“Development of Behavioral and Physiological Indices of the Welfare Status of Captive Fishes to Improve the Rearing Environment, Management and Handling”

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GENERAL RESEARCH OBJECTIVES

Fish have been traditionally considered as cognitively simpler animals relative to mammalian and even avian species. There is a general feeling that they fall below the phylogenetic line that delineates where consciousness and sentience begins. For example, fish are often employed in scientific experimentation in lieu of birds and mammals, as this is seen as more ethically acceptable (Huntingford, 1990). In the year 2000, over 400,000 fish were used in experimental procedures in Canadian research institutions. This amounts to approximately 21% of total research animals used (CCAC, 2000). With regard to the number of studies where death is the ultimate-end point, fish are tied with mice in first place. Also, anatomical, physiological, neuropharmacological and behavioural data suggest that fish can suffer in ways similar to 'higher' vertebrate animals (Chandroo et al., 2003; Sneddon et al., 2003 Chandroo et al., 2004). Therefore we ought to consider the welfare of fish as a serious matter of concern.

The objective of the study was to develop behavioural and physiological indices of the welfare status of captive fishes in order to improve the rearing environment, management and handling of these species. Fish such as rainbow trout and salmon are used extensively in the laboratory as they are considered to be excellent models for which to study teleost physiology and behaviour. Many of the stressors that affect fish in an aquaculture setting are similar to those stressors that can affect research fish kept in a laboratory setting (i.e. dense confinement, lack of environmental enrichment, perturbations in water quality, dietary insufficiencies, various treatment protocols, social stressors, human handling etc.).

We have been investigating the physiological and behavioural stress responses to common fish husbandry practices, as well as considering the more fundamental question of how to assess the capacity for suffering in the rainbow trout. This has been done through the non-invasive investigation of cognitive capacity and characteristics of sentience such as fear and frustration.

Since we cannot always apply behavioural constructs and other measures of ‘suffering’ known to apply to birds and mammals, there is a great deal to learn about how to assess suffering in fish. Fish do not always display the more traditional and obvious signs of suffering that we are familiar identifying in other animals. For example, they are not capable of facial expression, nor can most species make vocalizations, and given their general anatomical structure, changes in body postures are extremely limited. My research looks into many different aspects of behaviour, which should eventually give us an overall picture of how to assess fish welfare. The main objective is to figure out how to minimize stressful situations for fish held under captive conditions. However, the ultimate goal is to expand the scientific database relating to fish welfare in order to
improve the care and handling of fish in the laboratory. This in turn, may also serve as a foundation from which to build codes of practice for the care, handling and management of farmed fish at the industry level.

EXPERIMENTAL PROTOCOLS
(as described in original letter of acceptance)

There were two proposed experimental ideas. The first described an investigation into the nature of fear by use of the conditioned suppression paradigm. The other experiment proposed to investigate pain perception in trout. However, due to logistical constraints and experimental limitations that could not be rectified, it was not possible to proceed with the second experiment. Therefore, one full experiment has been completed and is in the process of being prepared for submission to a scientific journal. Also, as promised, there will be a statement at the end of the paper acknowledging the grant from the Johns Hopkins Center for Alternatives to Animal Testing and the Animal Welfare Institute.

INVESTIGATING ‘FEAR’ BY USE OF THE CONDITIONED SUPPRESSION TECHNIQUE

BACKGROUND

Conflicting arguments maintain the current debate about whether or not fish have the capacity for subjective experiences or conscious ‘feelings’. One side of the argument claims that fish lack the necessary neuroanatomical structures (i.e. the neocortex) that allow for the phenomenon of subjective experiences (Rose, 2002). Another side has shown that fish, such as the rainbow trout, do possess physical features have the biological function of sensing certain stimuli in order to be interpreted in a subjective way (i.e. nociceptors used for experiencing pain) (Sneddon et al., 2003). Somewhere in between is a plethora of literature, which covers cognitive capacity (Overmier and Hollis, 1990; Keiffer and Colgan, 1992) to avoidance behaviour (Pinckney, 1967; Gallon, 1972; Brown, 2001), to pain perception (Chandroo et al. 2004) to physiological stress responses (Mazeaud et al., 1977; Schreck, 1981; Barton et al., 1986; Sloman et al., 2001) – all of which are frequently referenced by those interested in investigating the possibility of sentience in fish. However, there are problems with interpreting sentience in each of these categories. It is often insinuated that more complex, higher cognitive function comes with a higher level of sentience (Århem and Liljenström, 1997; Rose, 2002). The opposing argument is that a high level of cognitive function is not needed to experience certain primitive feelings like fear (which has evolved as a biologically adaptive mechanism) (Dawkins, 2000); therefore the ability to suffer may not necessarily be dependent on cognitive capacity. Likewise, the physiological stress response can be easily misinterpreted. The association between stressors and endocrine changes is not direct. Sometimes the elevation of ‘stress hormones’ does not indicate an animal in distress. For instance, it has been found that cortisol levels increase during the act of mating in stallions (Colborn et al., 1991). Finally, interpreting simple avoidance
behaviour as an animal’s fear response is not always correct. An animal may take flight from a ‘frightening’ stimulus, not out of fear, but simply as a result of a reflexive response, much like the human knee-jerk reaction. Although the avoidance of a stimulus indicates that the animal finds the stimulus aversive, it would be more convincing if the response was thought to have been consciously performed. In this way, an intense avoidance response would more likely be motivated by fear instead of being evoked by a reflexive non-conscious mechanism. Deliberate behaviour will be investigated in more depth as it suggests that rainbow trout may have the capacity for sentient behaviour.

The conditioned suppression paradigm (also known as conditioned emotional response, CER) has been commonly used to assess levels of anxiety or fear in animal subjects (Estes and Skinner, 1941). This paradigm consists of the suppression of a stable and repetitive operant behaviour (e.g. lever-pressing) that is maintained by positive reinforcement (e.g. food reward), by a fear evoking conditioned stimulus (e.g. bell-ring). The aversive quality of the conditioned stimulus (CS) is acquired during the course of classical conditioning where the subject learns to associate the initially neutral stimulus with a brief inescapable fright (e.g. electric shock). The resulting suppression of the stable behaviour is empirically measured by calculating its rate of change in the presence of the CS. The suppression ratio is commonly used to measure the strength of the suppressive effect. The ratio takes the form of $A/(A+B)$, where $A$ is the response rate during the CS and $B$ is the response rate in the absence of the CS (usually measured immediately before the CS presentation). According to this formula, a CS which completely suppresses responding will score 0.0, one that has no effect will score 0.5 and in some rare instances, a stimulus which elevates responding will score between 0.5 and 1.0. However, the interest lies not in the rate of change of the on-going behaviour, rather than the reason for the change in behaviour. Estes and Skinner (1941) recognized early on that treating ‘emotion’ purely as a ‘response’ tended to overlook the motivation behind behavioural displays.

Therefore, if carefully designed, an experiment may be able to uncover some of the motivation behind behavioural responses – giving us a glimpse of the ‘black box’ that lies between inner ‘emotions’ and outward responses. Although this paradigm has been traditionally tested on rats and pigeons, to the author’s knowledge, the conditioned suppression method has never been tested in domesticated fish for the purposes of investigating the phenomenon of fear.

MATERIALS AND METHOD

- Subjects and apparatus

Twenty four domestic rainbow trout were held in 1000 L tanks with aerated, continuously-flowing water (9 ºC). Fish were fed every other day with commercial trout diet (Martin Mills, Elmira, ON, Canada). All fish were kept six to a tank and each fish was tagged for individual identification.

The operant behaviour chosen for the present experiment was ‘pendulum-pressing’ from a demand feeder for a food reward (Figure 1). The negative stimulus chosen as the unconditioned stimulus (US) was a plunging dip net. Rainbow trout find this stimulus highly aversive, as they have been known to actively avoid it if given the
chance (Yue et al., 2004). Lastly, the neutral cue (later known as the CS) used to signal the oncoming US was the illumination of a blue light.

**Procedure**

In Phase 1 (individual training), using the behavioural shaping technique (Skinner, 1953), each fish underwent pendulum-pressing training. A pellet of food would only be delivered if the fish displaced the pendulum with its mouth. Each fish received 11 1-hour sessions every other day. On the final session, each fish’s ‘prime rate’ or pre-classical conditioning rate of pendulum pressing was measured.

In Phase 2 (classical conditioning) fish learned to make an association between a neutral cue and a negative stimulus. A blue light was illuminated for an interval of 10 seconds, at the end of which a dip net was plunged into the water in the middle of the tank. Each fish received 10 trials of light-net pairings on each of 3 days.

In Phase 3 (establish baseline rate) each fish received one 1-hour session of pendulum pressing for food reward. At the end of the session, the rate of responding for each fish was measured because this ‘baseline rate’ or post-classical conditioning rate of responding was expected to differ from the prime rate.

In Phase 4 the conditioned suppression test was applied. Fish were allowed to pendulum press for approximately 45 minutes. At the 30th minute, the blue light was illuminated for a period of 3 minutes (i.e. the negative context was superimposed on the positive context). The rates of responding during the 3 minute interval prior to the light illumination and the 3 minute interval during the light illumination were used to generate the suppression ratio.
Figure 1. A rainbow trout touches the tip of the pendulum with its mouth. A correct ‘response’ to the operant task was defined as: a fish using its mouth to displace the pendulum from its vertical position. In addition, the fish must then immediately retrieve and ingest the food pellet.
RESULTS

Of the 24 fish that served in the experiment, data was generated from 16 subjects. Eight fish did not pass Phase 1 for reasons ranging from health problems to non-responsiveness to operant training. These fish were therefore omitted from the study.

The number of sessions required by each fish to acquire the pendulum-pressing task varied greatly. The rate of learning in Phase 1 ranged from 1 to 7 sessions, with an overall mean of 4.3 sessions. Regardless of the number of sessions it took fish to master the food-reinforced task all subjects were required to continue with it until the end of the 11th session (Figure 2). On the last day of Phase 1, prior to undergoing negative conditioning, the response rate was calculated for each fish; the mean ‘prime rate’ for all subjects was $3.6 \pm 0.4$ responses / min. The mean ‘baseline rate’ was $2.9 \pm 0.3$ responses / min. After negative conditioning, the average rate of pendulum-pressing significantly decreased ($t = -2.61, P < 0.05$). The lower baseline rate may have been due to an initial delay of behavioural responding. Fish started pendulum pressing within an average of $1.7 \pm 0.8$ min before the classical conditioning phase, however, after the exposure to the light-dip net pairings, it took fish an average of $4.8 \pm 1.6$ min to start responding. The average time latency to first response between prime and baseline rates significantly differed ($t = 2.15, P < 0.05$). Figure 3 shows the typical behaviour of a fish that is placed into its operant chamber containing the pendulum.

![Figure 2](image)

**Figure 2.** Number of sessions needed for fish (N = 16) to learn the operant task of pendulum pressing for a food reward.
Figure 3. The rate of pendulum pressing of a typical subject (T1G) before and after classical conditioning. T1G’s prime rate of response was 4.9 presses/min (upper graph) whereas its baseline rate of response was 3.5 presses/min (lower graph). Note the time lag before the first response is approximately 1 minute before negative conditioning and 5 minutes afterwards.
As shown in Table 1, the light stimulus given during the conditioned suppression test had a disruptive effect on the rate of responding as the number of pendulum presses shown before and during the conditioned stimulus significantly differ ($t = 16.74$, $P < 0.0001$). The mean number of pendulum presses in the 3 minutes prior to the illumination of the light was $14.3 \pm 0.9$ whereas the mean number of responses during the 3 minute light stimulus was $0.1 \pm 0.1$. Therefore the presentation of the conditioned light stimulus elicited complete behavioural suppression (mean suppression ratio = 0.0). Finally, upon termination of the light stimulus, fish were found to recover their pendulum pressing behaviour. The time taken to the first response varied greatly amongst fish (ranging from 25 seconds to 13 minutes: 40 seconds) but all fish regained pendulum-pressing for food reward within 14 minutes.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Responses before CS</th>
<th>Responses during CS</th>
<th>Suppression ratio</th>
<th>Time to response recovery (min:sec)</th>
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**DISCUSSION**

The results of the present study indicate that an initially neutral stimulus that had been consistently paired with an aversive stimulus caused the suppression an ongoing appetitively reinforced operant behaviour. When the CS (light) was presented, it disrupted the fish’s steady pendulum pressing. Although the fish have never experienced these two contexts simultaneously, their behaviour was consistent with what learning theories would predict.

In this case, the fish completely suppressed pendulum pressing during the CS presentation. Presumably, the CS evoked the memory of the dip-net along with the subjective experiences that accompanied its presentation. It could be argued that the
suppression of the operant behaviour was motivated by fear. The experience of ‘fear’ is a negative state which can disrupt an animal’s current behaviour because it is biologically adaptive to cease whatever behaviour it is performing in order to attend to the threatening situation. According to the ‘conflicting motivation hypothesis’, conditioned suppression can be explained in terms of underlying motivation (i.e. CS-US pairings induces a negative motivational state, which conflicts with the appetitive motivational state maintaining operant responding). It is reasoned that the negative motivation brought forth by the CS subtracts from the positive motivation supporting operant responding, therefore decreasing the ‘incentive’ to respond (Makintosh, 1974; Davey, 1981). It would seem that if the fish had no ability for subjective experiences, the CS presentation would have little effect on pendulum pressing. However, the CS did have an effect and in a manner consistent with results obtained from other animals subjected to this paradigm. Fish follow identical behavioural fear patterns as mice, for example; mice show flight, freezing or immobility, scanning of its surroundings, inhibition of non-defensive behaviours with gradual resumption of normal activities (Blanchard et al., 2001). Mice are deemed to be sentient animals with the capacity for a range of subjective experiences. Why then, can these same behavioural patterns under the same paradigm, not be employed as evidence towards the possibility for subjective experiences in fish?

In fact, various interesting things shown by many ‘higher’ animals during conditioned suppression have been likewise demonstrated by the fish in the present study. For example, it had traditionally been assumed that the rate of operant responding is maintained despite the introduction of CS-US pairing. However, many authors have found this to be untrue (Millenson and Devilliers, 1972; Baker and Mercier, 1982). As Hurwitz and Davis (1983) noted, there are three primary dependent measures of conditioned suppression: 1) the pre-CS-US, or ‘prime rate’ of operant responding 2) the post-CS-US or ‘baseline’ rate of responding and 3) the rate of responding during the CS presentation. Many have found that the baseline response rates of animals are usually lower than their prime rates. Likewise, the fish’s response rates were significantly reduced from their prime rates. This may be explained, in part, by ‘contextual conditioning’. The system that we used was a positive contingency (if CS – then US). This means that the probability of the US is higher in the presence of the CS than in its absence. However, the background cues, or context, can itself act as a type of weak and vague CS and take on ambiguous meanings because it is present during negative conditioning but also present during positive reinforcement. Because the classical conditioning was done in the same environment as the operant conditioning, it is possible that background cues (e.g. tank walls) from one situation carried over into the other. Some background factors related to the negative conditioning were still present when returned to the positive operant situation. It is believed that subjects experience general apprehension in situations that become associated with fear cues (Balleine, 2000) or that ‘fear’ subtly generalizes to the entire experimental situation. It is therefore not surprising that the fish’s level of operant responding decreased after aversive conditioning. This ‘apprehension’ may also explain why the latency to first pendulum press when initially exposed to the feeding apparatus, differed in sessions before and after light-net conditioning. Fish took significantly longer to start operant responding presumably
because the pervasive negative ‘feeling’ had somewhat of an inhibitory or delaying effect.

During the actual conditioned suppression test, pendulum pressing was completely suppressed (i.e. responses fell to zero) during the entire 3-minute CS interval in every fish with the exception of one (which responded once during the CS). It is also noteworthy that pendulum pressing recovered shortly upon CS termination in all fish in a similar manner that has been noted in other animal species undergoing conditioned suppression (Estes and Skinner, 1941; Spevack et al., 1974). Most interesting is the wide range in response recovery times between fish. The time taken to first response after CS termination ranged from under 30 seconds to almost 14 minutes. The individual variation contradicts the traditional view of fish as hard-wired organisms. Hard-wired theory would predict that all fish would recover operant responding at the same time. It would also predict that fish would all recover pendulum pressing behaviour immediately upon CS termination. However this was not the case. Most fish required at least a few minutes to begin. Conventional neobehaviourist learning theories contended that conditioned responses could themselves have a motivating effect on instrumental responses. It is now believed that these effects are due to the conditioning of affective states, which in turn influence instrumental performance (Balleine, 2000). If the light cue, in the present experiment, did generate negative affect, the affective state is unlikely to be triggered on and off in an instantaneous manner. This may be the reason why the fish did not resume pendulum pressing immediately after the light turned off. However, through the process of contextual conditioning, the stimuli in the background environment can sometimes elicit conditioned responses and the feedback from these responses serves to motivate ongoing behaviour (Petri and Govern, 2004). Therefore even if the context, through negative conditioning, served to suppress pendulum pressing, the same background cues also motivated pendulum pressing (the motivation strengthens as the period of CS absence lengthens). Therefore fish start to respond again.

Upon successful operant conditioning, this framework allowed fish to use a learned warning signal to actively avoid an aversive stimulus in a non-reflexive, purposeful manner. More importantly, the fish’s pattern of avoidance responses points toward some level of conscious (therefore sentient) behaviour. Conditioned suppression has been considered a potentially valuable technique for the study of fear (Estes and Skinner, 1941; Spevack et al., 1974). The results from the present study are in support of this suggestion. The type of behavioural variability shown in the operant learning process in addition to the results from the conditioned suppression test challenge the view that fish are unconscious, non-sentient animals.

This question of sentience is not a trivial one because the term ‘welfare’ can only apply to sentient animals. The present study aimed to investigate the trout’s capacity to ‘feel’ fear, a negative subjective experience. Fear is an important area of research in animal science and animal agriculture, since this intense stressor can seriously harm an animal’s welfare and biological performance (Jones, 1997). From this viewpoint, it is imperative to understand the trout’s ability to possess mental experiences, for it is this that allows an animal to suffer. Although it is impossible to measure feelings directly, we can measure them indirectly through the behaviour that the animal shows. However, there is clearly still much to learn about cognition, fear and suffering in captive fishes. Although these types of fundamental studies are of limited immediate practical value,
both at the farm-level in the aquaculture industry, as well as at the laboratory level in biomedical research, they will provide a foundation on which can be built humane husbandry recommendations. We envisage moving from understanding the fish’s psychology, to eventually developing management practices that will effectively address ethical concerns without compromising animal research, animal usage, or common sense.
REFERENCES


