ABSTRACT

Title: THE EFFECT OF AGONISTIC ENCOUNTERS ON AGGRESSIVE RESPONSE IN SOCIALLY ISOLATED SIAMESE FIGHTING FISH, *BETTA SPLENDENS*

Degree candidate: Debbie Charles

Degree and year: Master of Science, 2003

Thesis directed by: Professor Janet R.P. Halperin
Department of Animal and Avian Sciences

Male Siamese fighting fish were held for 7 to 10 weeks either in visually-social conditions (interact aggressively with a mirror image for 2 min, three times a week) or as visually-isolated fish (no aggression, saw only the back of a mirror). Phase I of this experiment investigated whether visually-isolated fish became hyper-aggressive, compared to visually-social fish. Aggression levels were tested with a series of models and mirrors. Social isolation did not affect duration or frequency of specific agonistic behaviors, nor of pooled behavioral categories (ANOVA, $P > 0.05$) within aggression tests. Phase II was to determine if aggression levels re-adjusted in visually-isolated fish experiencing live encounters among groups of 4 fish in naturalistic environments. Data was not significant unlike in previous isolation experiments (ANOVA, $P > 0.05$). However, several behavioral trends were observed that were consistent with previous isolation studies.

Keywords: Aggression; Aggressive display; Isolation; Siamese fighting fish

THE EFFECTS OF AGONISTIC ENCOUNTERS ON AGGRESSIVE RESPONSE IN SOCIALLY ISOLATED SIAMESE FIGHTING FISH, *BETTA SPLENDENS*

by

Debbie Charles

Thesis submitted to the Faculty of the Graduate School of the University of Maryland, College Park in partial fulfillment of the requirements for the degree of
Master of Science
2003

Advisory Committee:
Professor Inmaculada Estevez, Chair
Professor Janet R.P. Halperin, Advisor
Professor Larry W. Douglass
Professor William R. Stricklin

ACKOWLEDGMENTS

There are numerous people whom I would like to thank for helping me to achieve much success in my academic career. I would like to thank my advisor and graduate committee members. I would like to say a big thank you to Dr. Halperin for all of her effort, kindheartedness, and strength in trying to advise me and develop my research project. I would like to thank Dr. Estevez who was never too busy to take time out of her hectic schedule to encourage and give me many pointers in conducting my research project. I would like to thank Dr. Stricklin who was always there when I needed to talk about academics, career opportunities, or just life in general. I would also like to thank Dr. Douglass who always had his door open whenever I just dropped in to talk or had a question about statistics.

I would like to graciously thank the Halperin Lab. I would like to thank Kevin Donaldson who spent a lot of time and effort designing and building my Betta Population Tanks. I would like to thank Nicole Halperin who served as my sidekick whenever I needed an extra hand with fish maintenance and intensive observations. I would also like to thank my experimental animals, my Siamese fighting fish, for being patient with me whenever I teased them with a mirror or fish model.

A special thanks goes out to my University of Maryland family. I would like to thank the administrative staff for nurturing me when I needed comforting. I would like to thank my fellow graduate students for their sense of humor during the good and bad times of graduate life. I would like to thank special faculty members in the graduate school for answering my many questions and checking up on me to make

sure that my graduate process was a smooth one. A special thanks goes out to my friends from the Black Graduate Student Association. Thanks for all of your hospitality and making Maryland feel like a home away from home.

Last but certainly not least, I would like to thank the author and finisher of my faith- without you I would cease to exist. I would like to thank my family and friends, both old and new and far and near, who supported me in everything that I have done from this point on. Your encouragement, motivation, and prayers have helped me to achieve the very best that life has to offer. A special thanks goes out to my mother who molded me into the person that I am today, my sister who will one day follow in my footsteps, and to two very special men in my life for being in my corner whenever I needed them to be.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I. REVIEW OF LITERATURE

The review of literature in this thesis discusses various theories which question and study aggressive motivation within various laboratory animal species, primarily fish species. This review first provides background knowledge and a categorization of aggressive maneuvers that can generally be associated with Siamese fighting fish, *Betta splendens* and focuses on the theory of honest aggression and the implications of manipulating aggressive motivation with the use of social isolation. A discussion of popular hypotheses explaining the variation of aggression level as a result of social isolation is also included. Finally, a brief overview of several social reinforcement methods that have been proposed and used to quantify and measure an animal's aggression level is presented.

1.1 Background information on Siamese fighting fish, *Betta splendens*

Siamese fighting fish, *Betta splendens* originated in Siam, which is currently known as Thailand. These impressive fish are in the anabantoid family and have what is called a labyrinth, which serves as an accessory-breathing organ. The origin of the scientific name, *Anabantidae*, derives from the Greek verb, *anabaino*, meaning "to ascend" or "to go up", referring to the habit of such fishes as they swim to the surface to respire. This respiratory modification consists of a pair of irregular passages leading from the mouth cavity up behind eye, over the gill on either side, and apparently eventually to the swim bladder. Siamese fighting fish will rise to the surface to take gulps of air. The air bubbles pass through the labyrinth and provide supplemental oxygen to blood flowing through the vessels lining the membranes of the labyrinth and gills. The labyrinth organ enables Siamese fighting fish to live in warm, oxygen-depleted water habitats, such as rice paddies and ditches, under conditions that would considered to be lethal to most other fish (White, 1975).

Siamese fighting fish are bubble nest builders and nest guarders. The males build bubble nests at the surface of the water. These can be very large structures incorporated within pieces of vegetation. Males also take full responsibility for the eggs and fry. The male will display and chase the female to a spot below the nest. He eventually wraps himself around her in an embrace and she releases her eggs, which he will take up in his mouth and transfer to the nest in a bubble (Jaroenstasinee and Jaroenstasinee, 2000). Siamese fighting fish have been domesticated for many years, but there has been a very selective breeding process over the last decade. This was the result of breeding with the Plakat Thai, the true fighting fish of Thailand. This breeding process yielded Siamese fighting fish that have more solid colors, bigger longer flowing fins, and more exotic forms and variations (White, 1975).

The keen eyes of Siamese fighting fish can see color and are situated on their axis such that they can rotate outwards and provide excellent peripheral vision to the individual. The ability to see color and peripherally gives Siamese fighting fish a good advantage over other fish of relatively the same size in that they can avoid predation better. The ability to see color also affects the Siamese fighting fish habitat selection process (White, 1975). From the researcher’s point of view, the visual capability and reliance of visual social cues of fighting fish, serve as a great advantage for studying the physiology and behavior patterns of this species within laboratory settings.

1.2 Aggressive Behavior and Fighting Ability

The agonistic behavior of the Siamese fighting fish has long been a popular subject for ethologists. Siamese fighting fish do not fight to the death, as many people believe. If the fish have enough space the loser can withdraw. But the interactions are sometimes fierce where wounds are inflicted and the fins are sometimes torn. On occasion it may take several weeks for the fins to grow back and wounds to heal fully (Kuhn, 1970).

Siamese fighting fish are particularly well suited for investigations of aggressive behavior for several reasons. They engage in sequences of displays and attacks, which can easily be detected, and various components of the agonistic sequence are modifiable through learning. The detailed description of aggressive behavior and fighting ability found in Siamese fighting within this section was summarized and referenced from Simpson (1968).

An approach by one male to another that has just been placed in its tank is usually rapid and direct, especially over the last few fish-lengths. During the approach, the fish’s vertical fins are fully erect, except for the first few rays of the dorsal fins, which are sometimes laid back a little. The back may be humped slightly, the gill covers may be erect and the pelvic fins laid back for the whole approach or only over the last few inches of the approach. After an approach, a male may swim rapidly away in a slightly ‘zig- zag’, which can be referred to as thrashing, as if to show both sides of his profile to the fish immediately behind him.

When displaying fish are close to their partners and they turn to face, they withdraw their pelvic fins simultaneously, if the gill covers are not already lowered as the fish turns to face only when it is about to bite its partner. The broadside orientation is distinguished from facing by the position of the pelvic fin nearest to the partner. The other fin may be erect or it may lie along the belly.   Transitions between the broadside and facing orientations seldom take longer than a quarter of a second.

In the threat display of males, the body and especially the head darken until the body becomes intense in color. Fish that have been frightened go paler in tone, sometimes to a dull straw color, and they show black longitudinal stripes. The top stripe runs through the eye, and the lower one starts on the cheek. Both extend to the base of the tail. These stripes are sometimes visible even in fairly dark-toned fish. In a tank with fish that hold territories, single males without territories often have dark fins and a uniformly pale body. Pale-toned fish sometimes show a black spot at the base of the tail, and such spots are prominent in young and in wild fish.

Gill cover erection is a sudden increase in the distance between the distal edge of the operculum and the body. The end of a gill raising bout is marked by a sudden decrease in this distance, even if the opercula are not completely closed. In practice such changes are clear- cut, although different individuals have different degrees of opening for what are here recognized as ’open’ and ‘closed’ positions. When the gill covers are erected the brachiostegal membranes are more than four lengths from its partner, these changes in orientation are not necessarily accompanied by the changes in the pelvic fin positions, and are not so easily distinguished. Fish that make a series of short visits to a partner, or display-eliciting situation, seldom face and turn broadside more than once per visit. Fin spreading also occurs simultaneously with gill cover erection.   The dorsal fin is the last fin to be erected fully, probably because it has to be lifted the furthest against gravity.

Bites and nips are given by facing fish, and bites are likely to occur once a fish starts to lower its gill covers as it turns to face. A bite is a sharp opening and closing of the fish’s mouth, and it has been distinguished from a biting attempt where the mouth is held open for at least half of a second, and where the open mouth is not necessarily directed at the partner. Biting attempts, perhaps better termed biting threats, are likely early in display, especially when the fish is facing the front end of its partner.

Mouth fighting occurs sometimes after the fish have begun to bite each other, when one takes hold of the other’s upper or lower lip. They may spend as long as a few minutes pulling and tugging, often lying on the substrate, which may cause some risk of drowning. Tail beating has also been recognized by the suddenness and speed of a cupped tail moved in the direction of the partner. Tail beating has also been described in various cichlid fish.

1.3 Honest Aggression: A Zahavian theory

Conflict of interest occurs in many contexts in the lives of animals. A conflict of interest is a situation that arises when individuals act according to incompatible goals, interests or attitudes. It is because of these incompatibilities, individuals in conflict behave in ways that differ from their non-conflict driven behavior.   For many animals, conflict of interest, while unavoidable, may compromise the benefits of group living or relationships with neighbors, especially when it escalates into aggression. Conflict management includes both the behavioral mechanisms that prevent aggressive escalation of conflicts and those that mitigate or repair the damage caused by such escalation (Aureli et al., 2002).

For example, consider two equally matched opponents that both seek the same resource. They differ in the value that they place on this commodity. Should they end up fighting, it is likely that the animal that values the resource the most will fight harder and/ or longer and will eventually win. It might be optimal for each party to signal their perceived value of the resource at the outset. This signal would lead to the same outcome as if they had the fight but without all the risks and costs. However, this peaceful strategy could be easily invaded by a cheat that always signaled a very high value. One version of this strategy has been examined by Enquist (Enquist et al., 1985). In his model, information about valuation is provided by the sender through its choice of alternative actions. These could be pure displays or they could be a mixture of display and tactical actions, such as biting. Relevant sender costs could be necessary, incidental, or a mixture of both. Using this strategy for agonistic contests, honest signaling is likely only if costs and effectiveness are positively correlated. This strategy thus supports Zahavi’s handicap notion (Zahavi, 1975).

Zahavi argued that receivers ought not respond to signals unless they were honest. If a receiver does not respond to a signal, then there is no selection on senders to provide one. Receivers should thus have the upper hand in any arms race.

Given this control, the optimal strategy for a receiver would be to respond only to those signals that carry some guarantee of honesty. One way to do this is to require that signals impose a cost such that the sender could not afford to produce the signal, or would produce it in an ineffective manner, where the provided information is untrue. According to Zahavi's theory, costly signals are referred to as handicaps. Sender costs are necessary for honest signaling, and if these costs have the right relationship to benefits to make cheating uneconomical, honest signaling is the evolutionarily stable strategy (Bradbury and Vehrencamp, 1998). This was formalized by Grafen, who argued that a stable communication system would evolve only if cheating on the convention, displaying at an intensity typical of a stronger individual, increased the handicap associated with that particular display. If an escalation cost did not exist, a cheater could gain an advantage over honest competitors, and either a predisposition to ignoring the 'cheatable' signal would arise, or the 'cheater' strategy would invade the population. Since stable ritualized communication systems do exist, cheating must be costly, and honesty must pay (Grafen, 1990). In 1987, Popp focused on precisely this question using aggressive interactions between goldfinches at bird feeders during the winter. The results show a clear correlation between the risks of subsequent attack and the effectiveness of different displays. In addition, as the value of the resource increased, either because of decreased food or increased needs due to low temperatures, birds were more likely to adopt the riskier displays (Bradbury and Vehrencamp, 1998).

1.4 Social isolation and effects on honest aggression

Studies of socio-sensory deprivation and isolation effects have attracted students of animal behavior for more than one century. In this review of literature, isolation refers to the physical and visual separation of an individual from conspecifics. Generally, auditory and olfactory cues are also precluded, although it is possible that chemical or even auditory cues could have been present within some studies. In much of the literature on the effects of isolation, the concept of aggression is diversely defined. Most studies refer to the word in a broad sense and no distinction is made between attacks and displays. Different patterns of aggressive behavior may have different causes, which may serve a variety of functions or may be expressed in different contexts (Archer, 1988). In rodents, offensive and defensive forms of attack differ not just in motivation but also topography. Various species have been studied, including naturally territorial fish, and those, which are found in social groups for at least part of their lives. The experimental paradigms used to test fish after isolation periods have also been diverse, ranging from direct encounters, to mirror tests or the presentation of models, to a conspecific confined in a glass tube. Likewise, different aspects of aggressive behavior have been considered, but overt attacks (e.g. biting) and aggressive displays (e.g. circling, gill cover erection, lateral and frontal displays, fin spread) have been most commonly measured, and frequency and duration of these aggressive acts, as well as latency to approach and/or to attack a potential rival have been quantified (Gomez-Laplaza and Morgan, 1994).

Fish show a diversity of aggressive acts, whose motor patterns are rather stereotyped and simple to describe and measure. Nevertheless, little is known about the effects of isolation on these patterns. Much of the research on prior social experience and agonistic response has been done with Siamese fighting fish. The presence of considerable individual differences in aggressive behavior of male Siamese fighting fish has led to many investigators to speculate that this variability may be the result of wide differences in prior social experience, which can lead to varying levels of aggressive motivation (Cain et al., 1980). In 1974, Hinkel and Maier studied the recovery of aggressive responses in fish that had been habituated to their own images. The amount of aggressive display was greatest for animals that had not been presented with the mirror for four days and progressively less for animals deprived of the mirror for shorter periods (Hinkel and Maier, 1974). The results of other experiments have also suggested that fighting fish display response frequency not only increases with isolation but also with age and with highly responsive fish with which they are tested. (Miley and Burack, 1977). In a particular experiment, Miley and Burack (1977) found that visually experienced Siamese fighting fish showed longer latencies to conspecifics than did isolated Siamese fighting fish. Peeke and Dark (1990) also found that isolated male threespine sticklebacks, *Gasterosteus aculeatus,* were more aggressive than male threespine sticklebacks that had male neighbors. Several experiments conducted by Halperin and Dunham (1993) demonstrated that an ‘isolation effect’ was found in individually housed Siamese fighting fish. It was experimentally proven that socially and visually-isolated Siamese fighting fish became more aggressive than fish that were not socially and visually-isolated. Koyama (1993) also reported a similar isolation effect in several experiments conducted with lab mice that were individually housed in comparison with those that were group reared. Individually housed mice developed aggression levels that were significantly higher than that of group-housed mice. It was observed that isolated mice did not have a chance to learn how to use cut-off behaviors, social behaviors such as genital sniffing that need to be learned within a social environment, whereas group housed mice had learned how they could effectively function to avoid unnecessary escalation of interactions with another animal. These results appear to be in conflict with those generally found in male cichlid fishes isolated for similar periods of time. For example, in male *Haplochromis burtoni* the number of attacks increased only after they had been isolated for between 2 and 7 hours. After 3- 12 days in isolation, however, the rate of attacks decreased when first presented with a social partner (Ferno, 1978).

1.4.1 Hypotheses on the effects of social isolation

Why is the effect of isolation on aggression important to study? To explain the diverse effects occurring as a result of social isolation, a number of different explanations have been postulated. The investigations undertaken have often had different objectives, and essentially similar post-isolation behavior patterns have been interpreted in different ways. Attempts to explain post-isolation aggressive behavior generally assume that aggression is increased by such treatment (Gomez-Laplaza and Morgan, 1994).

One of the earliest hypotheses that have been forward to explain the increase in aggression following isolation is that of Lorenz (1966) who suggested that depriving an animal of the opportunity to attack results in the build-up of aggressive motivation. It has also been suggested that many results concerning increased aggression after isolation can be explained in terms of recovery from pre-isolation habituation. This is sometimes referred to as the dis-habituation hypothesis, which suggests an individual habituates to the continuous or repeated presence of conspecifics with a corresponding waning of the aggressive response and recovers after an isolation period, consequently increasing the aggressive response. It has been shown by Klein et al. (1976) that in adult male fighting fish, a 6 hour habituation period of threat behavior components, during which pairs of fish had visual contact, produced significantly fewer attack behaviors during subsequent encounters, than in those pairs kept visually-isolated during the same period of time. This habituation mechanism may account for some of the diverse outcomes of isolation effects on aggression, but more specific tests of hypothesis are necessary (Klein et al., 1976).

An alternative explanation for the high aggression shown in live encounters by previously isolated fish of some species, suggests an experience- leveling effect of isolation (Franck et al., 1985). This hypothesis states that the previous agonistic experiences of socially living animals are reduced by isolation, through a process of forgetting prior experiences, leading to an increased frequency of aggressive actions in subsequent encounters. Although, the social isolation of swordtails (*Xiphophorous helleri*) for 4 weeks resulted in decreased attack readiness. It was concluded that external stimuli from conspecifics are essential for maintaining the level of aggression readiness characteristic of socially living males. Franck and Ribowski (1987) also found that prior experience of dominance or subordination, winning or losing, can affect later aggressive encounters. Prior losing experience often decreases the likelihood of an individual winning a subsequent contest (Baenninger, 1970). The effect of a winning experience seems to vary among studies. Some studies detected an increase while others observed no significant difference in the probability of winning a subsequent contest after a winning experience. But the general result of these studies is that when previous fighting experience significantly affects an individual's probability of winning its next contest, it also affects the individual's likelihood of initiating the contest. In a recent study of cyprinodontid fish, *Rivulus marmoratus* conducted by Hsu and Wolf (1999), individuals were given different combinations of two fighting experiences to investigate how a recent experience (given 24 hours prior to the contest) and a penultimate experience (given 48 hours prior to the contest) were integrated to influence the outcome of a subsequent contest. The study showed that winning and losing experiences had opposite but equal effects on the fighting outcomes of subsequent contests and both winning and losing effects lasted for at least 48 hours (Hsu and Wolf, 2001).

An increase in aggressive responsiveness of isolates has also been attributed to an increased sensitivity to environmental stimuli resulting from stimulus deprivation. The major proponent of the hyper-reactivity hypothesis in fish was Davis (1975), who suggested that a lowering of the threshold for stimuli in general was a consequence of the low level of external stimulation in isolates. Davis (1975) found that social isolation of a few days caused an increased reactivity to diverse stimuli in the paradise fish, *Macropodus opercularis*, and proposed that isolation affects readiness for social display by having general effects on behavior. The increased intensity of display behavior in isolates is regarded as an expression of general reactivity and not necessarily a direct effect of isolation on aggressive motivation itself.

Two hypotheses have been proposed to explain the decreased aggression in fish that sometimes follows isolation. The first of these suggests that the repeated presentation of specific external stimuli from models or live conspecifics is necessary to maintain a high level of aggression in adult males of some fish species. Without such stimulation, a low rate of attacks follows a period of isolation. Eventually, the reversal of the reduced aggression is shown by Siamese fighting fish after prolonged visual isolation, by daily visual exposure to opponents (Meliska et al., 1980). An interesting explanation proposed by Meliska et al. (1980) for achieving such results can be attributed to the fact that in this particular experiment paired fish shared common water, which could have affected responses to visual isolation. Some fish release behaviorally potent pheromones, which can implicate social interactions in some fish. Similarly, Todd (1971) found reduced aggression in catfish exposed to water resided in by conspecifics that were non-combative. These studies suggest that pheromones released by neighboring conspecifics may modulate the effects of visual isolation and/or visual exposure by modifying thresholds for agonistic behaviors. The second hypothesis is that of Halperin and Dunham (1993), who suggested a ‘dual process hypothesis’, to account for the decrease in aggressiveness some times observed following isolation. According to this hypothesis, two dynamic processes occur simultaneously during social isolation in fish; an initial process that decreases the isolated animal’s readiness to respond to social stimuli and another stable process which progressively increases the motivation to perform social behavior, resulting in high rates of aggressive display. The former process is assumed to be rapidly reversible and disappears soon after social stimuli reappear.

1.4.2 Implications on the effects of social isolation

The potential for isolation to increase or even decrease aggressiveness in animals has important consequences. An animal's ability to assess an opponent's fighting ability, either through direct combat or displays correlated with fighting ability, allows the individual to avoid engagement in costly fights with opponents that it is unlikely to beat. To assess the fighting ability of opponents, animals often use signals produced during contests (Wagner, 1989). By altering the relationship between a signal and fighting ability, signalers can potentially exploit the behavioral rules of receivers through the production of misleading signals; for example, through the production of signals that receivers interpret as conveying a fighting ability greater than the signaler actually possess (Wagner, 1989). When hyper-aggressive fish were matched with fish with normal aggression levels, hyper-aggressive fish aggressively displayed more intensely when in combat. Hyper-aggressive fish that bluffed while in combat but did not have a high fighting ability to back up the displays usually lost their fights (Halperin et al., 1998). Similarly, in a study on colony formation, individually reared rats suffered from more serious physical injuries than socially reared rats. During exposure to an aggressor, rats that were individually housed during the play period took longer to display a submissive posture and did not emit the expected behavioral responses to reduce the number of further attacks. This inefficient behavior strategy resulted in more serious injuries (Luciano and Lore, 1975).

1.5 Studies on social reinforcement

A recurring problem in the study of aggressive behavior concerns the extent to which such behavior is under the control of internal factors in the animal. Hogan and Roper (1978) have suggested that the controversy surrounding this issue is similar to the controversy surrounding the issue of whether some behavior is innate or learned, and that little can be gained by insistently categorizing motivational systems in terms of their susceptibility from internal to external factors. The manipulations that are effective in changing the probability that a particular behavior will occur in a standard situation vary depending on the motivational system to which the behavior belongs. The passage of time with no opportunity to engage in a particular behavior leads to stable, predictable changes in the eating and drinking behavior of rats and many other animals, while deprivation of aggressive behavior has mixed and variable effects. The purpose of the experiments reported in several papers was to explore the effectiveness of a different manipulation in the changing the level of aggressive motivation with the use of social reinforcement or ‘priming’. Priming of aggressiveness is a well-documented phenomenon in fish. Siamese fighting fish willingly display to various types of mirrors and models (Kuhn, 1970). Several authors have noted that the effect of visual isolation is to increase the number of displays to a mirror image. According to Thompson (1966), if a male Siamese fighting fish that has been exposed to the visual image of another male along one wall of an aquarium is observed for some time following exposure, one will see the subject return to repeatedly to the area where the intruding male fish was encountered. As the subject periodically swims along the aquarium where the intruder was presented, the fish may display aggressively. Such observations suggested that presentation of this releasing stimulus might act as a reinforcing consequence for an operant response. It seems that the display behavior, the behavior occurring in the absence of the appropriate unconditioned releasing stimulus, might come under the control of other stimuli which had been associated with the behavior. This releaser can act as a positive reinforcer for an operant response since its contingent presentation increases the frequency with which the operant occurs. A releaser has the same functional properties as other stimuli, which are capable of acting as unconditioned stimuli in a classical conditioning paradigm ad functioning as reinforcers for operants, such as food.

According to Baenninger (1970), a variable that may affect the response rate in the visual reinforcement situation is the prior social experience of a subject. To a fish that has experienced being dominant in a paired encounter, the presentation of a mirror is more reinforcing than it is to a fish that has experienced being subordinate. It is possible that some difference in aggressiveness between male fish enables some to have experience as dominant members of pairs while others, lacking such aggressiveness, are relegated to subordinate roles. In 1966, Baenninger found that aggressive displaying by dominant male fish was suppressed less by catecholamines than was displaying by subordinate males. This difference suggests that a difference exists between dominant and subordinate fish in pharmacological tolerance. Studies conducted by Cain et al. (1980) showed that fish that eventually became dominant displayed more often than their subordinate opponents before dominance was established, although both eventually dominant and subordinate fish attacked at similar rates until submission occurred. Although submissive and non-aggressive behaviors of the opponent appear to inhibit attacks, they do not have the same effect on displays. Dominant fish did not show a significant decrease in displays once their opponent submitted, nor did fish display differentially to aggressive and non-aggressive opponents.

The results of other experiments have suggested that Siamese fighting fish display response frequency increases not only with isolation but also with age and responsiveness of the fish with which they are tested. For example, Hogan and Bols (1980) found that in fighting fish, swimming speed in a runway for a view of a conspecific was shown to be positively correlated with vigor of aggressive display, where reduced runway times reflect increased aggressive motivation. Priming also increased the choice of conspecific over food, but only in the presence of particular external stimuli. These results were interpreted to mean that the level of aggressive motivation is determined by a complex interaction of internal and external factors. The internal state can vary as a result of unspecified factors and a result of priming. The results also suggest a basic similarity among motivational systems, such as aggression, hunger, and thirst, in the relation between level of motivation and instrumental behavior; increasing level of motivation leads to better performance in terms of both choice and speed. These results confirmed what Hogan (1974) found. Fish that lived in a T-maze and were tested within their home environment swam much faster for food than for a mirror image, while fish tested in an unfamiliar environment swam the same speed for both reinforcers. However, Bronstein (1981) debates the previous findings by Hogan and Bols (1980) by stating that male fighting fish chosen for having the potential to show moderate or high levels of aggression will turn toward another aggressive fish located nearby. The mechanism of choice appears to be the subject's lunging at the opponent, rather than its learning either where the opponent or which of its movements will be followed by social contact. Also, conspecifics located within a maze become increasingly attractive when the animals located there are positioned so that subjects can probably see those animals prior to entering the maze themselves.

In 1992, Halperin et al. conducted an experiment that compared responses of fish after three weeks during which controls displayed to a conspecific for two minutes every other day, while isolates saw a complex visual stimulus, which did not elicit a display. Again, isolates had depressed display readiness, but higher display rates once they were primed with visual stimuli. These results support the dual process hypothesis, proposed a year later that social isolation has two effects on aggressive display in Siamese fighting fish; causing decreasing readiness to display, but leading to greater display rates once fish are primed. Priming of aggressive behavior is also clearly demonstrated in the golden hamster (Potegal and ten Brink, 1984).

All of the topics that were previously presented in this review of literature provide a foundation of information in which we sought to build upon within our research study.

CHAPTER II THE EFFECT OF SOCIAL ISOLATION ON AGGRESSIVE BEHAVIOR IN SIAMESE FIGHTING FISH, *BETTA SPLENDENS*

2.1 Abstract

The objective of this study was to investigate whether social isolation altered the aggressiveness of Siamese fighting fish compared to fish that had not been visually-isolated. One hundred twenty-eight male Siamese fighting fish were balanced into a visually-social group and a visually-isolated group. Visually-social fish could aggressively interact with a mirror image for 2 min, three times a week, but visually-isolated fish saw only the back of a mirror, which did not release aggressive display for a period of 7 to 10 weeks. After isolation, aggression levels were tested using a sequence of mirrors and models. Social isolation did not have an effect on duration and frequency of aggression and on the duration of aggressive behaviors displayed within each behavioral category (ANOVA; duration of behaviors, $P > 0.05$), (ANOVA; frequency of behaviors, $P > 0.05$), (ANOVA; duration of behaviors within behavioral category, $P > 0.05$).

Keywords: Aggression; Aggressive display; Isolation; Siamese fighting fish

2.2 Introduction

The aim of most studies dealing with the effects of isolation on aggressive behavior in fish is to determine the conditions that promote or reduce its behavior, so as to increase our understanding of how social interactions are regulated. In this sense, isolation has proven to modify aggressive behavior, having quantifiable effects on aggression level in many fish species (Gomez-Laplaza and Morgan, 1994). Fish show a diversity of aggressive acts and motor patterns, which are rather stereotyped and simple to describe and measure. Nevertheless, little is known about the effects of isolation on these patterns. Much of the research on prior social experience and agonistic response has been done with Siamese fighting fish. The presence of considerable individual differences in aggressive behavior of male Siamese fighting fish has led to many investigators to speculate that this variability may be the result of wide differences in prior social experience, which can lead to varying levels of aggressive motivation (Cain et al., 1980).

Ethologists have long been interested in the effects of social deprivation on aggression. The emotion-charged reaction to Lorenz's statement (Lorenz, 1966) that "Intraspecific aggression is in man just as much as a spontaneous instinctive drive as in most higher vertebrates", has made the question of whether aggressive tendency builds up during social isolation, an issue of political contention as much as a matter for scientific enquiry. However, literature on various animals report many effects of social isolation on aggression, including depression, elevation, and mixed effects.

In 1992, Halperin et al. conducted an experiment that compared responses of fish after three weeks during which controls displayed to a conspecific for two minutes every two days, while isolated fish saw a complex visual stimulus which did not elicit a display. Isolated fish had depressed display readiness, but higher display rates once they were primed. Halperin and Dunham (1993) demonstrated that an ‘isolation effect’ was found in individually housed Siamese fighting fish that were visually-isolated. It was demonstrated that visually-isolated fighting fish became more aggressive than fish that were not visually-isolated.

The focus of this current research was to investigate whether social isolation altered or perhaps even increased the aggressiveness of visually-isolated Siamese fighting fish compared to that of visually-social fighting fish as it was determined in the previous study (Halperin and Dunham, 1993). It was necessary to check whether social isolation had increased aggressiveness in our study. Theory suggests that social isolation can possibly increase fear during isolation as well (Halperin and Dunham, 1993).

We hypothesized that social isolation would affect the aggressiveness of Siamese fighting fish due to the lack of aggression releasing stimuli that are present in the social environment in which they live. As a result of this, aggression levels observed in visually-isolated fish will be altered when compared to that of fish that were visually-social.

2.3 Animals, Materials, and Methods

2.3.1 *Study Location and Animals*

This experiment took place during Spring and Summer 2003. Approximately 128 male Siamese fighting fish were obtained from a local commercial supplier. The 128 fish were chosen on the basis of four distinct colors (32 blue fish, 32 red fish, 32 iridescent- turquoise fish, 32 brightly- colored) with the intention of being able to easily identify each individual when they were grouped together. Each fish was individually housed in a 25cm x 12.7cm x 12.7cm plexiglas tank in room B of Lab 0148 in the Animal & Avian Sciences Building. The temperature was maintained between 21-23º C and the lighting cycle was maintained on a 12:12 h light and dark cycle. The 128 fish were divided into four separate experimental groups of 32, which were housed and tested at different time periods within the study (See Appendix- Table 4.1). The fish were fed TetraMin Betta Bites daily.

2.3.2 *Group Balancing*

After arrival, each fish was exposed to a mirror for 2 min. Each individual's aggressive display to its mirror image was timed and recorded. The latency to display was used to balance the fish into two experimental groups: (control group- visually- social and experimental group- visually-isolated). Latency was defined as the time interval between the placement of the mirror in front of the individual until the individual had performed an accumulated duration of gill display, a behavior highly associated with aggression, for approximately 5 s. The purpose of this method was to ensure that both fast-responding and slow-responding fish, as a result of seeing their mirror image, would be equally distributed in the visually-social and visually-isolated group.

2.3.3 *Experimental Treatment (Differential Isolation)*

For a period of 7-10 weeks, fish were differentially socially isolated for either 7 or 10 weeks in room B of Lab 0148. Opaque paper dividers were placed between each tank to prevent individuals from seeing one another. During the 7-10 week period of social isolation, a visual stimulus, a mirror, was shown to the ‘visually-social’ group. The 12.7cm x 25cm mirror was placed at one side of the tank for a 2-min period. Individuals in the visually-social group typically displayed to their mirror image for the 2-min period. Simultaneously, individuals in the visually-isolated group were exposed to the ‘back of a mirror’ for the same length of time. Showing the back of a mirror did not provide individuals in the visually-isolated group with an opportunity to see their mirror image and therefore were not given the same opportunity to display to their mirror image as the visually-social group. Visual stimulation was shown 3 times a week. The times at which visual stimulation was shown to both the visually-social and visually-isolated group varied on each day so the fish would not become conditioned to expect stimulation at a particular time of day. The purpose of this phase of the experiment was to ensure that individuals in the visually-isolated group would become hyper-aggressive as a result of being exposed to the back of a mirror and not being given the opportunity to aggressively display to their mirror image for an extended period of time. Neutral stimuli were also presented to the both groups during the last 2 weeks of isolation. Neutral stimuli consisted of various objects such as pen caps, mesh backing, black paper, which did not resemble a fish stimulus in any shape or form. This was done to prevent fish from associating the researcher with only aggression releasing social stimuli. This isolation effect on aggressive behavior was documented in an experiment conducted by Halperin and Dunham (1993).

2.3.4 *Aggression Test*

At the end of the 7-10 week period of differential isolation, the aggression levels for all of the fish were tested. Aggression tests consisted of showing a specific sequence of stationary neutral stimuli, fish models, and mirrors over a 2- day period. It has been noted in previous experiments that social isolation has two effects on aggressive display in Siamese fighting fish; decreased readiness to display and greater display rates once fish were primed. Priming consists of showing potentially aggression-releasing stimuli. Day 1 served as a priming day. Each stimulus used for priming purposes was shown to each fish for 5 min, while on Day 2 the stimuli served to measure aggression levels and were shown for 2 min (Table 2.1).

Table 2.1. Order arrangement and timing schedule of priming stimuli.

Order (Fixed or Random)	Stimulus	Duration (min)
1. Fixed	crumpled –beige paper	5
2a/b. Random	green plant model and red (broadside) fish model	5 5
	or	
3a/b. Random	red plant model and green (broadside) fish model	5 5
4. Fixed	mirror	5

Priming on Day 1 also served the purpose of familiarizing fish to testing situations so that aggression levels could be more accurately measured. Priming stimuli were shown in an ascending order based upon the ability of the stimulus to evoke an aggressive response from each fish. For example, the fish were not expected to display to the crumpled beige paper because it was a neutral stimulus and was not intended to release aggression. However, it was expected for all of the fish to display to their mirror images because it simulated an encounter with a live conspecific. There was a 10-min break between the presentation of each priming stimulus because it is still undocumented how long it takes for priming to occur.

On Day 2, a similar set of stimuli was used to conduct aggression tests and measure aggression levels for each fish (Table 2.2).

Table 2.2. Stimulus sequence and timing for aggression test.

Stimulus	Duration (min)
Priming mirror	2
BREAK	2
Test mirror	2
BREAK	6
Small blue (facing) fish model	2
Large blue (facing) fish model	2
Medium white (facing) fish model (with light scale markings)	2

The first mirror on Day 2 served as a priming mirror and the second mirror was used as an aggression test stimulus. Again, this was done to ensure that each fish was primed sufficiently before the aggression tests were conducted. There was only a 2-min break between the presentation of the first mirror stimulus and the second mirror stimulus, since it was presumed that priming would have been largely complete as a result of showing stimuli on Day 1. The 6-min break between the test mirror and model sequence was chosen only for scheduling purposes and was not expected to have a large impact on the efficacy of the test itself. Each fish model (small blue facing fish model, large blue facing fish model, and medium white facing model) was shown consecutively in the listed order. The model sequence was prevalent for trying to determine if an individual fish's response was hyper- aggressive or hyper-fearful.

For example, we presumed that all of the fish would display to the small blue facing fish model because it represents an easily dominated rival that can be actively threatening. A hyper-aggressive fish should aggressively display to the small blue facing fish model and the large blue facing fish model as well. However, if an individual fish displayed to the small blue facing fish model but did not respond to the large blue facing fish model, it may have simply habituated to the model or it might be aggressive but have its aggression masked by fear. To distinguish this we immediately showed fish a medium sized white facing fish model. The white fish model represented a mild aggression-releasing stimulus because a white color is not expected to yield an aggressive display from an observing fish but the ‘fish-like’ shape of the model would have some capacity to release a display. The size of the response can therefore be affected by any traces of aggressive motivation induced by the large blue fish model. So while we expected a hyper- fearful fish to avoid or not aggressively display to the large blue fish (facing) model; a model that was designed to elicit fear as well as aggression; if it was hyper-aggressive as well as hyper-fearful, it should display again to the white (facing) fish model because it would not be intimidated by the mild stimulus and so could express any aggressiveness that had been aroused by the large blue model.

Aggression tests were videotaped in room A of Lab 0148, which was also maintained between 21-23º C and the lighting cycle was maintained on a 12:12 h light and dark cycle. Displays such as fin down, gills out and orientations such as broad close, broad far, face close, face far, avoidance, thrashing, and other were recorded and documented with the Noldus Observer 4.1 program (refer to glossary of terms for definitions). Behaviors that were recognized as displays or orientations were considered to be mutually exclusive. The Noldus Observer 4.1 program was used to calculate frequency and duration of both displays and orientations.

2.4 Statistical Analysis

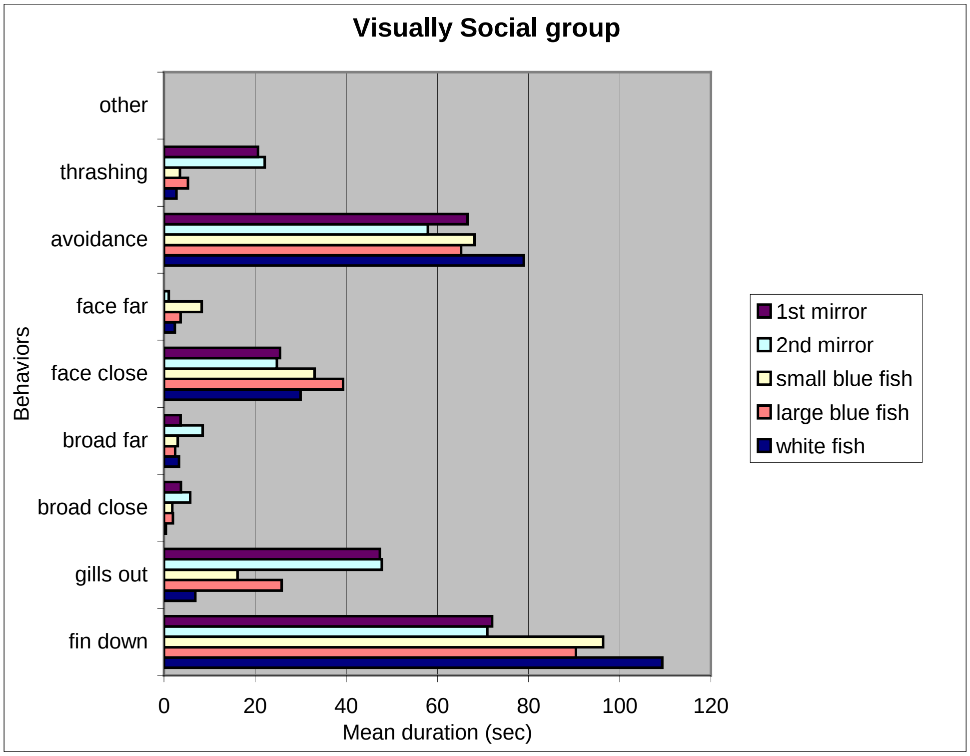
Duration and frequency of aggressive response to each stimulus used within aggression tests were analyzed using analysis of variance (ANOVA). The effects of the visually-social treatment and visually-isolated treatment on aggressive response to the repeated presentation of stimuli were analyzed using a mixed model ANOVA with repeated measures for stimulus (The SAS System for Windows V8). Sources of variation included the fixed effects for treatment and stimulus. Repeated measure structures in SAS were also used to fit a correlation among the ordered repetition of stimuli. The model residuals indicated homogenous variances and were analyzed without transformation. Due to the low frequency of behaviors observed during aggression tests, data for behaviors were averaged and coded into four specific behavioral categories; high aggression (face close, gills out), high fear/aggression (face far, gills out), high fear (avoidance, fin down), high aggression/high fear (gills out, face far, thrashing, fin down, avoidance) (refer to glossary of terms for definitions). Categorical durations were then separated into 3 levels, by dividing the 2-min duration for each stimulus into 3 intervals, and ranked from 0 to 2: (0-40s: 0=low display; 41-80s: 1= medial display; 81-120s: 2= high display). A logistic analysis of variance (ANOVA) was performed using the generalized mixed model macro of SAS, version 8 (GLIMMIX). The error option was set to Poisson to compensate for the multivariate coded categories being analyzed. Statistical significance was accepted as $P<0.05$.

2.5 Results

In this study, social isolation did not have an effect on aggressive motivation, which was measured by the duration of behaviors to a series of models and mirrors following the 7-10 week isolation period (ANOVA, fin down P=0.1936; gills out P=0.1176; broad close P=0.5981; broad far P=0.0821; face close P=0.6754; face far P=0.1050; avoidance P=0.6362; thrashing P=0.3371; other P=0.3284) (Figures 2.1a and 2.1b). (Standard errors for behaviors; fin down : ± 14.03, gills out: ± 14.41, broad close: ± 2.27, broad far: ±4.65, face close: ±13.29, face far: ±2.69, avoidance: ± 15.76, thrashing: ±9.30, other: ±1.15). Social isolation was also shown not to have an effect on the frequency of behaviors displayed by fish represented in both the 'visually-social' and visually-isolated group to deviate from one another during the 7-10 week isolation period (ANOVA, fin down P=0.5101; gills out P=0.0820; broad close P=0.9195; broad far P=0.0045; face close P=0.1121; face far P=0.9463; avoidance P=0.8610; thrashing P=0.8735; other P=0.7499) (Figure 2.2a and 2.2b). (Standard errors for behaviors; fin down : ± 14.03, gills out: ± 13.77, broad close: ± 2.27, broad far: ±4.65, face close: ±13.29, face far: ±2.69, avoidance: ± 15.76, thrashing: ±9.30, other: ±1.15).

The categorical data reiterated that in all of the behavioral categorical, with the exception of high fear, at least 92% of the displays were ranked at level 0, indicating a low level of display for each category. Within the high fear category, 57% of the displays were ranked at level 0. Once again, social isolation did not have an effect on the duration of behaviors displayed in any behavioral category (ANOVA, high aggression P=0.2804, high fear/aggression P=0.5011, high fear P=0.5375, high aggression/high fear P=0.9091) (Figure 2.3a and 2.3b).

Figure 2.1a and 2.1b . Mean duration of behaviors within the visually-social and visually-isolated group to stimuli used in aggression tests. Duration of behaviors (ANOVA P>0.05) (refer to section 2.5 for standard errors).



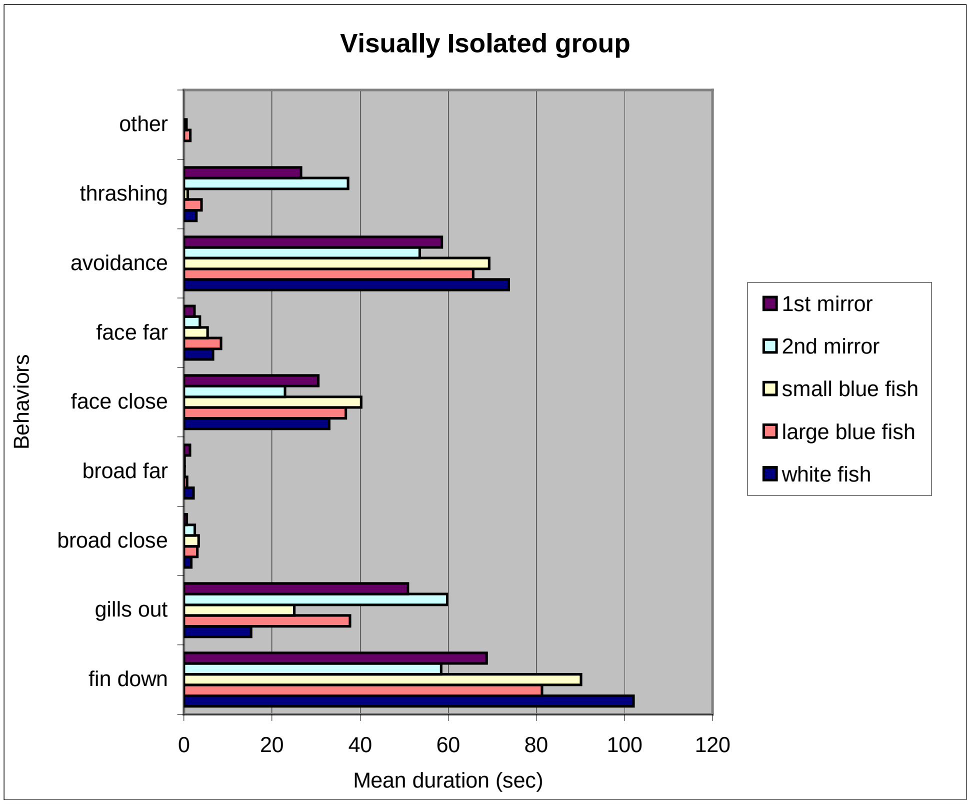


Figure 2.2a and 2.2b. Mean frequency of behaviors within the visually-social and visually-isolated group to stimuli used in aggression tests. Frequency of behaviors (ANOVA P>0.05) (refer to section 2.5 for standard errors).

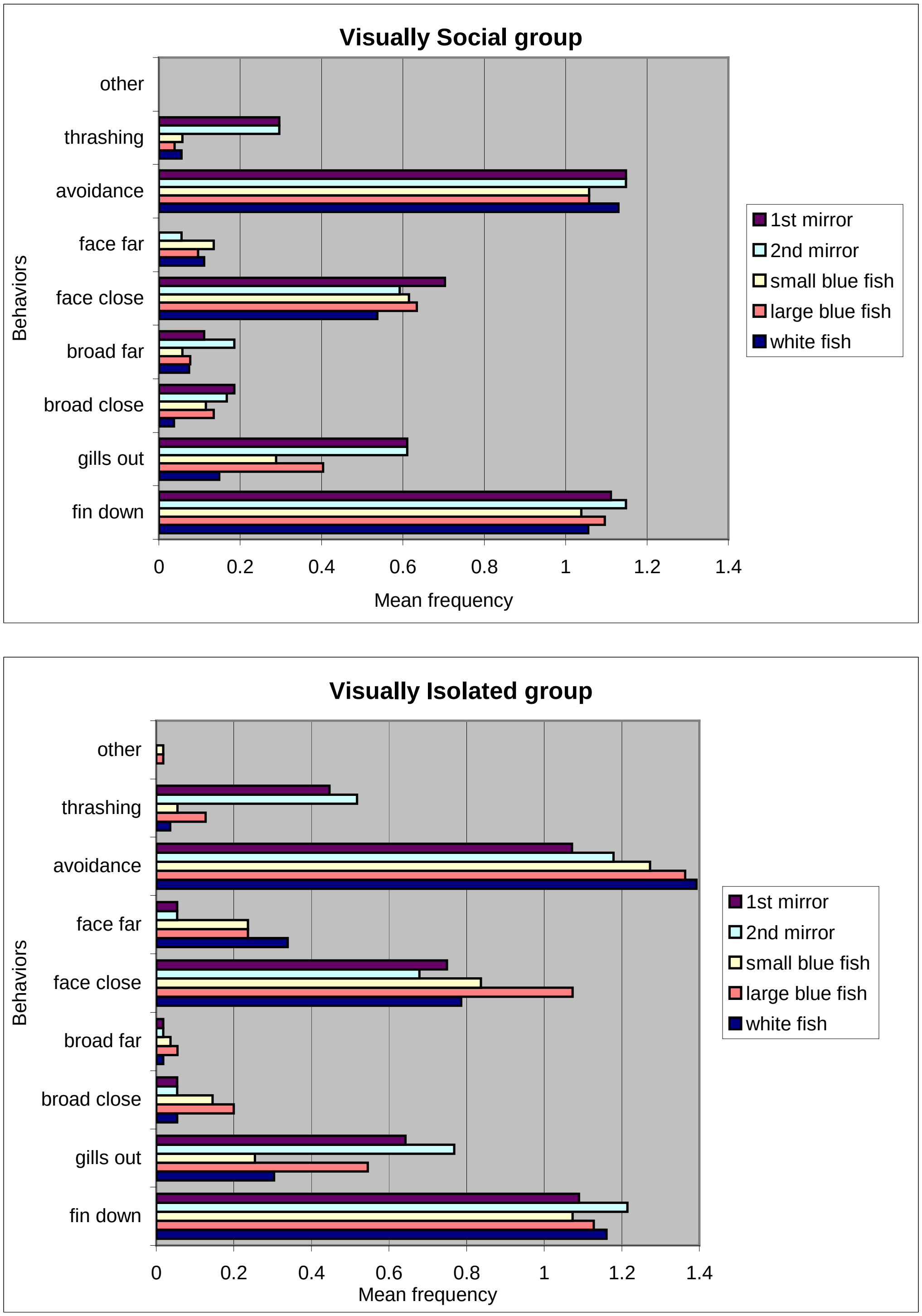
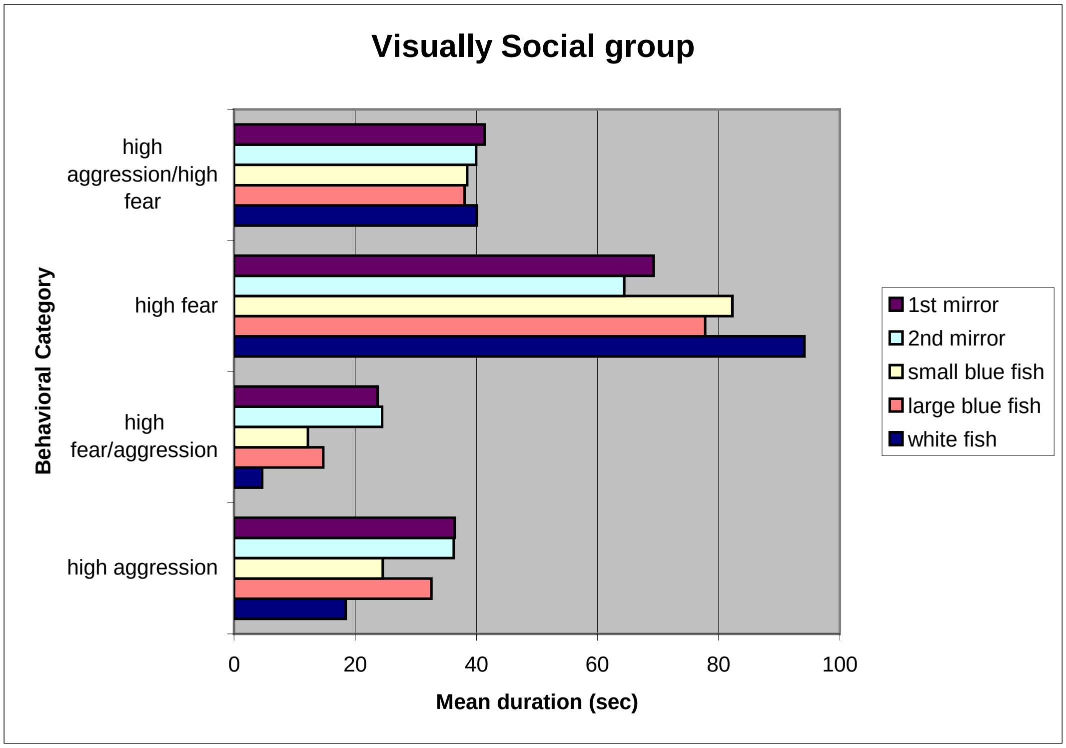
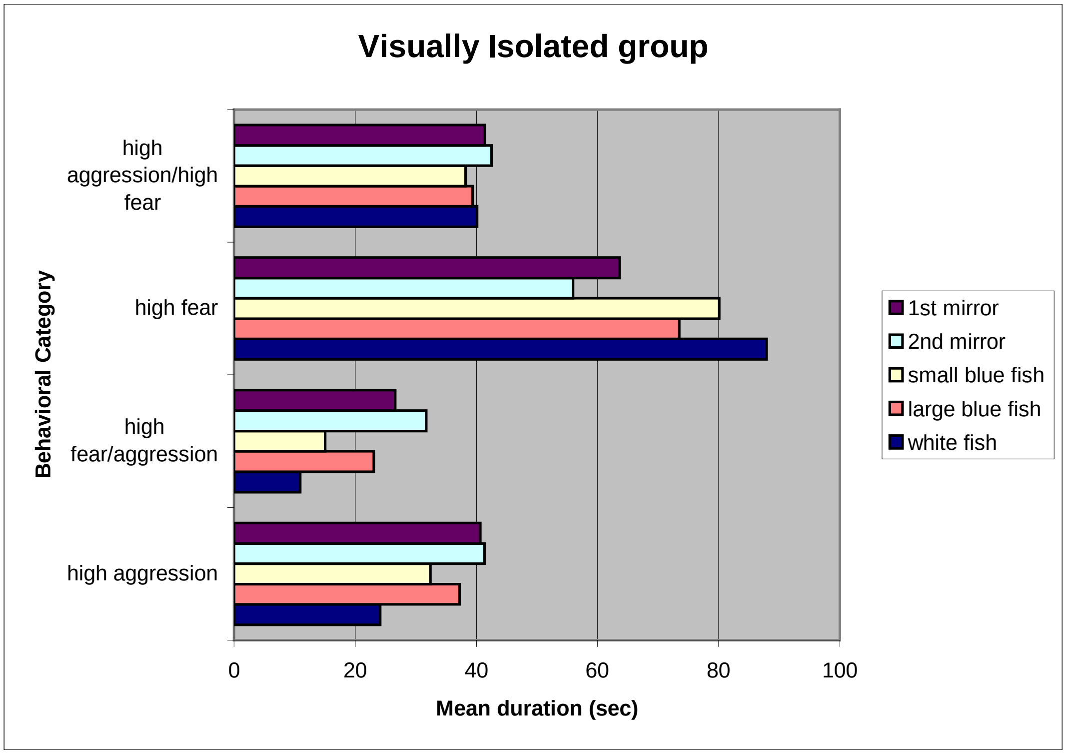


Figure 2.3a and 2.3b. Mean duration of behaviors within behavioral categories for visually-social and visually-isolated group to stimuli used within aggression tests. Durations of behaviors within categories (ANOVA P>0.05).





2.6 Discussion

This experiment was designed to investigate whether social isolation altered the aggressiveness of visually-isolated Siamese fighting fish compared to that of visually-social fighting fish. We hypothesized that the visual isolation treatment would affect the aggressive motivation of Siamese fighting fish due to the lack of aggression releasing stimuli that were present in the social environment in which they were housed. As a result of this, we expected that aggression levels observed in visually-isolated fish would be altered compared to that of fish that were visually-social. Unfortunately due to the lack of a treatment effect, we were not able to sufficiently conclude anything from the aggression levels that were measured and compared. Data showed that there was not a significant difference in the duration and frequency of display between both the visually-social and visually-isolated groups.

According to Thompson (1966), if a male Siamese fighting fish that has been exposed to the visual image of another male along one wall of an aquarium is observed for some time following exposure, one will see the subject return to repeatedly to the area where the intruding male fish was encountered. As the subject periodically swims along the aquarium where the intruder was presented, the fish may display aggressively. Such observations suggested that presentation of this releasing stimulus might act as a reinforcing consequence for an operant response. It seems that the display behavior, the behavior occurring in the absence of the appropriate unconditioned releasing stimulus, might come under the control of other stimuli which had been associated with the behavior. This releaser can act as a positive reinforcer for an operant response since its contingent presentation increases the frequency with which the operant occurs. A releaser has the same functional properties as other stimuli, which are capable of acting as unconditioned stimuli in a classical conditioning paradigm ad functioning as reinforcers for operants, such as food. This theory was consistent with our observations, however certain stimuli seem to be more successful for serving as an aggression releaser. The 1st and 2nd mirror yielded the longest duration response for gills out and face close behaviors, which are viewed to be highly aggressive, in comparison to each of the fish models. According to results from an experiment conducted by Bols (1977), it was observed that a responsive Siamese fighting fish evoked the most vigorous display followed by a paradise fish and a non-displaying Siamese fighting fish, which were of approximately equal effectiveness, and finally a marble, which evoked no display whatsoever and failed to sustain performance. The various stimuli used, in descending order of their effectiveness as reinforcers, were a live male Siamese fighting fish, a mirror, a moving model and a stationary model. This order corresponded to the order of ability of the stimuli to evoke display. The same was apparent within this study. The mirrors were responsible for evoking the highest duration of aggressive display. The large blue fish model, which was intended to yield both an aggressive and fearful response, evoked a high level of display, whereas the small blue and white fish models evoked very little response by both groups of fish.

Most studies on the habituation of threat displays in Siamese fighting fish have defined constant conspecific exposure as continual or prolonged visual exposure and have used mirrors, models, and the opportunity to view live conspecifics as reinforcers (Cain and Baenninger, 1980). Unlike fish in an unrestrained semi-naturalistic environment, fish in these situations cannot attack, elude, or drive their opponents away. It has been suggested that the waning of threat display in these situations may reflect the fact that the experimental fish come to associate the stimuli of its opponent with a lack of consequences for aggression (Goldstein, 1975). This theory of associative learning may also apply to the behavioral patterns noticed within our study, but a definite conclusion cannot be defined until similar studies are conducted.

When behaviors were combined to make up the four behavioral categories that were analyzed in our study, we found that both the visually-social and visually-isolated groups displayed more behaviors that corresponded to the high fear category to all of the stimuli shown. This trend can possibly explain the lack of a treatment effect present for highly aggressive behaviors. In Hogan and Bols' experiment (1980), it was also suggested that aggression was not the only motivation determining the reinforcing efficacy of a stimulus. Both the paradise fish and the non-displaying Siamese fighting fish induced a fair amount of escape behavior in the subjects. This was evident by the high degree of thrashing, immobility, turning back, and in some cases avoidance. 'Face far' behavior can possibly also be interpreted as an escape behavior as well. Fish with erected gills and also choose to display at a distance from a stimulus send a message that they are not only aggressive but fearful and wish to keep some distance from their opponent if they need to flee at any given moment.

In conclusion, there were several trends that were apparent within this study. However, because our objective was not clearly met it is difficult to come to any conclusions on why the trends occurred and if they were related to the lack of a treatment effect being present.

CHAPTER III THE EFFECT OF AGONISTIC ENCOUNTERS ON AGGRESSIVE RESPONSE IN SOCIALLY ISOLATED SIAMESE FIGHTING FISH, *BETTA SPLENDENS*

3.1 Abstract

The objective of this study was to observe aggression during live agonistic encounters in a naturalistic environment comparing populations of visually-isolated fish with similar populations of visually-social fish and to determine if the aggression levels of visually-isolated fish adjusted over a 2-week period. After 7 to 10 weeks of isolation, fish in both the visually-social and visually-isolated groups were divided into populations of 4 and placed in population tanks. Aggression was measured in focal animals during initial agonistic encounters. Each fish was moved back to its original tank and the aggression levels were re-tested. Social isolation did not have an overall effect on the duration and frequency of agonistic encounters and on the duration of behaviors displayed in each behavioral category (ANOVA-duration of agonistic encounters, $P > 0.05$), (ANOVA-frequency of agonistic encounters, $P > 0.05$), (ANOVA, duration of behaviors within behavioral category, $P > 0.05$). However, significant trends for particular behaviors were observed.

Keywords: Aggression; Agonistic encounters; Isolation; Siamese fighting fish

3.2 Introduction

The social environment is a component of an animal's habitat that can have extensive effects on development. For many animals, interactions with conspecifics are predictable and stable sources of information. The social world in which an animal develops can provide opportunities for learning about food, habitat, mates, and communication (Wagner, 1989). Within a social environment, variation in densities and assortments of peers, mates, and relatives, can change the environmental landscape dramatically, and with it the costs and benefits of interacting with others (White et al., 2001).

In 1992, Halperin et al. conducted an experiment that compared responses of fish after three weeks during which controls displayed to a conspecific for two minutes every two days, while isolated fish saw a complex visual stimulus which did not elicit a display. Isolated fish had depressed display readiness, but higher display rates once they were primed. Halperin and Dunham (1993) demonstrated that an 'isolation effect' was found in individually housed Siamese fighting fish. It was experimentally proven that visually-isolated fighting fish became more aggressive than fish that were not visually-isolated.

Halperin et al. (1998) later observed that hyper-aggressive fish, as a result of being visually-isolated, aggressively displayed more when in combat. Hyper-aggressive fish that bluffed, or displayed vigorously, while in combat did not have a high fighting ability to back up displays and usually lost their fights. The possibility that exhaustion due to hyper-aggression may have made the delayed retaliation by fish that were not visually-isolated particularly successful. Koyama (1993) also reported a similar isolation effect in several experiments conducted with lab mice that were housed individually in comparison with those that were group reared. Individually housed mice developed aggression levels that were significantly higher than that of group-housed mice.   It was observed that isolated mice did not have a chance to learn how to use cut-off behaviors, social behaviors such as genital sniffing that need to be learned within a social environment, whereas group housed mice had learned how they could effectively function to avoid unnecessary escalation of interactions with another animal.

The focus of this research was to investigate if aggression levels of visually-isolated fish adjusted when they are housed in a naturalistic physical and social environment, compared to adjustments in visually-social fighting fish.

We hypothesized that social encounters would affect the aggressive motivation of Siamese fighting fish when introduced to a naturalistic social environment with other Siamese fighting fish. As a result of this expected difference in the expression of aggression in live encounters, aggression levels of visually-isolated fish may be altered as they remain in a different environment and have a long period of time to interact with other individuals.

3.3 Animals, Materials, and Methods

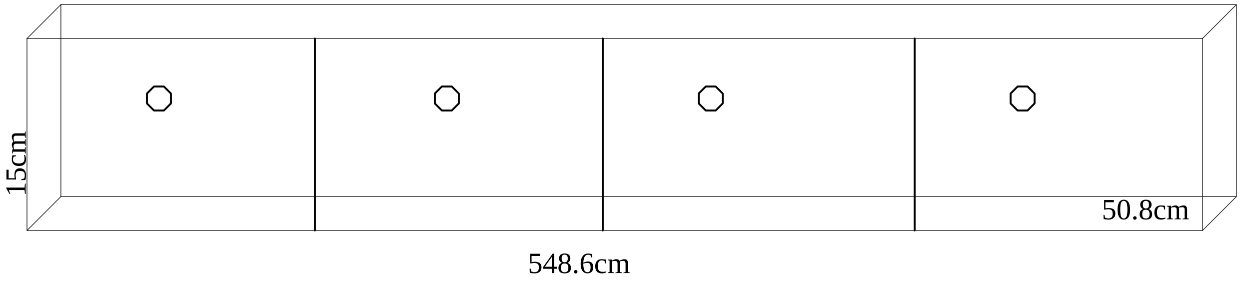
Refer to section 2.3

3.3.1 *Social Interactions (agonistic encounters)*

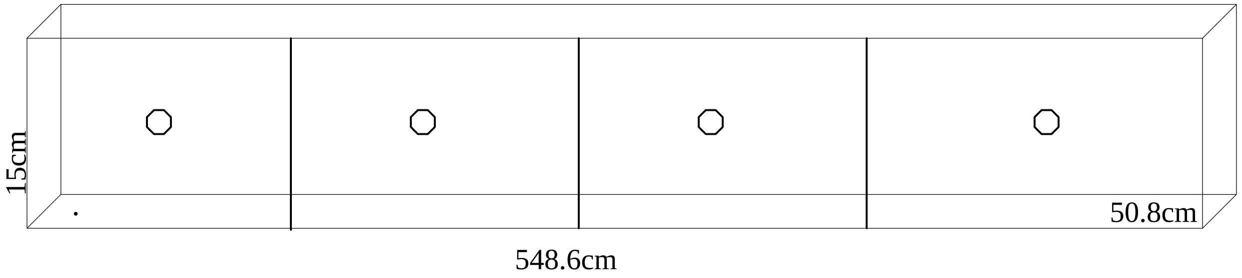
For phase II of the study, the fish were transported to rooms 0356 and 0358 of Animal Wing 3 the day after aggression tests were conducted. Both rooms were regulated at a temperature range between 24-25.6ºC and the lighting cycle was maintained on a 12:12 h light and dark cycle. This phase of the study lasted 2 weeks. On Day 1 of the 2-week period, a group of 32 fish were divided by their color identity and experimental treatment and housed in populations of 4 fish in eight long 548.6cmx50.8cmx15cm population tanks. A population tank of four visually-isolated fish and a population of tank of four visually-social fish made up one replicate, with a total of 4 replicates within each group of 32 fish. Each population tank was set up with artificial aquatic plants, gravel, and shallow water (7.6cm" deep) to resemble a rice paddy that serves as a naturalistic habitat for Siamese fighting fish (Jaroenstasinee and Jaroenstasinee, 2000) (Figures 3.1 and 3.2). The fish were initially separated by opaque dividers and were given the remainder of the day and early morning of the following day to habituate to their new environment before any interactions took place.

Figure 3.1. Grouping of fish in 548.6cm x 50.8cm x 15cm population tanks.

4 visually-isolated fish



4 visually-social fish



○-Represents a red fish, blue fish, iridescent-turquoise fish, and a brightly colored fish placed in random order in areas separated by opaque dividers.

Figure 3.2 Arrangement of 548.6cm x 50.8cm x 15cm population tanks.

3.3.2 *Observational Techniques*

On the Day 2 of phase II (after 12pm), the opaque dividers in each population tank were removed simultaneously and each individual was given the opportunity to interact with one another. Agonistic encounters were focally observed and recorded in each tank. The fish were observed and recorded for 5-min intervals in rotation, each observed twice, for a total observation period of 40 min for each population tank. The intensive observation was repeated twice for each population tank (from 0-40 min and 80- 120 min). Behaviors, which were recorded included approach high, approach low, chase high, chase low, swim away, mouth fight, tail beat, biting, display, avoiding, and not interacting (refer to glossary of terms for definitions).

Body pattern such as color and stripes was also recording for each fish during agonistic encounters and were considered to be mutually exclusive from behaviors. The duration and frequency of these behaviors within agonistic encounters were documented and analyzed using the Noldus “Observer 4.1” program.

After each population tank was intensively observed, it was then checked periodically throughout the day. For the remainder of the 2-week period, each population tank was periodically checked daily, in the morning and the afternoon.

3.3.3 *Aggression Retest*

After the 2- week co-habitation in the population tanks, each fish was moved back to room A of Lab 0148 and placed in its original tank. On the following day, aggression retests were conducted using the same format for aggression tests. However, slightly modified fish models were used to prevent fish from habituating to fish models that were previously used in the aggression test (Table 3.1). Displays such as fin down, gills out and orientations such as broad close, broad far, face close, face far, avoidance, thrashing, and other were recorded and documented with the Noldus Observer 4.1 program (refer to glossary of terms for definitions). Behaviors that were recognized as displays or orientations were considered to be mutually exclusive. The Noldus Observer 4.1 program was used to calculate frequency and duration of both displays and orientations.

Table 3.1. Stimulus sequence and timing for aggression retest.

Stimulus	Duration (min)
Priming mirror	2
BREAK	2
Test mirror	2
BREAK	6
Small blue and red (facing) fish model	2
Large blue and red (facing) fish model	2
Medium white (facing) fish model (no scale markings)	2

3.4 Statistical Analysis

The duration and frequency of agonistic encounters in the population tanks and response to each stimulus used within aggression retests within visually-isolated and visually-social groups were analyzed using analysis of variance (ANOVA). The effects of the visually-isolated treatment and the visually-social treatment on the response to the repetition of stimuli used in aggression tests and retests were taken into account by calculating the differences of responses and were analyzed using a mixed model ANOVA with repeated measures for fish (The SAS System for Windows V8). Sources of variation included the fixed effects for treatment within agonistic encounters and treatment and stimulus for aggression retests. Repeated measure structures in SAS were also used to fit a correlation among the ordered repetition of stimuli. The model residuals indicated homogenous variances and were analyzed without transformation. For further analysis, the duration of behaviors observed within agonistic encounters were averaged and coded into specific behavioral categories; high fear (hiding/avoiding, not interacting) and high aggression (mouth fight, tail beat, bite, display, chase high) (refer to glossary of terms for definitions). Categorical durations of behaviors were then separated into 3 levels, by dividing the 10-min duration for each intensive observation period into 3 intervals, and ranked from 0 to 2: (0-200s: 0=low display; 201-400s: 1= medial display; 401-600s: 2= high display). A logistic analysis of variance (ANOVA) was performed using the generalized mixed model macro of SAS, version 8 (GLIMMIX). The error option was set to Poisson to compensate for the multivariate coded categories being analyzed. Statistical significance was accepted as $P<0.05$.

3.5 Results

3.5.1 Social Interactions (Agonistic Encounters)

Duration of most behaviors observed within agonistic encounters that took place in the population tanks were not affected by social isolation. However, there were particular behaviors that were significantly different with in the visually-social and visually-isolated group. (ANOVA, approach low $P< 0.0001$; chase high $P=0.0017$; swim away $P=0.0251$;mouth fight $P=0.0202$; tail beat $P=0.0176$) (Figure 3.3). (Standard errors for behaviors; approach low: ± 0.18, chase high: ±0.47, swim away: ±0.52, mouth fight: ±0.81, tail beat: ±1.29).

The frequency of two behaviors in particular were observed within agonistic encounters were found to be significantly different within both groups (ANOVA, chase low P=0.0019; mouth fight P=0.0174) (Figure 3.4). (Standard errors for behaviors; chase low: ±0.46, mouth fight: ±11.51). Behaviors observed within agonistic encounters were further analyzed with the generalized mixed model macro. However social isolation was not found to have an effect on the duration of behaviors displayed in each behavioral category (ANOVA, high aggression P=0.0660, high fear P=0.7038) (Figure 3.5).

3.5.2 Aggression Retest

When aggression levels were re-tested and compared to those measured within the first round of tests, it was not sufficiently proven that social isolation had a significant effect on aggressive displays (ANOVA, fin downdiff P=0.2404; gills outdiff P=0.7777; broad closediff P=0.6138;broad fardiff P=0.7664; face closediff P=0.6743; face fardiff P=0.5060; avoidancediff P=0.5480; thrashingdiff P=0.4449; otherdiff P= 0.7856) (Figure 3.6a and 3.6b) (Standard errors for behaviors; fin downdiff : ± 149.82, gills outdiff: ± 147.35, broad closediff: ± 22.14, broad fardiff: ±10.23, face closediff: ±162.44, face fardiff: ±33.01, avoidancediff: ± 161.23, thrashingdiff: ±75.56, otherdiff: ±0.74). It was also not determined whether social isolation affected the frequency of behaviors after the agonistic encounters had taken place compared to those immediately following the 7-10 week isolation period (ANOVA, gills outdiff P=0.8687; broad closediff P=0.9492; face closediff P=0.9210; face fardiff P=0.4265; avoidancediff P=0.4247; thrashingdiff P=0.5015; otherdiff P= 0.6876) (Figure 3.7a and 3.7b). (Standard errors for behaviors; fin downdiff : ± 0.02, gills outdiff: ± 0.03, broad closediff: ± 0.02, broad fardiff: ±0.01, face closediff: ±0.05, face fardiff: ±0.01, avoidancediff: ± 0.04, thrashingdiff: ±0.02, otherdiff: ±0.01).

Figure 3.3. Mean duration of behaviors within agonistic encounters in the population tanks. Approach low, chase high, swim away, mouth fight, and tail beat ANOVA P< 0.05) (refer to section 3.5.1 for standard errors).

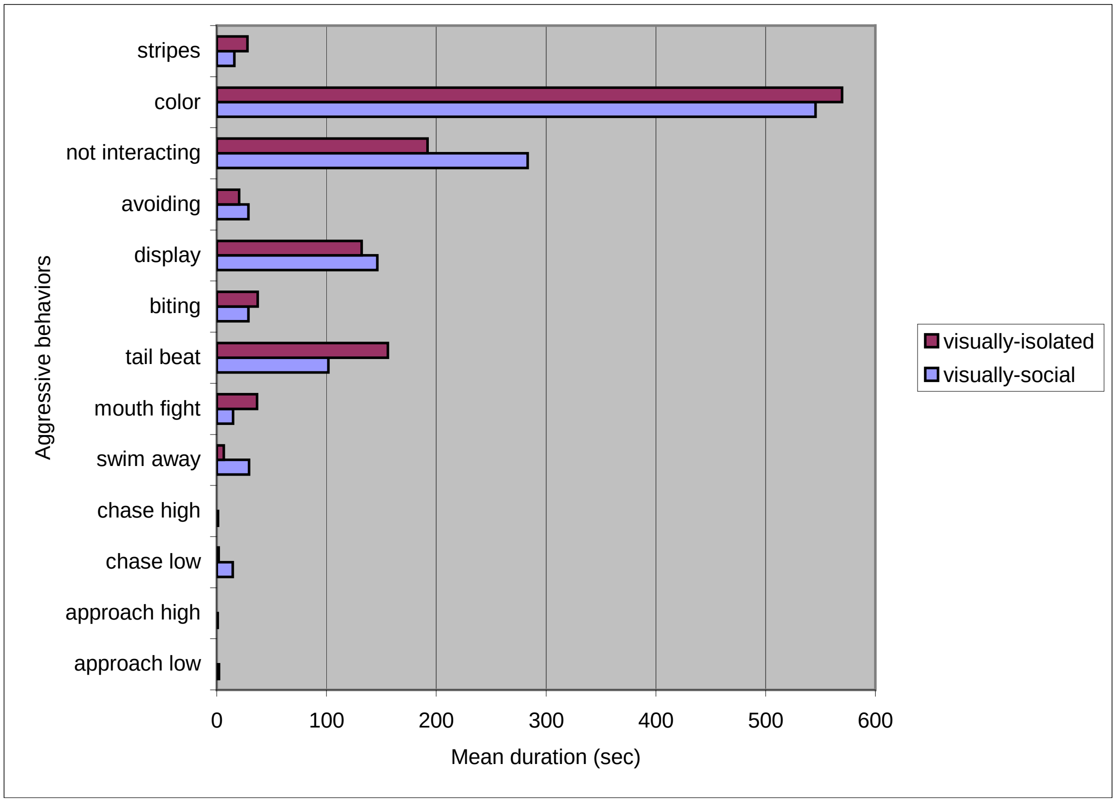


Figure 3.4. Mean frequency of behaviors within agonistic encounters in the population tanks. Chase low and mouth fight (ANOVA P< 0.05) (refer to section 3.5.1 for standard errors).

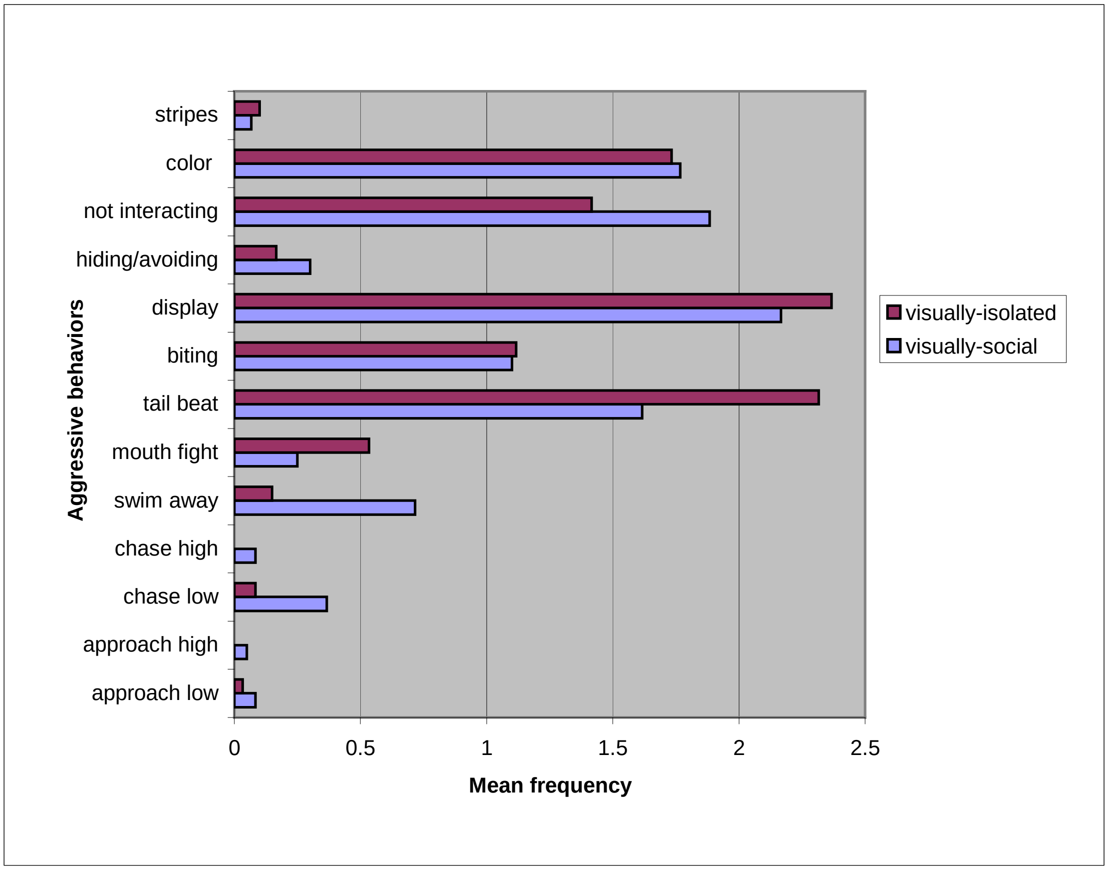


Figure 3.5. Mean duration of behaviors within behavioral categories observed within agonistic encounters in the population tanks. Duration of behaviors within behavioral category, (ANOVA P > 0.05).

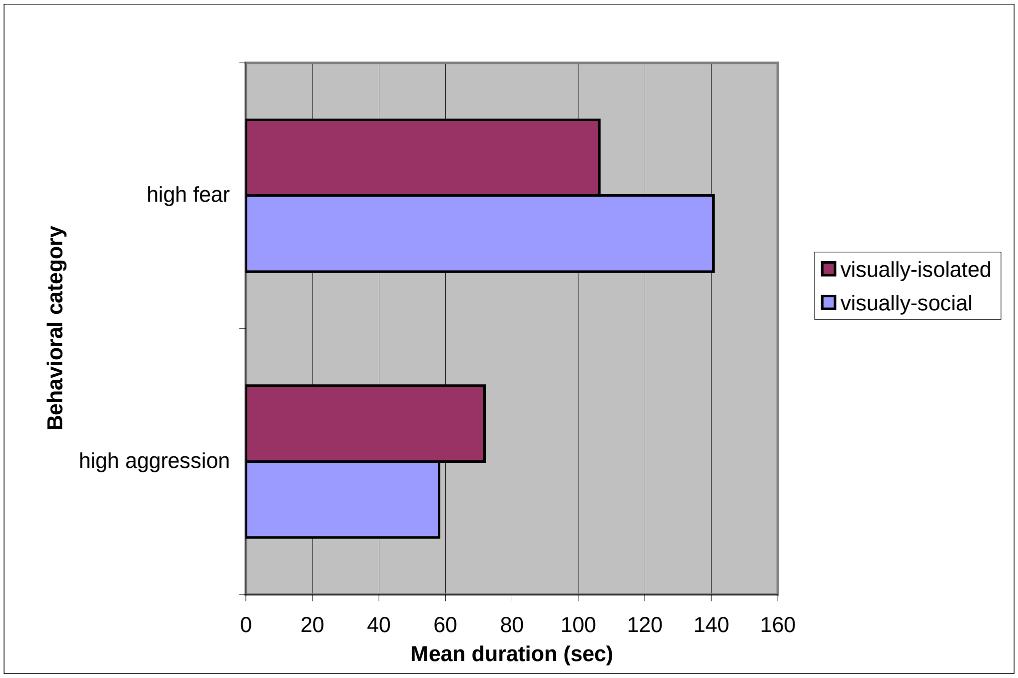
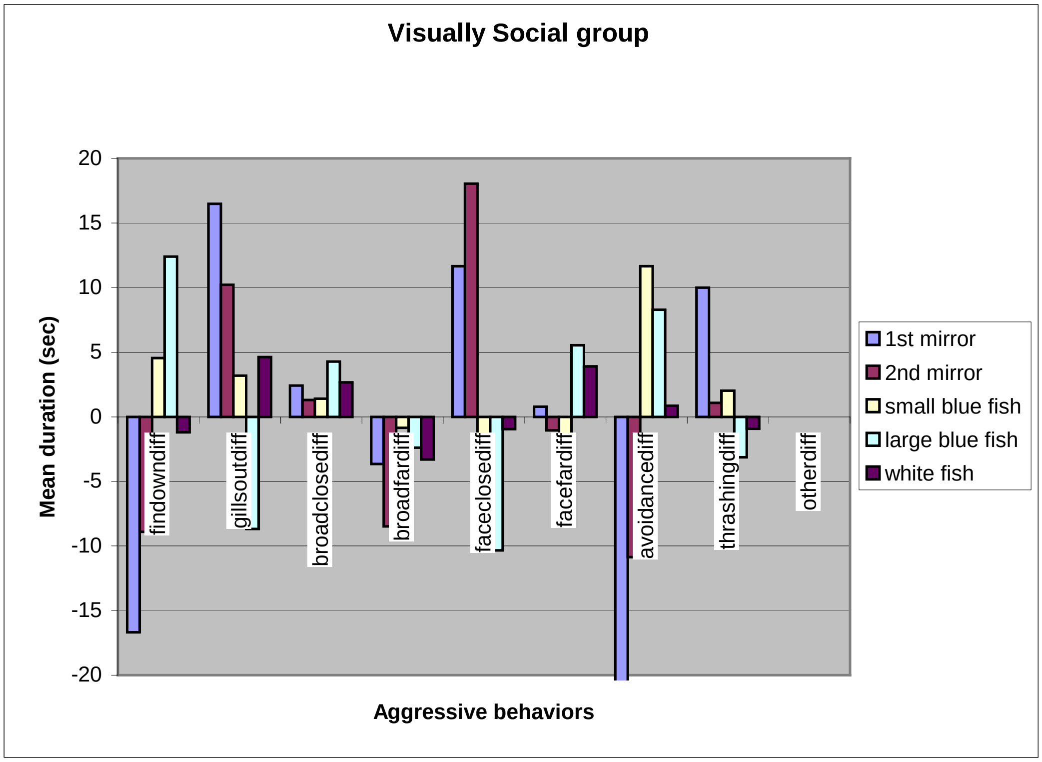


Figure 3.6a and 3.6b. Mean comparison of duration of behaviors (aggression retest minus aggression test for the visually-social and visually- isolated group). Duration of behaviors (ANOVA P>0.05) (refer to section 3.5.2 for standard errors).



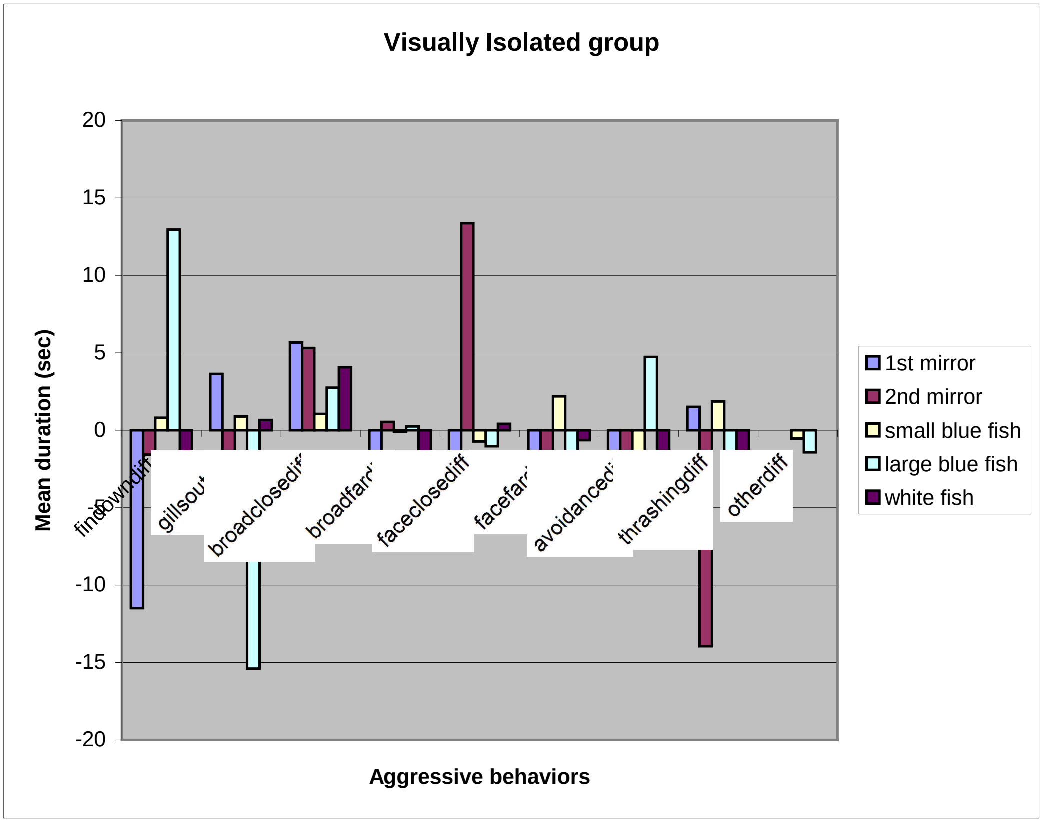
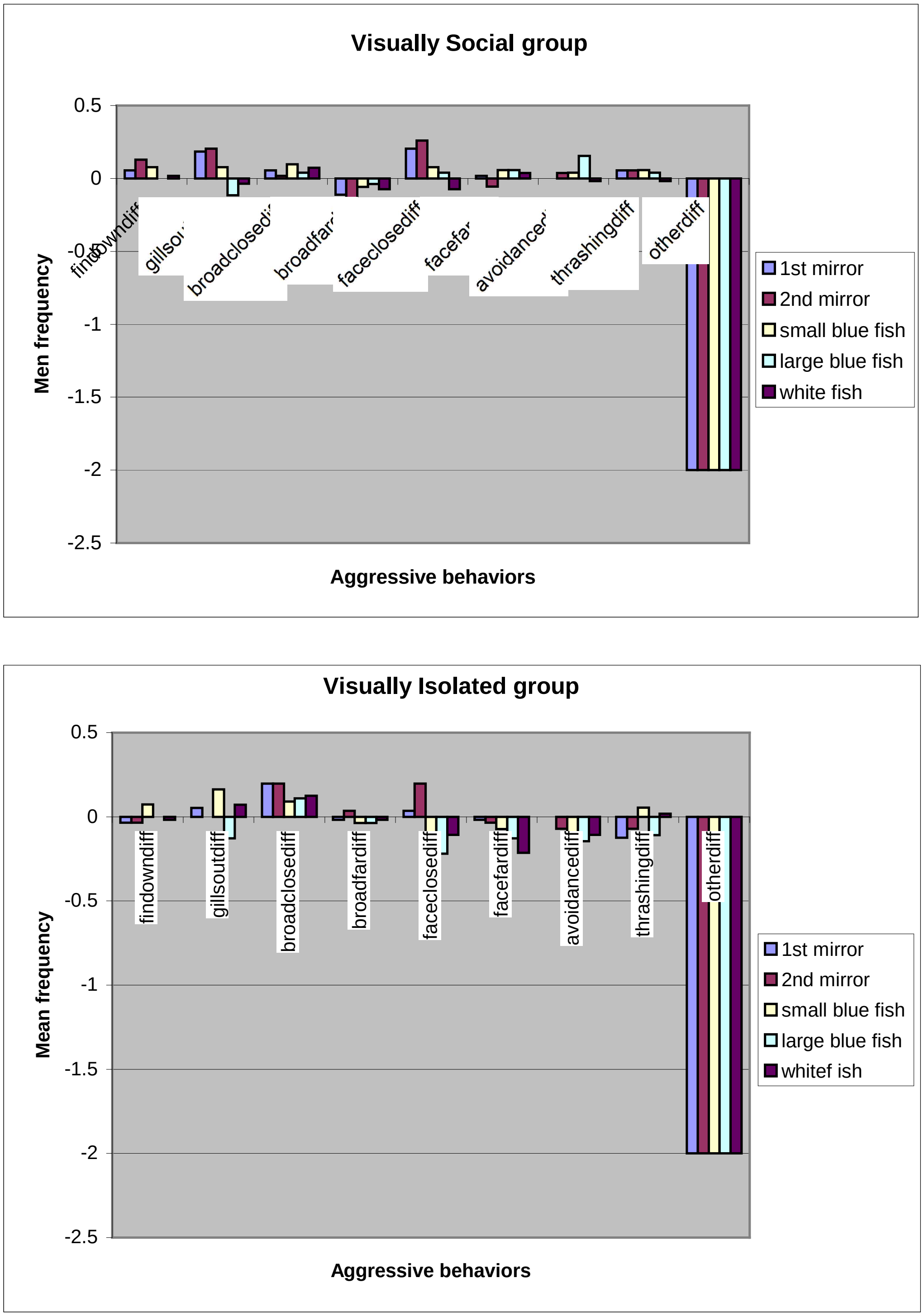


Figure 3.7a and 3.7b. Mean comparison of frequency of behaviors (aggression retest minus aggression test for the visually-social and visually-isolated group). Frequency of behaviors (ANOVA P>0.05) (refer to section 3.5.2 for standard errors).



3.6 Discussion

This experiment was designed to determine if aggression levels of visually-isolated fish adjusted when they were housed in a naturalistic and social environment compared to any changes in aggressiveness in visually-social fighting fish. We hypothesized that agonistic encounters would affect the aggressive motivation of Siamese fighting fish when introduced to a naturalistic social environment with other Siamese fighting fish. As a result of this, we predicted that aggression levels observed in visually-isolated fish would be altered when they were placed in a different environment and given a long period of time to interact with other individuals. Due to the fact that there was no treatment effect present within the former part of our study, we concluded that the chances of getting a treatment effect in the latter portion were slim. Our data supported this conclusion which showed that there was a significant difference in the duration of particular aggressive behaviors displayed by the visually-social and visually group, although there was a low occurrence of agonistic encounters when fish were moved to the population tanks. However, these highly aggressive behaviors were observed in both groups. For example, mouth fighting and tail beat behaviors were mostly displayed by the visually-isolated group and chase high and approach low behaviors were frequently displayed by the visually-social group. Frequency data reiterated similar findings in both groups, mouth fighting was displayed mostly in the visually-isolated group whereas chase high was displayed mostly in the visually-social group. It seems as if the visually-isolated group tended to display behaviors that were often long in duration and generally consumed high levels of energy in comparison to that of the visually-social group. However, this trend was not apparent for many of the other behaviors that were observed for both groups.

For further analysis, behaviors were combined to make up two behavioral categories, high aggression and high fear, which were analyzed in our study. We found that there was no significant difference in the behaviors that were displayed between the visually-social and visually-isolated group within the high aggression (mouth fight, tail beat, bite, display, chase high) and high fear category (hiding/avoiding, not interacting). The ranking data within each behavioral category revealed that at least 75% of the observations in both the high fear and high aggression category were ranked at 0. This trend can possibly also explain the lack of a treatment effect for behaviors displayed during agonistic encounters for both groups.

Unfortunately due to the lack of a treatment effect for aggression tests, we were not able to conclude anything from the aggression re-tests that were measured and compared. Data showed that there was not a significant difference in the duration and frequency of display between both the visually-social and visually-isolated groups. However, one behavioral trend was apparent for the visually-social group. It was evident that visually-social fish were more prone to aggressively interact with the 1st and 2nd mirror after the two week social interaction period. This trend is consistent with the findings within aggression tests (refer to section 2.6). Data for the models were very inconsistent and non-conclusive.

CHAPTER IV IMPLICATIONS

There may be an explanation for why isolation did not have an effect in our study. One factor pertinent to some experiments dealing with consequences of isolation is the transfer of individual fish to and from isolation. This is potentially traumatic and may have short and/or long term effects on the behavior of experimental subjects. The pattern of aggressive behavior shown by previously isolated fish may also be influenced by the effects of transfer and confinement (Gomez-Laplaza and Morgan, 1994). Fish, like most animals, are sensitive to a variety of physical disturbances, such as handling, transfer, and confinement. When this occurs, physiological changes are produced as a result of these disturbances, and considerable time is needed to return to normality. In brown trout, *Saluma trutta*, it was observed that the recovery after transfer between two aquaria took at least 3-4 days in terms of total changes in activity, heart rate, and ventilation rates (Laitien and Voltonen, 1994). On the other hand, there have been other studies that have noted that responses to outside disturbances were short lived. For example, juvenile coho salmon, *Oncorhynchus kisutch*, experienced a great increase in oxygen consumption following diverse types of handling, but the effect was short term. The metabolic rate returned to normal within 1 hr after manipulation (Davis and Schreck, 1997).

This effect may have been true for our experimental animals as well. Our fish were transferred to a different room for each of the phases listed within the experiment. We tried to compensate for this change in environment by allowing a habituation period for at least 12 hrs before testing for aggression levels. It is very possible that the habituation period may have been more beneficial had it been extended for a longer time period. Also, throughout the 7-10 week isolation period, the fish were handled frequently when their tanks needed to be cleaned, siphoned, or topped up with water. Several students assisted with these activities instead of having a single fish care provider. So it is also very possible that these fish were frequently in a chaotic state due to the fact that there were a few handlers involved with regular tank maintenance. Theoretically, a period of isolation during which fish experienced mildly fearful stimuli should induce behaviors correlated with fear (Halperin et al., 1998). Thus, these disturbances may also explain why fish in both the visually-social group and the visually-isolated group displayed more behaviors that were correlated with being fearful rather than aggressive.

CHAPTER V SUMMARY AND CONCLUSION

The aim of most studies dealing with the effects of isolation on aggressive behavior in fish is to determine the conditions that promote or reduce this behavior, in hope of increasing one's understanding of social interaction regulation. In this particular study, we were hoping to investigate if social isolation would affect the aggressive motivation of Siamese fighting fish due to the lack of aggression releasing stimuli that are present in the social environment in which they live in. We also hypothesized that social encounters with other fish would affect the aggressive motivation of Siamese fighting fish when introduced to a naturalistic social environment. Due to the lack of a treatment effect in this study, we were not able to provide a definitive answer to add to our understanding.

However, we observed certain behavioral trends that were documented in previous studies. During aggression tests, the 1st and 2nd mirror elicited longer durations of fin up, gills out, and face close behaviors, which are viewed to be highly aggressive, than did any of the fish models. In an experiment conducted by Bols (1977), it was observed that a responsive Siamese fighting fish evoked the most vigorous display, followed by a paradise fish and a non-displaying Siamese fighting fish, which were of approximately equal effectiveness, and finally a marble, which evoked no display whatsoever and failed to sustain performance. This order corresponded to the order of ability of the stimuli to evoke display. The same was apparent within this study. The mirrors were responsible for evoking the highest duration of aggressive display. The large blue fish model, which was intended to yield both an aggressive and fearful response, evoked a high level of display as well.

When behaviors were combined into four behavioral categories that were analyzed in our study, we found that both the visually-social and visually-isolated groups displayed more behaviors that corresponded to the high fear category to all of the stimuli shown. This trend can possibly explain the lack of a treatment effect present for highly aggressive behaviors. In Hogan and Bols' experiment (1980), it was also suggested that aggression was not the only motivation determining the reinforcing efficacy of a stimulus. Both the paradise fish and the non-displaying Siamese fighting fish induced a fair amount of escape behavior in the subjects. This was evidenced by the high degree of thrashing, immobility, turning back, and in some cases avoidance. There is a real possibility that the frequent disturbances that occurred throughout the isolation period were to blame for this finding of enhanced fearfulness in the visually-isolated group.

Data from the live agonistic encounters that were staged in the population tanks indicated significant differences in the duration or frequency of display for specific behaviors that were prevalent for both the visually-social and visually-isolated groups. Significant trends between the groups were apparent after the 2-week social interaction period, when the visually-social fish responded more aggressively to the 1st and 2nd mirrors than they had in their first aggression tests. This was a higher level of aggression than the visually-isolated fish showed, as they were no more responsive in their aggression retests than in their original aggression tests.

Despite the lack of expected results, since the creation of a population of hyper-aggressive fish was not achieved in our study, we were satisfied with our basic experimental design. Perhaps, if we had better system for keeping fish in social isolation with minimal sensory deprivation and, especially, minimal disturbances that could increase fearfulness, we would have obtained feasible results.

APPENDIX

Table 4.1. Calendar and Schedule for Phase I and II of the experiment (Fall 2002-Spring 2003).

Date	**Group 1** **32 fish** **(4 reps)**	**Group 2** **32 fish** **(4 reps)**	**Group 3** **32 fish** **(4 reps)**	**Group 4** **32 fish** **(4 extra reps)**
Dec 30	Isolation			
Jan 6	Isolation			
13	Isolation			
20	Isolation			
27	Isolation			
Feb 3	Isolation			
10	Isolation			
17	Isolation			
24	Isolation	Isolation		
Mar 3	Aggression tests	Isolation		
10	Social interactions	Isolation		
17	Social interactions	Isolation		
24	Aggression retests	Isolation		
31		Isolation		
Apr 7		Aggression tests	Isolation	

14		Social interactions	Isolation	
21		Social interactions	Isolation	
28		Aggression retests	Isolation	
May 5			Isolation	Isolation
12			Isolation	Isolation
19			Isolation	Isolation
26			Isolation	Isolation
Jun 2			Aggression tests	Isolation
9			Social interactions	Isolation
16			Social interactions	Isolation
23			Aggression retests	Isolation
30				Aggression tests
July 7				Social interactions
14				Social interactions
21				Aggression retests

GLOSSARY OF TERMS

Aggressive response- Any purposive action of an organism toward another organism or stimulus with the actual or potential result of harming, limiting, or depriving it.

Agonistic - Refers to any activity performed in the context of an aggressive interaction. It encompasses the actions of both the instigator and the victim.

Approach high- Occurs when an individual moves towards another individual with fully erect fins and expanded gills.

Approach low- Occurs when an individual moves towards another individual with fully erect fins and no expansion of the gills.

Avoidance/ avoiding- Occurs when an individual does not respond by means of not approaching or escaping when a stimulus is shown or an individual displays.

Biting- Involves a sharp opening and closing of an individual’s mouth directed toward the tail of another individual.

Broad close- Occurs when an individual aligns itself in a lateral orientation within close proximity, in the front portion of the tank adjacent to the stimulus to the halfway point of the tank, in relation to a stimulus.

Broad far- Occurs when an individual aligns itself in a lateral orientation, from the halfway point of the tank to the back portion of the tank, away from a stimulus.

Chase low- Occurs when an individual lunges at another individual with fully erect fins and no expansion of the gills.

Chase high- Occurs when an individual lunges at another individual with fully erect fins and expanded gills.

Color- Occurs when an individual expresses a dominant status with vivid and intensified body coloration.

Conspecific - Pertaining to individuals belonging to the same species.

Display- A communicative and visual representation of a behavioral characteristic within animal species. Displays within agonistic encounters were measured in response to an individual expanding its gills to another individual in a stationary orientation.

Display readiness- The frequency of occurrences of social displays that are performed during a brief encounter with a live conspecific or stimulus.

Dominant- An individual exercising authority or influence within a social environment.

Face close- Occurs when an individual positions itself in a frontal orientation within close proximity, in the front portion of the tank adjacent to the stimulus to the halfway point of the tank, in relation to a stimulus.

Face far- Occurs when an individual positions itself in a frontal orientation, from the halfway point of the tank to the back portion of the tank, away from a stimulus.

Fin down- Occurs when an individual does not extend its fins in response to a stimulus.

Fear- An expression of escape behaviors or threats caused by the perception of danger.

Gills out- Occurs when an individual extends its gills in response to a stimulus.

Habituation- A simple form of learning involving a relatively permanent reduction or elimination of a response in the absence of overt reward or punishment.

High aggression- A behavioral category representing a combination of behaviors that correspond to individuals expressing an increased level of aggression relative to another group of individuals.

High aggression/ high fear- A behavioral category representing a combination of behaviors that correspond to individuals expressing an increased level of aggression and fear relative to another group of individuals.

High fear- A behavioral category representing a combination of behaviors that correspond to individuals expressing an increased level of fear relative to another group of individuals.

High fear/ aggression- A behavioral category representing a combination of behaviors that correspond to individuals expressing an increased level of fear, but also expressing traces of aggression relative to another group of individuals.

Hyper-aggressive- An exaggerated level of aggressive response or display behavior to a stimulus or another individual.

Hyper-fearful- An exaggerated level of fearful response or inactivity to a stimulus or another individual.

Latency- The time between the presentation of a stimulus and the beginning of a response.

Motivation- A tendency to perform a given behavioral action or a group of behavioral actions.

Mouth fight- Occurs when 2 individuals take hold of each other’s upper and lower lip and tug at one another. The idea behind this is to hold the opponent under water to see who can last the longest without having to take a breath of air.

Mutually exclusive- Behaviors, displays, and/ or events that cannot occur simultaneously.

Not interacting- The absence of any behavioral interchange between 2 or more individuals.

Other- Any displays and/or orientations that were not easily classified or distinguishable.

Priming- An increase of aggressive motivation with the presentation of social releasers such as mirrors, pictures, or models.

Stereotype- Frequent repetition of a particular posture or movement with no obvious function.

Subordinate- An individual seen as being inferior or below another individual in rank.

Social isolation- To separate an individual from social or visual contact with others and/or external stimuli.

Stimulus- An object that can rouse or incite a specific activity within an individual.

Stripes- Occurs when an individual expresses a subordinate status with dull but visual stripes on its body.

Swim away- Occurs when an individual retreats from another individual with laid back fins. Gills may or may not be expanded.

Tail beat- Recognized by quick movements of an individual’s cupped tail in the direction of another individual.

Thrashing- Occurs when an individual performs an exaggerated and alternated surging of the body in response to a stimulus.

Unconditioned stimulus- A stimulus that can evoke a response within an organism without undergoing any previous learning.

Visual stimulation- The act of showing a stimulus with the intent of arousing a behavioral response from the individual viewing the stimulus.

REFERENCES

Archer, J., 1988. The Behavioral biology of aggression. Cambridge University Press, Cambridge.

Aureli, F., Marina, C., and Van Schaik, C.P., 2002. Conflict resolution following aggression in gregarious animals: a predictive framework. *Animal Behaviour* 64, 325-343.

Baenninger, R., 1966. Waning of aggressive motivation in *Betta splendens. Psychonomic Science* 4, 241-242.

Baenninger, R., 1970. Visual reinforcement, habituation, and prior social experience of Siamese fighting fish. *Journal of Comparative and Physiological Psychology* 71 (1), 1-5.

Bradbury, J.W. and Vehrencamp, S.L., 1998. Principles of Animal Communication. University of California, San Diego, pp. 655-658.

Bronstein, P.M., 1981. Social reinforcement in *Betta splendens*: A reconsideration. *Journal of Comparative and Physiological Psychology*, 95 (6), 943-950.

Bols, R.J., 1977. Display reinforcement in the Siamese fighting fish, *Betta splendens:* Aggressive motivation or curiosity? *Journal of Comparative and Physiological Psychology* 91 (2), 233-244.

Cain, N.W. and Baenninger, R., 1980. Social organization and the maintenance of aggressive behavior in community-housed male Siamese fighting fish *(Betta splendens). Animal Learning and Behaviour* 8(1), 171-176.

Cain, N.W., Anderson, R., Stein, L., Jessen, C., 1980. Effects of prior social experience on agonistic responding by Siamese fighting fish *(Betta splendens). Animal Learning and Behaviour* 8(3), 491-496.

Davis, L. and Shrek, C., 1997. The energetic response to handling stress in juvenile coho salmon. *Journal of Fish Biology* 126, 248-258.

Davis, R.E., 1975. Readiness to display in the paradise fish *Macropodus opercularis, L., Belontidiidae:* The problem of general and specific effects of social isolation. *Behavioral Biology* 15, 419-433.

Enquist, M., 1985. Communication during aggressive interactions with particular reference to variation in choice of behaviour. The problem of general and specific effects of social isolation. *Animal Behavior* 33, 1152-1611.

Ferno, A., 1978. The effect of social isolation on the aggressive and sexual behaviour in a cichlid fish, *Haplochromis burtoni*. *Behaviour* 65, 43-61.

Ferno, A., 1986. The aggressive behavior between territorial Astatotilapia burtoni (Pisces, Cichlidae) with unlimited or only visual contact emphasizing low-intensity aggression during free interaction. *Behavioural Processes* 13, 367-384.

Franck, D., Hannes, R.P., Lanffermann, H., and Ribowski, A., 1985. Effects of social isolation on aggressiveness in fish with special reference to the swordtail *(Xiphophorus helleri). Behavioural Processes* 10, 415-427.

Franck, D. and Ribowski, A., 1987. Influences of prior agonistic experiences on aggression measures in the male swordtail *(Xiphophorus helleri). Behaviour* 103, 217-240.

Grafen, A., 1990. Biological signals as handicaps. *Journal of Theoretical Biology* 144, 517-546.

Goldstein, S.R., 1975. Observations on the establishment of a stable community of adult male and female Siamese fighting fish, *Betta splendens*. *Animal Behaviour* 23, 179-185.

Gomez-Laplaza, L.M. and Morgan, E., 1994. Laboratory studies of the effects of short term isolation on aggressive behaviour in fish. *Marine and Freshwater Behaviour and Physiology* 33, 63-102.

Halperin, J.R.P., Dunham, D.W., and Ye, S., 1992. Social isolation increases social display after priming in *Betta splendens* but decreases aggressive readiness. *Behavioural Processes* 28, 13-32.

Halperin, J.R.P and Dunham, D.W., 1993. Increased aggressiveness after brief social isolation of adult fish: A connectionistic model which organizes this literature. *Behavioural Processes* 28, 123-144.

Halperin, J.R.P, Giri, T., Elliot, J. and Dunham, D.W., 1998. Consequences of hyper-aggressiveness in Siamese fighting fish: cheaters seldom prosper. *Animal Behaviour* 55, 87-96.

Hinkel, T.J and Maier, R., 1974. Isolation and aggression in Siamese fighting fish (*Betta splendens*). *Psychological Reports* 34, 1323-1326.

Hogan, J.A., 1974. On the choice between eating and aggressive display in the Siamese fighting fish *(Betta splendens). Learning and Motivation* 5, 273-287.

Hogan, J.A and Roper,T.J., 1978. A comparison of properties of different reinforcers. *Advances in the Study of Behavior* 8, 155-255.

Hogan, J.A and Bols, J., 1980. Priming of aggressive motivation in *Betta splendens*. *Animal Behaviour* 28, 135-142.

Hsu, Y., and Wolf, L.L., 1999. The winner and lose effect: integrating multiple experiences. *Animal Behaviour* 57, 903-910.

Hsu, Y., and Wolf, L.L., 2001. The winner and lose effect: what fighting behaviours are influenced? *Animal Behaviour* 61, 777-786.

Jaroenstasinee, M and Jaroenstasinee, K., 2000. Bubble nest habitat characteristics of wild Siamese fighting fish. *Journal of Fish Biology* 58, 1311-1319.

Klein, R.M., Figler, M.H., and Peeke, H.V.S, 1976. Modification of consummatory (attack) behavior resulting from prior habituation of appetitive (threat) components of the agonistic sequence in male Betta splendens (Pisces, Belontidae). *Behaviour* 58, 1-25.

Koyama S., 1993. Isolation effect in Mice *(Mus musculus). Journal of Ethology* 11(2), 117- 130.

Kuhn, D.J., 1970. Display patterns of Siamese fighting fish. *The American Biology Teacher* 2, 102-104.

Laitien, M and Voltonen, T., 1994. Cardiovascular, ventilatory and total activity Responses of brown trout to handling stress. *Journal of Fish Biology* 45, 933-942.

Lobb, M.L. and McGain, G., 1976. Procedurally related differences in the aggressive behavior of *Betta splendens. Animal Learning & Behavior* 4, 167-171.

Lorenz, K., 1966. *On Aggression.* Methuen, London, pp. 83-100.

Luciano, D. and Lore, R., 1975. Aggression and social experience in domesticated rats. *Journal of Comparative and Physiological Psychology* 88, 917-923.

Peeke, H.V.S and Dark, K.A., 1990. The effects of isolation on the sensitization and habituation of aggression in the threespine stickleback (*Gasterosteus aculeatus*). *Ethology* 85 35-42.

Potegal, M. and ten Brink, L., 1984. The behavior of attack-primed and attack satiated female golden hamsters, *Mesocricetus auratus. Journal of Comparative Psychology* 98, 66-75.

Meliska, C.J., Meliska, J.A., and Peeke, H.V.S, 1980. Threat displays and combat aggression in *Betta splendens* following visual exposure to conspecifics and one-way mirrors. *Behavioral and Neural Biology* 28, 475-486.

Miley, W.M. and Burak, G., 1977. Strength of aggressive display in Siamese fighting fish, (*Betta splendens)* toward a conspecific, an alien species (*Macropodus opercularis* L.), and a mirror image as affected prior conspecific visual experience. *Behavioral Biology* 21, 267-272.

Simpson, M.J.A., 1968. The display of Siamese fighting fish, *Betta splendens. Animal Behaviour Monographs* 1, 1-73.

Thompson, T., 1966. Operant and classically- conditioned aggressive behavior in Siamese fighting fish. *American Zoologist* 6, 629-641.

Todd, J.H., 1971. The chemical languages of fishes. *Scientific American* 224(5), 98-108.

Wagner, W.E., 1989. Fighting, assessment, and frequency alteration in Blanchard's cricket frog. *Behavioral Ecology &Sociobiology* 25, 429-436.

White, D.J., King, A.P., and West M.J., 1968. Facultative development of courtship and communication in juvenile male cowbirds *(Molothrus ater). Behavior Ecology* 4, 487-496.

White,W., 1975. The Siamese Fighting Fish: its life cycle. Sterling Publishing Co., Inc., New York, pp. 11-32.

Zahavi, A., 1975. Mate selection: A selection for a handicap. *Journal of Theoretical Biology* 53, 205-214.