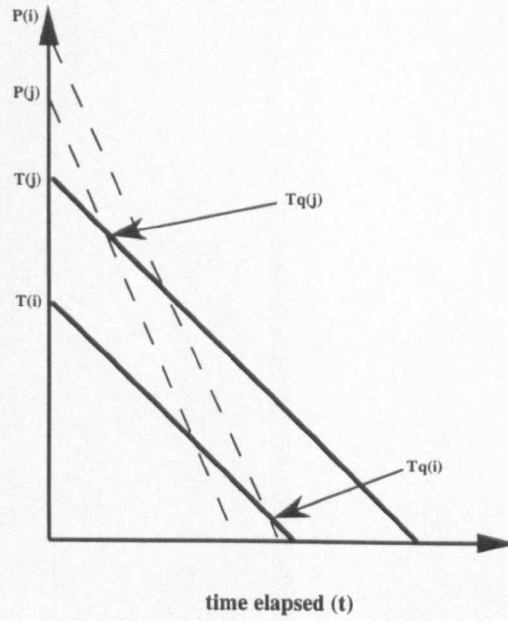B_i = the effect of absolute body size

G_i = the effect of GSI

c and $c1$ are both constants

$\alpha(B_j)t$ = body size-dependent physiological time

Estimated time to win (T)

Physiological time prepared to incur (P)

(top graph axes: P(i), P(j), T(j), T(i), Tq(j), Tq(i); x-axis: time elapsed (t))

(bottom graph axes: P(j), P(i), T(j), T(i), Tq(i), Tq(j); x-axis: time elapsed (t))

Figure A3.4.1 (top) Graphical illustration of the model. A pair of fish (*i* and *j*) differ in their body size and their GSI which determine the time it will take them to win (*T*) and the time that they are prepared to fight for before giving up (*P*) versus the time (*t*) since the carouselling phase began. Both fish persist until one of them reaches its quit time (T_q) when $T = P$. The first to quit is the loser, thereby defining the duration of the fight. In this case this is fish *j*. **Figure A3.4.2** (bottom) shows that by increasing the GSI of the smaller fish (*j*) and thereby raising P, the effect is to increase its quit time beyond that of the larger fish (*i*) resulting in fish *j* winning the fight.

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Each fish should then fight until its time-dependent physiological function exceeds the time it will take it to win the fight. Thus, each fish has a quit time Tq_i where:

$$T_i(t) = P_i(t)$$

This will be at the intersection of the two lines in Figure 7.4.1. The fight lasts the smaller of Tq_i and Tq_j (in Figure 7.2 this is Tq_j and fish i wins). Figure 7.4.2 shows how fish j could win a fight if it had a larger GSI and fish i had a lower GSI. However, there will come a point where body sizes are so disparate that the larger fish wins irrespective of GSI.

The model represents a number of ideas that I had formulated regarding how fights are resolved. These are;

1) That body size does confer an advantage (larger fish can expect to win sooner and have a greater amount of physiological time which 'runs out' slower).

2) That difference in GSI can cause a smaller individual to win, despite its size disadvantage.

3) That there will be a limit to what degree of body size advantage can be overridden.

4) The longest fights will be those between fish that both have high GSIs.

A3.5 Simulation of the model with generated data

Predictions of the model regarding which fish of a pair would win and how long the fight would last were further investigated by writing a computer program allowing fights to be simulated. The programming was done by Prof. Marc Mangel. The program generated a population of fish that vary randomly and independently with respect to body weight and GSI. It then picked, at random, pairs of fish from this population and simulated fights between them by calculating a quit time for each fish on the basis of the equations for T and P. The fish with the shortest quit time gives up and the output of the simulation is a figure for the duration of the fight and information as to whether the fish that won was larger or smaller.

It is desirable to be able to illustrate the output from the simulation such that the effects of body size and GSI difference on fight duration and outcome can be seen. One way of doing this is to calculate an index of GSI-dependent weight difference. This index is: the weight of the fish with the lesser GSI minus the weight of the fish with the greater GSI. This means that the smaller fish of the pair can have a negative weight difference (when it also has a lesser GSI) and a positive weight difference (when it has a greater GSI). Conversely, the larger fish of the pair can also have a negative weight difference (if it has a greater GSI) and a positive weight difference (if it has a lesser GSI). Figure 7.5.1 shows the general pattern of fight durations generated by the simulations. Figure 7.5.2 shows how the probability of the smaller fish winning changes as a function of weight difference.
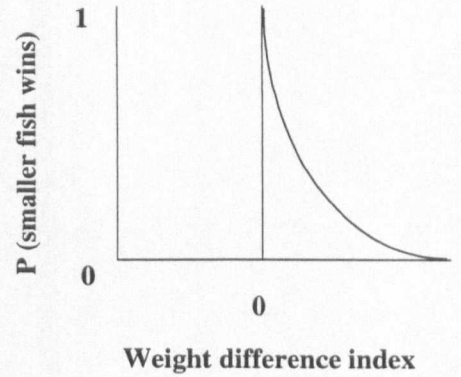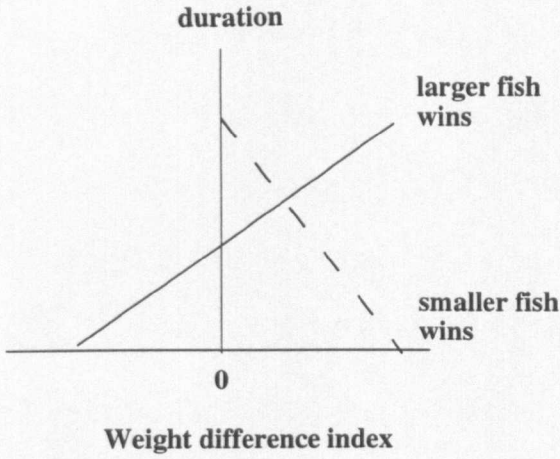
Figure A3.5.1 (left) Results of the simulation for fight durations as a function of GSI-dependent weight difference. **Figure 7.5.2** (right) Results of the simulation for the probability of the smaller fish winning as a function of GSI-dependent weight difference.
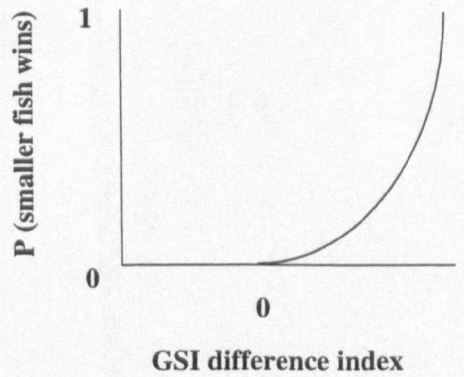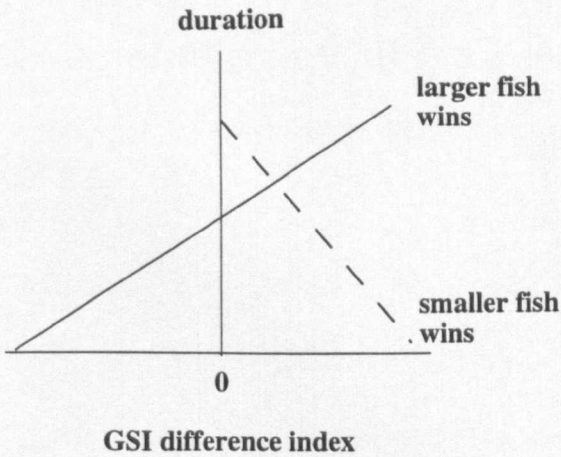
Figure A3.5.3 (left) Results of the simulation for fight durations as a function of weight dependent GSI difference. **Figure A3.5.4** (right) Results of the simulation for the probability of the smaller fish winning as a function of weight dependent GSI difference.

Using a similar notation, one can look at how weight dependent difference in GSI affects fight duration. For each pair, the difference in GSI is calculated as the GSI of the fish with the smaller body minus the GSI of the fish with the larger body. The smaller fish of the pair can have a negative GSI difference (when it has a lesser GSI) and a positive GSI difference (when it has a greater GSI). Conversely, the larger fish has a negative difference in GSI (when it has a greater GSI) and a positive GSI difference (when it has a lesser GSI). Simulated fight durations as a function of weight-dependent GSI difference are shown in Figure 7.5.3 and the probability of the smaller fish winning is shown in Figure 7.5.4.

In terms of when smaller fish are expected to win, a number of patterns are clear;

1) The smaller of the pair never wins if has a lesser GSI (Figures 7.5.2 and 7.5.4).

2) The probability that a smaller fish wins when it has a greater GSI starts to decrease as the difference in weight increases (Figure 7.5.2)

3) The probability that the smaller fish wins when it has a greater GSI increases as the difference in GSI increases (Figure 7.5.4).

In relation to the duration of the carouselling phase a general pattern emerges. The very long fights occur firstly and as would be expected, between pairs of fish that are closely matched for both weight difference and GSI difference. However, there is also the counter-intuitive suggestion that fights are very long where a larger fish is fighting a much smaller fish. Such circumstances are likely to be rare but arise because the smaller fish has a much greater GSI (see Figures 7.5.1 and 7.5.4).

A3.6 Testing the qualitative predictions of the model

The model makes a number of qualitative predictions regarding the duration of the carouselling phase and the probability of winning as illustrated in figures 7.5.1 - 7.5.4. The simplest way to compare the model's predictions with the experimental results is to calculate from the real data the weight and GSI difference indexes (see section 7.5) and plot these against the duration of the carouselling phase (see Chapter 2 for definition). These data are shown in Figures 7.6.1 (weight difference) and 7.6.2 (GSI difference).
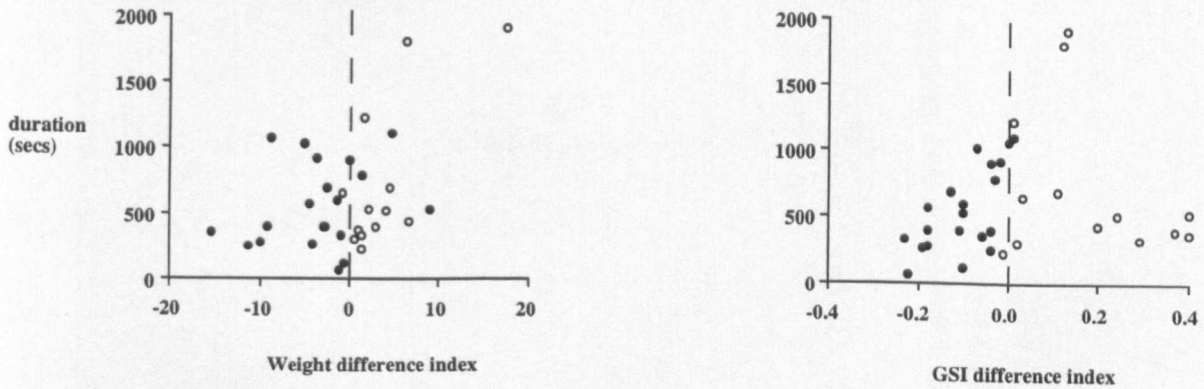
Figures A3.6.1 (left) The duration of the carouselling phase plotted against the weight difference index (weight of the fish with the lesser GSI minus fish with the greater GSI). Unfilled circles are those fights won by the smaller fish of the pair and filled cicles are those won by the larger fish of the pair. **Figure A3.6.2** (right) the duration of the carouselling phase plotted against the GSI difference index (GSI of lighter fish minus the GSI of the larger fish). Legend is as Figure. A3.6.1.

A3.7 Testing the quantitative predictions of the model

The experimental data (from Chapter 2) on GSI and body size differences in relation to duration can be used to estimate the parameters in the model and test whether the model fits observed values. Half of the data set was used to estimate the parameters in the model and the other half was then used to test if the model could predict the observed patterns. Parameter fitting was done by Prof. Marc Mangel. Model predictions generally did not provide a good match to experimental observation (see Table A3.6.1). Including a stochastic element to the model (to accommodate the fact that there may be some observational error) did not improve the fit to the data.

Table A3.6.1 A summary of the results of attempting to fit the model to real data. Parameters were estimated by using half of the data. Predicted durations were then calculated for the remainder of the data set and compared to the observed values.

	Number of fights in the range	
Time range of fight	Observed	Predicted
0 -300	5	9
300 - 600	14	8
600 - 900	6	14
900 - 1200	5	1
1200 - 1500	1	0
1500 - 1800	1	1
1800 - 2100	1	0

A3.8 Discussion

As discussed in Chapter 2, the resolution of animal fights is complicated. There is much evidence that asymmetries, such as the difference in body size between contestants, are used to resolve fights. In the case of *T. zillii*, asymmetries in gonadal state are more important than size asymmetries for the outcome of fights. This raises the question as to whether asymmetries such as this, that are not directly observable, can be assessed. A simpler alternative is that there is no assessment, and that fights are resolved by a 'which individual is prepared to pay the highest cost to retain its territory' rule. The purpose of this modelling exercise was to test this specific idea regarding the resolution of fights. The objective was to model persistence in the carouselling phase with a threshold giving-up time that is a function of asymmetry in GSI and body size, such that GSI could cause a smaller fish to win.

The model predicted that GSI could override the advantage of body size and this agrees well with the experimental results. However, the qualitative predictions from the model regarding the duration of the carouselling phase were not matched by the observations. There was no correspondance between the model and the the experimental results in relation to weight difference (compare fig A3.5.1 with fig. A3.6.1). Although there was a better fit between predicted and experimental patterns for GSI difference (compare fig. A3.5.3 with fig. A3.6.2), the correlations were not significant. That the model failed to account for the observations was further confirmed by the results of the quantitative test of the model (table A3.6.1). This could be for a number of reasons;

1) That fights are not in fact resolved by GSI dependent persistence with respect to a cost threshold. Detailed analysis of behavioural exchanged during the carouselling phase (Chapter 2) support this view. Winners and losers showed clear differences suggesting that assessment of something other than body size is occurring in the carouselling phase. The next logical step would be to consider a model in which GSI is assessed. However, modelling the complex process of assessment (see for example, Enquist & Leimer, 1983) is beyond the scope of project.

2) That the model's assumption that time is equivalent to cost is incorrect. As shown in Chapter 5, the costs of fighting has many components, some of which may be unrelated to the time spent fighting. A different measure of the cost of fighting may have been more appropriate to test the model with.

3) That there may be a large amount of observational noise in the data set because it is impossible to know when the assessment of body size finishes and carouselling 'War of Attrition' started. The measure I used for the duration of the carouselling phase was methodologically consistent but may not have been biologically consistent.

A3.9 Conclusions

The main benefit of modelling the resolution of fights in *T. zillii* has been that to make explicit the idea in question, namely that fights may be resolved by a simple GSI-dependent persistence rule. Simple behavioural rules have an inherent appeal, because the mechanisms underlying their execution are biologically conceivable as well as relatively easily modelled. As it turned out, the model suggested that fights

are not resolved in the hypothesised way. As with a negative experimental results, a negative theoretical result is an advance in its own right because it potentially allows one to reject one option of many and suggests the way forward for further analysis. In this case, it appears that the fish have a more sophisticated set of assessment rules than they were given credit for in the model.