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Effect of hypoxia on opercular displays: evidence for an honest signal?

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One of the most common displays made by fish is the opercular display. This display appears in virtually all taxa of fish, and is typically associated with aggressive contests between individuals. In addition, the frequency and duration of this display is often correlated with success in aggressive contests. Despite the ubiquitous nature of this display, no explanation has been provided to explain why this display is used. Here, we test the hypothesis that the opercular display functions to honestly convey information about physiological condition. While making this display, fish are unable to pass water efficiently over their gills and hence, display duration may be dependent upon underlying body condition. We tested this hypothesis by measuring the duration and frequency of opercular displays of Siamese fighting fish, *Betta splendens*, displaying to their mirror images in normoxic or hypoxic water. We observed that fish significantly reduced their displays under hypoxic conditions. This result is consistent with the hypothesis that opercular displays provide an honest indication of current physiological condition.

Communication and the underlying veracity of information exchanged between individuals have long fascinated biologists. Until the application of game theory, it was assumed by students of ethology that communication was a means of providing information to benefit both the sender and receiver (Johnstone 1998). With the advent of game theory and its application to the study of animal communication, it became apparent that individuals could benefit significantly through deceit (Parker 1974; Maynard Smith 1982, 1994). Indeed, game theory models provide a different perspective on animal communication, recognizing it as an arms race between the receiver and sender. Senders may misinform potential receivers for personal benefit while receivers attempt to extract reliable information that will allow them to benefit from the interaction (Johnstone 1998).

To elicit the appropriate response from the receiver, the majority of signals tend to be honest most of the time, particularly where production of the signal imposes some cost (Maynard Smith & Harper 2003). Signals based upon physical characteristics of the sender (i.e. indexes sensu Maynard Smith & Harper 2003) cannot be falsified (Clutton-Brock & Albon 1979; Reby & McComb 2003), although honesty may also occur where signal production is economically feasible only for individuals of very high quality (Bradbury & Vehrencamp 1998), consistent with the handicap principle (Zahavi 1975, 1977). The roaring display of red deer, *Cervus elaphus*, which accurately communicates size to opponents is an example of a signal that cannot be bluffed (Clutton-Brock & Harvey 1979; Clutton-Brock et al. 1982; Reby & McComb 2003). Similarly, the mating call of the male túngara frog, *Physolaemus pustulosus*, is based upon the physical size of the caller, allowing females to obtain accurate information about the relative size and hence, quality, of potential mates (Ryan 1980). More recently, Buchanan et al. (2003) showed that European starlings, *Sturnus vulgaris*, produce songs that accurately reflect their nutritional status as fledglings in the preceding year. Individuals that had been energetically stressed for 3 months postfledging spent less time singing, took longer to start singing, and sang fewer song bouts of shorter duration than control individuals.

The maintenance of honest communication in aggressive displays in fish is less well known. One of the best studied species is the Siamese fighting fish, *Betta splendens*. Aggressive contests between male *Betta* typically start with a head-on (facing) opercular flare display. As contests escalate, the frequency and length of the opercular flare increase dramatically. Of the display elements manifested

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during male–male contests, the duration of opercular flaring is among the most predictive of whether an individual will win or lose an encounter. Winners are reported to spend more time in the head-on opercular flare position than are losers of contests (Simpson 1968). Ultimately, displays may escalate into a fight involving tail beating and biting (Halperin et al. 1997, 1998), and can result in severe injury or death (Simpson 1968). The primacy of the opercular flare in the display sequence, its repeated use as a contest escalates, and its reliability in predicting the outcome of contests suggest that it may provide a means of assessing one's opponent throughout an aggressive encounter.

Oliveira et al. (1998) and McGregor et al. (2001) have reported that male Siamese fighting fish glean information on the competitive ability of potential rivals from visual observation of interacting conspecifics, and adjust their level of initial aggression relative to the past performance of their opponent. Furthermore, males are known to assess contestants in situ. Halperin et al. (1998) reported that hyperaggressive 'cheater' males, primed to escalate aggressive encounters with nonprimed opponents, seldom prevailed in aggressive encounters. Thus, selection has favoured an exacting assessment of opponents in this species. But what information is being acquired?

To appreciate the cost of making a sustained opercular display, it is important to recognize the function of the operculum and its role in respiration. For the gills of fish to function, they must be adequately ventilated via a unidirectional flow of water to allow gas exchange. This is particularly important within an aquatic environment because water contains 1/30 the amount of oxygen by volume as air (Hughes 1963). To ventilate the gills, bony fish use a dual pumping system. The buccal pressure pump forces water across the gills, while the pumping action associated with the movement of the operculae generates a suction force that draws water over the gills (Hughes 1963). The combined action of these two pumps results in a nearly continuous flow of water. When fish are making an opercular display, their ability to ventilate their gills is significantly compromised, and if this display is accompanied by an open mouth, there will be no ventilation of the gills.

While holding the opercular spread in either the facing or broadside positions (sensu Halperin et al. 1997), individuals must forego gas exchange via active surface breathing, or by passing water across the gill surface. Males in better body condition would be able to withstand the absence of O_2 uptake and CO_2 elimination for a greater period of time, and thus would be able to maintain an opercular spread longer than conspecifics in poorer body condition. In that sense, the duration of the opercular display should represent an honest signal of a male's body condition.

We tested the hypothesis that gas exchange limits the duration of male *Betta splendens* opercular displays by comparing the display durations of laboratory-held, mirror-image stimulated fish under normoxic versus hypoxic water conditions. If gas exchange limited the duration of displays, we predicted that fish stimulated to display under hypoxic conditions would manifest significantly shorter aggressive display durations than they did under normoxic conditions. Results conforming to that prediction would suggest that hypoxic stress associated with opercular displays will impact other aggressive displays and will therefore provide an honest signal of male body condition in Siamese fighting fish.

METHODS

Twenty individual fish were used for these experiments and were purchased from a local supplier. Their length and body depth were measured upon completion of these experiments. One fish died before body measurements were made, so we have data for 19 of the 20 fish used (see Table 1). All fish were photographed to quantify their physical size and colour, and each fish was maintained individually within a 3-litre aquarium. Fish were held within the laboratory on 12:12 h light:dark cycle at 20°C, and each fish was isolated from all others by an opaque partition. Fish were fed TetraMin (Tetra, Melle, Germany) flake food and frozen brine shrimp (*Artemia salina*) daily, and unconsumed food was removed every second day. Due to the small size of the aquaria, manipulations of individuals took place while each remained within its holding tank, eliminating the need to handle, and hence stress fish during these experiments.

We employed a repeated measures two-way ANOVA design to determine the influence of dissolved oxygen (DO) concentration (normoxic or hypoxic) and the presence or absence of an intruder (the individual's mirror image) on the frequency of opercular displays over a 5-min period. These two factors generated four unique treatment combinations to which each fish was exposed. To run these experiments, we randomly selected an individual to receive one of the four treatments. If the fish were to be exposed to a hypoxic environment, DO concentration was reduced to 3 mg/litre by bubbling nitrogen gas

Table 1. Morphological measures of fish used in this experiment

Fish	Standard length (mm)	Body depth (mm)	Fin/body colour
1	39.3	11.4	Dark red/purple
2	33.8	9.3	Dark red/green
3	32.3	10.2	Light blue/red
4	37.8	11.2	Dark red/green
5	35.6	11.4	Dark red/dark green
6	35.8	11.8	Red/light red
7	35.7	11.2	Dark red/purple
8	38.7	9.9	Dark blue/purple
9	38.0	10.0	Red green/green
10	No measurement		
11	35.2	11.3	Red/purple red
12	38.6	12.1	Dark red/green
13	40.0	11.7	Red/red
14	35.4	11.0	Blue/blue
15	40.6	12.7	Dark red/purple
16	30.4	10.5	Red/red
17	38.9	12.4	Light blue red/blue
18	34.2	12.2	Red green/red
19	42.1	13.4	Red green/green
20	35.5	9.3	Red green/green

through the holding tank water (measured by a YSI dissolved oxygen metre). For the normoxic treatment, air was bubbled through the tank for the same amount of time (approximately 2 min).

Once the desired DO level was established within the fish's holding tank, plastic was placed over the surface of the water to prevent individuals from obtaining additional oxygen through surface air respiration. An opaque partition was then removed from one end of the holding aquarium. If the treatment included an intruder, removing this partition revealed a mirror and an image that stimulated aggression. If the treatment involved no intruder, removing this partition exposed two sides of the test aquarium to the laboratory environment.

Removal of the partition was also the beginning of the recording phase of these experiments. Fish behaviour was monitored and recorded using a Sony digital video camera during 10-min trials. Once the experiment was complete, air was bubbled through the aquarium water for 5 min to return the DO levels to normoxia regardless of the treatment conditions. The tank was then returned to a holding location that contained an opaque partition on all four sides of the tank. The behaviour of fish during these trials was assessed during subsequent analysis of the videotapes. We used a computerized event recorder to measure the frequency and duration of opercular displays, lateral displays, combined opercular and lateral displays, and attempted surface respiration.

Fish used in this experiment were allowed to view only their mirror image, and aggression occurred in a context where there was no chance of physical injury. The hypoxic environment to which these fish were exposed is not unusual for natural conditions in which these fish live. To further ensure fish safety, individuals were monitored continuously for signs of distress and aquaria were equipped to allow DO levels to be raised without moving, and further stressing individuals. Prior to beginning these experiments, the methods were reviewed by the University of Manitoba Animal Care Committee and were deemed to conform to the guidelines established by the Canadian Council on Animal Care.

RESULTS

Technical problems with the videotape meant that complete data were not obtained for all 20 fish used in these experiments. One trial each in the mirror/normoxic and no-mirror/normoxic treatments were lost. Two trials in the mirror/hypoxic and three trials in the no-mirror/hypoxic treatments were also lost. Beyond these technical difficulties, all fish were healthy and responded vigorously to the stimuli provided for the duration of the experiments.

During these experiments, we observed three main types of behaviour: opercular, lateral, and opercular lateral displays. The opercular display was the most common (Fig. 1) and involved flaring of the operculae while oriented towards the opponent. The lateral display involved flared fins while the fish was oriented perpendicular to the opponent. The opercular lateral display was a combination of both displays and represented the most escalated display (Simpson 1968).

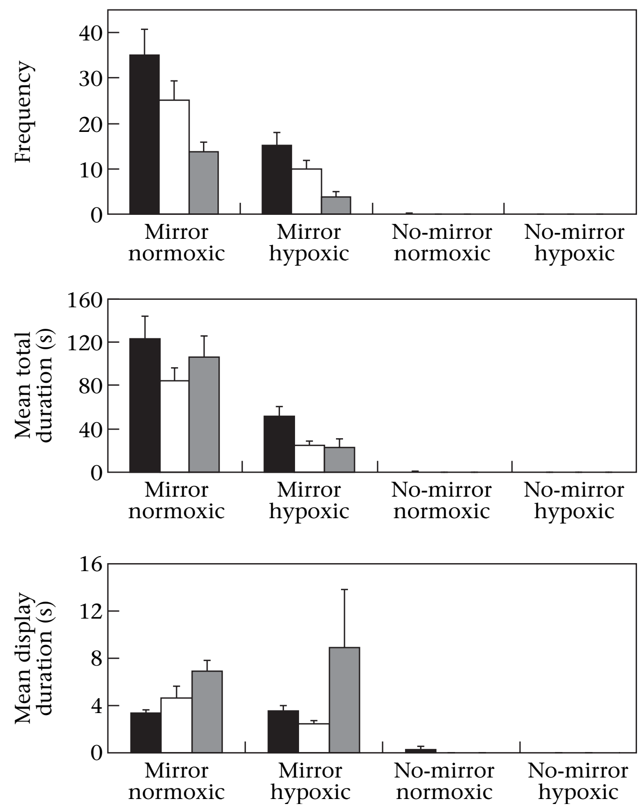

Figure 1. Measures of different aggressive displays to our manipulation of dissolved oxygen level and presence or absence of a mirror (mean + SE). ■: opercular displays; □: opercular lateral displays; ▩: lateral displays.

In only two instances did we observe any displays in the absence of the mirror, so we restricted all statistical analyses to aggressive displays when the mirror was present. As already noted, there were three instances in which we lost data with the mirror present, providing us with a total of 17 paired observations. The proportion of time spent in display was significantly affected by our manipulation of DO (Table 2). The reduction of DO reduced the proportion of time spent in display from approximately half of the total time under normoxic conditions to approximately 20% of the time in hypoxic

Table 2. Summary of the paired *t* tests associated with manipulations of dissolved oxygen

Dependent parameter	*t*	*P*
Proportion of time displaying	4.88	<0.0001
Opercular duration	3.69	0.001
Opercular frequency	3.99	0.0005
Lateral duration	3.71	0.001
Lateral frequency	4.14	0.0004
Opercular/lateral duration	5.20	<0.0001
Opercular/lateral frequency	3.73	0.0009

All tests were one tailed and had 16 degrees of freedom.

conditions (Fig. 2). In addition, every behavioural parameter associated with aggression was significantly affected by manipulations of DO, resulting in fewer displays and a total reduction in display duration (Table 2).

The mechanism probably responsible for the change in the proportion of time spent in display is illustrated in Fig. 3. At the beginning of each session, display rates of fish in both hypoxic and normoxic conditions were similar. After approximately 3 min, the display rates of fish under hypoxic conditions were significantly reduced, whereas there was no obvious influence of time on display rates for these same fish under normoxic conditions (Fig. 3).

We also observed fish behaving in a manner that would increase their oxygen levels by increasing the number of attempts at aquatic surface respiration in response to the hypoxic manipulations (Table 3). Interestingly, this behaviour was unaffected by the perceived presence of an intruder because the presence of the mirror had no impact upon these results (Table 3).

DISCUSSION

The results of these experiments are consistent with the hypothesis that opercular displays convey honest information about the physiological status of an individual. In making an opercular display, fish significantly compromise their ability to ventilate their gills and therefore suffer self-imposed hypoxic stress. We simulated this hypoxic stress by placing fish within a hypoxic environment to determine whether this stress was communicated during aggressive encounters with their mirror image. This change was not subtle. Under hypoxic conditions, fish reduced their display frequency and total display duration of all measured aggressive behaviours by approximately half that observed under normoxic conditions (Fig. 1). We assume that an effect so obvious within our data should be apparent to a potential combatant interested in determining their chances of bettering an opponent.

In our opinion, the opercular display cannot be used to bluff the physical condition of the sender and is consistent in that sense with displays used in mate choice that accurately convey information about potential mate quality. To breathe, fish must ventilate their gills, and

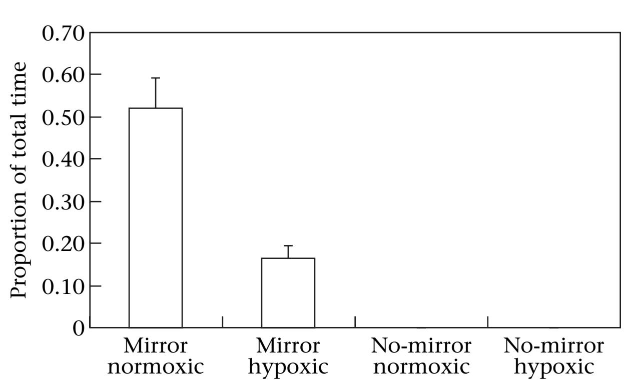

Figure 2. The proportion of time spent displaying by *Betta splendens* males under all experimental conditions.

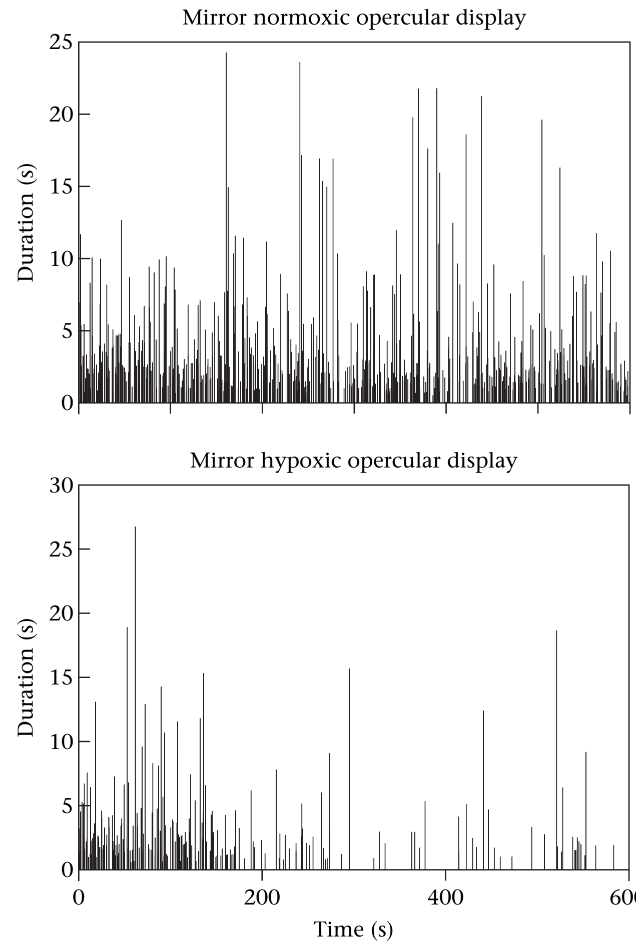

Figure 3. A representative sample of the temporal pattern of opercular displays within a single trial for one fish when confronted with its mirror image.

when not in motion, they do so through the combined actions of the branchiostegal and opercular pumps. The branchiostegal pump brings water through the mouth and into the branchiostegal cavity; the opercular pump then draws water from this cavity and passes it over the gills. Although the action of one pump in the absence of the other can pass water at a relatively slow rate over the gills, it will do so in a severely compromised fashion and we believe it reasonable to assume that flaring the operculae is the functional equivalent to a terrestrial vertebrate holding its breath. The point at which breathing must resume will be dependent upon a number of factors. However, in the short term, metabolic rate and other components

Table 3. Repeated measures ANOVA on the frequency of aquatic surface respiration as influenced by the presence or absence of a mirror (M), dissolved oxygen level (DO), and their interaction

Source	*F*	*P*
M	0.94	0.34
DO	9.71	0.0038
M*DO	0.10	0.75

Error degrees of freedom for this analysis was 32.

associated with stress will likely limit the time for which ventilation of the gills can be reduced via opercular displays. In this light, however, stress associated with the intrasexual contest and both the energetic and potential survival advantages of avoiding escalated contests with superior competitors would provide the selective impetus necessary for the ventilation of the gills via the operculae to become ritualized into a display. Full support of the honest signal hypothesis also requires showing that, in free-ranging animals, natural variation in the display behaviour is closely correlated with physical condition. Additionally, it remains to be shown that information is conveyed by the honest signal. This requires showing that animals respond appropriately to variation in the signal, in this case to variation in display duration and frequency.

The ability to respond to hypoxic stress has been posed as the underlying mechanism influencing communication in other animals. Brandt (2003) has argued that threat displays in male side-blotched lizards, *Uta stansburiana*, involve lateral compression of the thorax that may interfere with aerobic metabolism. As a consequence, individuals must therefore rely primarily on anaerobic metabolism while maintaining this display. The implication is that the duration of this display will then correlate with individual endurance capacity. Sneddon et al. (1998) showed that shore crabs, *Carcinus maenus*, were capable of showing their full repertoire of aggressive acts during a dominance contest, but that the duration of these contests was significantly reduced under hypoxic conditions. Under severe hypoxic conditions, the physiological response of shore crabs is to switch to anaerobic metabolism. This is a more energetically demanding form of metabolism and results in an accumulation of L-lactate in the blood and tissues. Sneddon et al. (1998) found that under hypoxic conditions, the larger crabs won 93% of all contests, compared to 78% of fights under normoxic conditions. They interpreted these results as evidence that smaller crabs are less able to withstand the costs of anaerobic metabolism, either due to reduced anaerobic capacity or greater sensitivity to the by-products of anaerobic metabolism. Similarly, Briffa & Elwood (2000) have shown that hypoxic manipulation can significantly alter the outcome of contests between hermit crabs, *Pagurus bernhardus*, contesting access to a shell by altering the vigour of the rapping display used to evict a resident crab.

It is not necessary that opercular displays merely reflect the oxygen status of the individual in question. Indeed, physiological condition per se will influence the ability to sustain such a display (sensu Buchanan 2000). Individuals whose physiological status is compromised by disease, poor nutritional status, or some other form of stress would not be able to sustain a long opercular display because reduced physiological condition will inherently place greater demands on their standard metabolic rates and will require that they resume breathing sooner (Schreck 1990). Any chronic condition that imposes stress upon individuals should ultimately be apparent through the duration of displays that limit the ventilation of the gills.

The hypothesis that opercular displays impose a hypoxic cost that manifests itself in behaviours that represent an honest signal of body condition provides a functional explanation for the existence and widespread occurrence of this display. It is known that acute physical stress can reduce by approximately one-quarter the energy available for other activities (Barton & Schreck 1987). Our data show that conspecifics should be able to use this display to accurately assess the physiological condition of a competitor. Enacting an opercular display reduces or prevents the passage of water over the gill surface, essentially requiring that the fish 'hold its breath'. This, in combination with the physical requirements of other behaviours associated with aggression, means that all activity must be supported by existing glycogen stores of the fish. A fish that is energetically stressed or in poor physical condition should therefore be less able to maintain this display, and will honestly convey this information to a potential competitor.

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