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Preferential Association Among Kin Exhibited in a Population of Atlantic Spotted Dolphins (*Stenella frontalis*)

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While it is widely accepted that dolphins form associations with conspecifics based primarily on similarities in age class and reproductive status, perhaps equally important is the investigation into secondary influences such as kinship. Preferential association among kin is well-researched in numerous terrestrial species, but has only recently been investigated in cetaceans. This study brings another species into the body of work being formed on the influence of relatedness on cetacean relationships. The association indices of 26 individuals with known relatives in a population of Atlantic spotted dolphins, *Stenella frontalis*, were compiled from encounters in the Bahamas from 2002-2006. Analysis demonstrated that there is preferential association among kin in this population. Mean association indices were found to be significantly higher within families than between families, and there was a positive correlation between relatedness and coefficient of association. Also, the effects of social segregation based on sex and age class, which were evident in the sample population as a whole, were absent in kin dyads.

Coefficients of association, or COAs, have been used frequently in bottlenose dolphin studies (Gero, Bejder, Whitehead, Mann, & Connor, 2005; Rogers, Brunnick, Herzing, & Baldwin, 2004; Wells, Scott, & Irvine, 1987; Connor, Heithus, & Barre, 1999; Rossbach & Herzing, 1999) and also in studies of Atlantic spotted dolphins (Herzing & Brunnick, 1997). Analyses of individual patterns of association provide insight into the social structure of a population (Whitehead & Dufault, 1999; Hinde, 1976). Specifically, investigation into whether an individual is either associated with preferentially or actively avoided on the basis of its sex, relatedness, or dominance can be extremely informative.

Since 1985, a unique, non-invasive, and long-term assessment of the life history, communication, and behavior of a population of free-ranging, habituated Atlantic spotted dolphins (*Stenella frontalis*) has been conducted off Grand Bahama Island (Welsh, 2007; Herzing, 2005; Miles & Herzing, 2003; Herzing & Brunnick, 1997; Herzing & Johnson, 1997; Herzing, 1996). Like *Tursiops* species of dolphins, this population of spotted dolphins lives in a fission-fusion society in which group size, composition, and membership vary. However, strong and stable long-term bonds are often formed. It is known that close dolphin associations are formed between some individuals which are similar in age class and/ or reproductive status, and that mothers and calves form the strongest associations (Rogers et al., 2004; Herzing & Brunnick,

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1997; Wells et al., 1987), but still unknown is whether relatedness has any role in the formation of preferential associations among juveniles and adults. Inclusive fitness theory, which proposes that individuals will cooperate with or aid genetic relatives to increase their own fitness (Hamilton, 1964), may be applicable to the association patterns of related dolphins.

Reciprocal altruism (Trivers, 1971) is, by definition, directed towards non-relatives and can arise only when there are many opportunities for reciprocation. Therefore, in societies of highly social animals, we can expect frequent associates to demonstrate altruistic behaviors towards one another (Goodall, 1986a; Connor & Norris, 1982). Inclusive fitness and reciprocal altruism are generally regarded as distinct mechanisms for the emergence of altruism. However, although reciprocal altruism is not necessarily based on kinship, the evolutionary conditions leading to altruism are the same as those for kin selection (Fletcher & Zwick, 2006; Goodall, 1986a; Connor & Norris, 1982). Mutual support among non-relatives can benefit the cooperating individuals indirectly by decreasing the reproductive success of a rival, but a more direct or obvious benefit can be seen when fitness is increased inclusively through the mechanism of kin selection.

Within every society, there are numerous selective pressures driving the formation of relationships. It is important to consider these pressures at every level: from how individuals recognize one another to how the population is ultimately affected by the relationships of its members. To begin with, preferential associations, and avoidance, can occur only when there is some form of recognition or familiarity. Recognition can be accomplished from chemical (Blaustein & O'Hara, 1986), visual, or vocal cues (Tyack, 1999; Caldwell, Caldwell, & Tyack, 1990; Caldwell, Caldwell, & Miller, 1973). Familiarity is developed socially from frequent or significant interactions with recognized conspecifics (Smith, Alberts, & Altmann, 2003). Goodall (1986a) states that among higher social mammals, the degree of familiarity is primarily *socially* based on close, prolonged associations. Spotted dolphins though, unlike these other social mammals, do not remain in family units throughout their lives. In fact, a significant decrease in association values between mothers and calves coincides with the birth of a sibling (Herzing & Brunnick, 1997).

Studies of various animals, including dolphins, have shown familiarity to be more important than relatedness (Curry, 1988), lack of kin recognition in the absence of familiarity (Blaustein & O'Hara, 1986), and a lack of preferential behavior towards either maternal siblings (Mitani, Merriwether, & Zhang, 2000) or paternal siblings (Erhart, Coelho Jr., & Bramblett, 1997). For example, genetic analysis of bottlenose dolphins in Sarasota Bay, Florida demonstrated that strongly associating male pairs are not closely related (Owen, 2003).

Furthermore, there is uncertainty as to which animals even have the capacity to recognize paternal siblings at all. Mitani et al. (2000) for example, argue that this is likely prevented by internal fertilization in a promiscuous society. However, numerous studies have found that some animals can not only recognize paternal kin (Alberts, 1999), but preferentially associate with them as well (Smith et al., 2003; Widdig, Nürnberg, Krawczak, Streich, & Bercovitch, 2001; Blaustein & O'Hara, 1986).

Still, many male alliances are known to be based on relatedness (bottlenose dolphins: Krützen et al., 2003; Parsons et al., 2003; lions: Girman,

Mills, Geffen, & Wayne, 1997; dogs: Grinnell, Packer, & Pusey, 1995; and chimpanzees: Goodall, 1986b). Krützen et al. (2003), for example, found that primary alliance (groups of two or three individuals) members of bottlenose dolphins (*T. aduncus*) in Shark Bay, Australia were significantly more closely related to one another and to their secondary alliance partners than randomly expected. It has also been shown that female baboons bias social behaviors towards both maternal and paternal half-sisters (Smith et al., 2003) and female striped dolphins tend to form groups with their kin (Gaspari, Azzellino, Airolid, & Rus Hoelzel, 2007). Even tadpoles have been found to prefer to associate with full siblings to half siblings, maternal siblings to paternal siblings, and paternal siblings to half siblings (Blaustein & O'Hara, 1986).

A number of other factors contribute to the variability found in studies of affiliative bonds in highly social mammals, one of which is gene flow (Haig, 2000; Haig, 1999; Haig, 1997). Genetic flow in most dolphin communities is male-mediated (Krützen, Barre, Connor, Mann, & Sherwin, 2004; Wells et al., 1987) however, Parsons (2002) found unique evidence of female-mediated gene flow in the bottlenose dolphins in the Bahamas. Demographics also affect kin associations. For example, the options for choosing associates for individuals in a small, closed population are restricted so that they may be required to differentiate between paternal and maternal kin (Smith et al., 2003). Also, relatively long inter-birth intervals make it less likely that maternal siblings will become close affiliates, as they would be dissimilar in age and reproductive status (Goldberg & Wrangham, 1997). Additionally, kinship may have less influence on associations when there are more mature males than there are females or when there is a low level of genetic variance. In these cases, alliance formation is beneficial regardless of relatedness (Parsons et al., 2003).

For this project, COAs of Atlantic spotted dolphins were compiled from encounters in the Bahamas from 2002-2006. This study investigates the relevance of Hamilton's theory to this population of spotted dolphins by analyzing COAs of relatives and non-relatives. To address the issue of preferential association with kin in this population of spotted dolphins, the following questions were addressed: 1) Do dolphins have significantly stronger associations with kin than with non-kin?, 2) Are strong associates that differ greatly in age more likely to be related?, 3) Is age difference correlated with the strength of kin associations?

Method

This population of free-swimming Atlantic spotted dolphins, *Stenella frontalis*, has been the focus of scientific observation for over 20 years. Identities of individual dolphins are known, as are the maternal relationships of individuals born after 1985. Eighteen of the known mother-calf relationships have been genetically confirmed through mitochondrial sequencing and none have been refuted (Green, Herzing, & Baldwin, 2007).

This study is an analysis of data from annual surveys from May through early September of 2002-2006 conducted by the Wild Dolphin Project (WDP) on board the *RV Stenella*, an 18.9m power catamaran. *Ad lib* sampling (Altmann, 1974) was conducted daily from 0700 to 2000 hours. An observer was stationed on the bridge to visually survey the water surface for the presence of dolphins. When dolphins were sighted, the vessel was steered in the direction of the dolphins and brought to idle. A group was defined as all individuals moving in the same direction and generally involved in the same activity (Rogers et al., 2004; Shane, 1990). Individual dolphins were identified from underwater photographs and video documenting the morphologies of the dorsal fin, flukes, scars, and spotting patterns (Herzing & Brunnick, 1997).

Sex was determined by direct underwater observation of the genital area, erections, or observational confirmations of pregnancy. Age classes were determined using the ontogenetic classification of the pan tropical spotted dolphin, *Stenella attenuata*, which correlates spotting patterns with age (adjusted for *Stenella frontalis* by Herzog, 1997; from Perrin, 1970). There is individual variation in the duration of the age classes, but *S. frontalis* generally follow the pattern of two-tone (0-3yrs), speckled (4-9yrs), mottled (10-16yrs), and fused (17+yrs).

In over 3600 survey hours there were 261 encounters with spotted dolphins, with the average encounter time lasting 56.8 minutes (SE = 44) and 76.34 minutes (SE = 50) for spotted-only and mixed species encounters, respectively. On average, 85% of the individuals in each encounter were identified (median = 94%, quartile range = 25%). Unidentified animals were not used in the analyses. The sighting criteria for calculating COAs of cetaceans in past studies have ranged from two (Slooten, Dawson, & Whitehead, 1993) or three (Rogers et al., 2004) sightings per individual to 10 (Quintana-Rizzo & Wells, 2001) to 30 (Gero et al., 2005) sightings per individual. Ninety-three individuals in this population were used in this study based on the criteria that each was seen at least four times between 2002 and 2006, and was born before 2002. The 93 individuals (Table 1) that met the criteria made up 81% of non-calf individuals identified since 2002. Calves were not included in this analysis due to the influence a calf's strong dependence on its mother potentially has on its associations with other individuals.

Table 1
Sex and age class composition

	Male	Female	Unknown	Total
Speckled	9	13	2	24
Mottled	10	15	0	25
Fused	20	24	0	44
Total	39	52	2	93

Study Site

The primary study area is centered north of West End, Grand Bahama Island, on the western edge of Little Bahama Bank. The entire study site is approximately 480 km², spanning 60km north to south, from 27°22' N to West End (26°29' N), and 8 km east to west. Little Bahama Bank (LBB) is an unprotected, shallow (6-16m) sand bank with patches of turtle grass, rock, and reef on the sandy bottom. The western border of the study area is a steep drop-off of over 500m in the Gulf Stream.

Data Analysis

COAs ranging from 0.00 (two dolphins never seen together) to 1.00 (two dolphins always seen together) were determined for each dolphin in the study using a half-weight index (HWI):

$$\text{Half-weight index} = N_{ab} / [N_{ab} + 1/2(N_a + N_b)],$$

where N_{ab} is the number of encounters where both individuals were present, N_a is the number of encounters that included dolphin a but not b, and N_b is the number of encounters that included dolphin b but not a. The half-weight index accounts for bias from pairs being more likely to be scored when separate than when together (Cairns & Schwager, 1987). HWIs were calculated and analyzed using Socprog version 2.3 (Whitehead, 2006) in Matlab[®] version 7.1 (The Mathworks, Inc., 2006). These programs were also used to determine the level of social differentiation, generate and compare randomly permuted data, and to run and analyze results from Mantel tests, principal coordinates analysis, and matrix correlations. All other statistical analyses were performed by SPSS 15.0.

To test the null hypothesis that individuals were associating randomly, a statistical comparison of randomized association matrices generated by Socprog to the observed association matrices was accomplished with a modification of the Manly/Bejder permutation test (Whitehead, Bejder, & Ottensmeyer, 2005; Whitehead & Dufault, 1999; Bejder, Fletcher, & Bräger, 1998; Manly, 1995). Mantel tests were then performed within and between age and sex

classes on all individuals to provide a baseline for comparison with association patterns of only kin dyads. Observed association matrices were randomized 20,000 times with 100 flips per permutation within daily sampling periods.

Kinship Associations - whole study group

Of the 93 dolphins in this study, there were 23 individuals with known relatives that were also in the study sample from ten matrilineal lines, or families. These individuals comprised 17 sibling pairs and five aunt/uncle-nephew pairs. To test for a correlation between relatedness level and association value, the observed association matrix was compared to a relatedness matrix with a Mantel test. The relatedness matrix was made by assigning a value of 0.00 to dyads not known to be related, a 0.25 to the 17 known sibships, and a 0.125 to the five known aunt/uncle-nephew dyads. These coefficients of relatedness represent the approximate proportion of genes shared by the relatives (Alcock, 1998). The values used here were based on the conservative assumption that all of the sibling pairs in this study were half-siblings. Next, analyses were restricted to mottled-speckled pairs. Mottled-speckled dyads should theoretically show weaker associations, as they are farther apart in age than dyads of individuals in the same age class. Since the more than half (55%) of kin dyads are mottled-speckled, the association index distribution of non-maternally related mottled-speckled pairs was compared to the association index distribution of maternally related mottled-speckled pairs.

Kinship Associations - restricted to individuals that have known relatives

Only individuals with known relatives were used for this portion of the analysis. The data set was restricted in this way to ensure that individuals associating with known kin exhibited the same general association patterns as the whole population, such as gender and age segregation. A Mantel test was then used to compare the HWIs of relatives between and within families.

Results

The null hypothesis of no preferred or avoided associations was rejected ($CV_{\text{observed}} = 1.11$, $CV_{\text{random}} = 1.02$, $p < 0.01$). The mean HWI of the dyads formed by the 93 individuals was 0.12 (SE = 0.13). Association indices were significantly higher in same-sex associations than in mixed-sex associations ($t = 7.89$, $p < 0.01$, matrix correlation = 0.15). They were also significantly higher within age class than between ($t = 3.8333$, $p < 0.01$, matrix correlation = 0.08, Table 2). As mentioned earlier, mother-calf associations are the strongest associations and, therefore, the mean HWIs reported here are relatively low since those dyads were not included in the analyses.

Table 2
Mean HWIs show segregation based on sex and age class

	Same-sex	Mixed-Sex	Same Age Class	Different Age Class
Mean (SE)	0.14 (0.04)	0.10 (0.04)	0.13(0.04)	0.11 (0.04)
Max (SE)	0.54 (0.17)	0.39 (0.13)	0.48 (0.14)	0.51 (0.15)

Kinship Associations - whole study group

Association indices were significantly higher within families than between families ($t = 2.74$, $p < 0.01$, matrix correlation = 0.15, Table 3). The mean within-family index (0.23, SE = 0.11) was much higher than the value of the between family index (0.14, SE = 0.07). When the data set was restricted to

mottled-speckled dyads, thirteen of the 592 possible dyads were kin. Where the majority of non-maternally related mottled-speckled dyads (n = 580 dyads) were below the population average of 0.12, the majority (58.3%) of the related mottled-speckled dyads (n = 12 dyads) were high, at above 0.25.

Table 3
Mean HWIs within and between families

	Mean (SE)	Max (SE)
Within	0.23 (0.10)	0.26 (0.12)
Between	0.14 (0.07)	0.42 (0.15)
Overall	0.15 (0.06)	0.44 (0.13)

Kinship Associations - restricted to individuals that have known relatives

There was a significant positive relationship between the association matrix and the relatedness matrix according to the Mantel correlation test. With 10,000 permutations, the mean association was 0.12 for non-relatives (n = 4257 dyads), 0.24 for aunt/uncle-nephew pairs (n = 5 dyads), and 0.22 for sibships (n = 16 dyads). The overall mean association was 0.12 (Mantel z-test, $p < 0.01$, matrix correlation = 0.06). There was no significant correlation between the number of years separating the siblings and their association indices (n = 17 dyads, $r = 0.24$, $p = 0.18$). The same held true for all kin dyads (n = 22 dyads, $r = 0.14$, $p = 0.27$). Results from analysis of same-sex and mixed-sex associations of kin with non-relatives were consistent with results from the rest of the sample population. However, in kin dyad associations, there was no difference between same-sex and mixed-sex associations (Mann-Whitney U, $U = 45$, $p = 0.77$). This set included thirteen same-sex dyads, though only one was male-male. Considering only female-female associations versus mixed-sex, the mean of the same-sex associations of kin dyads (0.19) was lower than the mixed-sex associations (0.23, SE = 0.13, Table 4).

Table 4
Sex class segregation in non-kin dyads vs kin dyads

Mean HWIs within and between sex		
Class	Non-kin	Kin
Male-Female	0.13 (0.06)	0.23 (0.13)
Male-Male	0.14 (0.15)	0.39*
Female-Female	0.16 (0.08)	0.19 (0.14)
Within	0.15 (0.07)	0.22 (0.13)
Between	0.13 (0.06)	0.23 (0.13)
Overall	0.14 (0.06)	0.23 (0.13)

*one dyad only

There was also deviation from the population trends in within age-class and between-age class association of kin dyads. Their average between-class associations were lower than their average within-class associations with non-relatives, but the opposite was seen for associations of kin dyads. In that

case, the between-class associations were actually higher than the within-class associations, although not significantly (Mann-Whitney U, $U = 29.5$, $p = 0.73$, Table 5).

Table 5
Age class segregation in non-kin dyads vs kin dyads

Mean HWIs within and between age class		
	Kin-Nonkin	Kin-Kin
Speckled-Speckled	0.19 (0.09)	0.21 (0.14)
Mottled-Speckled	0.15 (0.07)	0.26 (0.13)
Mottled-Mottled	0.13 (0.06)	0.23 (0.06)
Within	0.17 (0.08)	0.22 (0.11)
Between	0.15 (0.07)	0.26 (0.13)
Overall	0.16 (0.07)	0.25 (0.12)

Discussion

This study builds upon support for Hamilton's (1964) theory of inclusive fitness previously found in studies of primates (Smith et al., 2003; Widdig et al., 2001; Alberts, 1999; Goodall, 1986b). There is also newly found support for the theory in other species of dolphins (*S. coeruleoalba*: Gaspari et al., 2007; *T. aduncus*: Krutzen et al., 2003, *T. truncatus*: Parsons et al., 2003) and now this study contributes supporting data from *S. frontalis*. Association indices were significantly higher within families than between families, demonstrating preferential association among kin. This finding was supported by a positive correlation between relatedness and association indices of dyads.

General social trends in this study population were typical of other well-studied dolphin societies in that associations were stronger in same-sex pairs than mixed-sex pairs, and stronger between individuals in the same age class than between individuals in different age classes (Smolker, Richards, Connor, & Pepper, 1992; Wells et al., 1987). The most intriguing aspect of the results reported here is that sex segregation and age segregation were not observed in kin dyads as they were in the whole population. Although individuals in different age classes constituted the majority of the kin dyads in this study, their association values remained high. This effect is very interesting and warrants further investigation into the question of preferential association among kin.

Relatedness may be an important factor in the formation of relationships that define dolphin societies, and one that is overshadowed by more well-known factors. Factors such as reproductive status and age acting as primary influences on social structure are well documented (Wells et al., 1987). It may be valuable to now examine the effects of less predominant factors such as kinship. It is also important to note that the results from this project were drawn conservatively, as no paternal relatedness was assumed and not all individuals in the analysis were seen in each year. It is possible not only that some of the kin dyads analyzed were full siblings or were paternally related, but also that some of the dyads to which the kinship dyads were compared were paternally related. Paternally related dyads in the comparison

group of individuals of known maternal relations could have diluted the results.

If it was to be expected that related individuals should associate preferentially with one another based on the theory of inclusive fitness (Hamilton, 1964), there should also have been lower associations among aunt/uncle-nephew dyads than among sibling dyads. There was, however, no difference. In fact, aunt/uncle-nephew associations were higher on average. This observation could be a result of a greater degree of relatedness (paternally related individuals) among the aunt/uncle-nephew dyads than was assumed. Alternatively, this observation, along with the lack of age segregation in kin dyads, lends support to the idea that the mixed age-class groups observed in this society may be a means for cultural transmission (Bender, Herzing, & Bjorklund, 2008; Herzing, 2005; Rendell & Whitehead, 2001). In addition to forming mixed age-class groups, the individuals in this population are long-lived and are often observed in multi-generational family groups. It would follow from inclusive fitness theory that some groups are formed based on relatedness so that young dolphins are exposed to important behaviors by their relatives.

High associations among grandmothers and grandchildren should also be expected, according to inclusive fitness theory. These associations were not included in this analysis because three of the four grandchildren were calves and one was only observed in two encounters. The average HWI of the grandmothers, Blotches and Paint, with their three grandchildren that were sighted at least four times, was 0.31 (SE = 0.10), which is higher than the population mean index of 0.12.

Future Research

Analysis of a long-term data set may provide a more definitive answer to the question of whether dolphin kin preferentially associate. Long-term associations can be examined to determine if, for example, associations with kin are longer-lasting than non-kin associations. Another avenue of investigation may be to determine whether, as is the case in male bottlenose alliances in the Bahamas (Parsons et al., 2003), male spotted dolphin relatives form coalitions with one another. Unfortunately, the only male-male kin dyad in this study comprised two young individuals, Kai and Brulee, neither yet of age to join an alliance. Their association index during this study was strong however, at 0.39. Future investigations could look into the alliance participation of this dyad.

Research efforts in the future could also be put towards investigating further the relationships of related individuals that are far apart in age, including but not limited to grandparental relationships. Concentrating on those dyads could elucidate the results of this study by reducing the variable of reproductive status, which seems to predominately shape dolphin social structure. Genetic investigations of paternal relatedness in this population are currently underway (Green et al., 2007). The results from that study can be used in the future to research association between paternally related individuals and to detect significant differences or similarities between paternal and maternal sibling associations.

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Manipulation and Tool Use in captive Yellow-Breasted Capuchin Monkeys (*Cebus xanthosternos*)

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In this short report we test, at an individual level, the prediction that tool use abilities and manipulative tendencies should be correlated, derived from hypotheses in the literature which connect them at a cognitive and evolutionary level. We recorded manipulative events of six captive yellow-breasted capuchin monkeys and later compared these results to their performance in a tool using task. The frequency of time each animal was involved with manipulative events was not correlated to the number of tool-using events displayed by them, even when we analyzed the males only (the most frequent manipulators). This result goes against the idea that tool use in *Cebus* is a product of both manipulative propensities and tendency to use objects. Most likely, the evolution of tool use in *Cebus* was due to a complex combination of factors, belonging to various behavioral systems, not only to the foraging one.

In spite of the phylogenetic distance to the great apes and men, capuchin monkeys are capable of extremely versatile tool use, both in captivity (reviewed in Fragaszy, Visalberghi, & Fedigan, 2004; Tomasello & Call, 1997) and in the wild (Fragaszy, Izar, Visalberghi, Ottoni, & Gomes de Oliveira, 2004; Moura & Lee, 2004; Ottoni & Mannu, 2001). Capuchins have recently been shown to exhibit further behaviors formerly restricted to great apes; researchers found that they use anvil and stone pounding tools in the wild (Fragaszy, Izar, Visalberghi, Ottoni, & Gomes de Oliveira, 2004), besides showing, in at least one site, frequent, and quite diverse tool use, including the use of tool sets, that is, more than one tool for a single task (Mannu & Ottoni, 2006; Moura & Lee, 2004).

Additionally, capuchins deal with the environment in a vigorous and persistent way, harshly exploiting any potential food resource, so much so that this behavior was labeled as "destructive foraging" (Terborgh, 1983). Such aspect of their behavior allows them to exploit a variety of food resources not accessible to other sympatric monkey species, including the extraction of embedded foods.

Three decades ago, Parker and Gibson (1977) somewhat linked these two aspects of their ecology (tool use and extractive foraging), and argued that intelligent tool-use and complex object manipulation arose independently in the ancestors of *Cebus* monkeys, and the ape-man clade as a consequence of the evolution of tertiary sensorimotor intelligence (*sensu* Piaget) as an adaptation for extractive foraging on a number of seasonally available embedded foods.

Although their hypothesis is proposed at the evolutionary level, the authors suggest some lines of enquiry in terms of comparative studies in order to test predictions from it. Of particular interest to us here, they stress the need

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to investigate individual variation in complex object manipulation schemata. Admittedly, their main concern is with variety (of schemata, of contexts, and of objects employed), which would reflect differences in tertiary sensorimotor intelligence. Nonetheless, if there is such variation, one could expect that this be reflected in other aspects of complex object manipulation and tool use as well, such as the time devoted to it.

In a different view that bears some relation to the above, it has been suggested more generally that “tool use in captive capuchins arises from their strong tendency to interact with objects over an extended time in a variety of ways” (Visalberghi, 1993, p. 128), namely from their manipulative propensities (note, however, that Visalberghi strongly disagrees with the underlying cognitive basis of capuchin tool use suggested by Parker and Gibson). More recently, this view was reinforced and somewhat expanded in a comprehensive review of the genus (Fragaszy, Visalberghi, & Fedigan, 2004), in which the authors suggest that their “great variety of explorative and manipulative behaviors” (p. 177), as well as their persistent and interested manipulation of them is one of the two characteristics which are relevant to tool use.

However, these ideas (or parts of them) are not of universal acceptance, and some researchers have shown results which disagree with various aspects of the reasoning. King (1986), for example, has shown that there are various primate species which do not exhibit (or rarely do so) complex object manipulation, yet they do show extractive foraging. She argues that extractive foraging is linked neither to special cognitive skills nor to the evolution of intelligence.

Furthermore, Fragaszy and Adams-Curtis (1991) did not find an ontogenetic link between manipulation and tool use, thus rejecting a prediction from Parker and Gibson’s (1977) theory that both activities were indicative of cognitive abilities of the 5th and 6th stages of a sensorimotor intelligence scheme based on Piaget’s. However, the authors do propose a correlation between the generative aspects of manipulation and the tool using abilities of different species, regardless of underlying cognitive capacities. They supported such a view with a comparison between age classes, but did not extend their reasoning to the evolutionary origin of tool-using behavior. Lastly, Jalles-Filho (1995) found evidence that manipulative tendencies were not correlated to propensity to use tools in an experiment with captive *Cebus apella*.

Here we report the results of an experiment designed to test the hypothesis, expanded from Parker and Gibson’s central thesis and some hypotheses in the literature, that tool use in captive *C. xanthosternos* monkeys is a function of their overall manipulative tendencies. The particular prediction that will be tested here is that more manipulative animals should also be more frequent tool users. The underlying principle of this prediction is that both ‘tool use’ and ‘manipulative behaviors’ share a common basis. Note, however, that we treat this common basis as a black box and do not address its nature, if an expression of tertiary sensorimotor intelligence, of propensities to interact with objects, or any other factor.

Cebus xanthosternos is an endangered capuchin monkey species inhabiting some areas in a restricted region on Northeastern Brazil (Rylands, Kierulff, & Mittermeier, 2005), whose behavior and ecology are just starting to be known. As far as we know, no study on tool use has been conducted on this species, either in captivity or in the wild.

Method

Experimental subjects

Six (2 adult and 2 juvenile males, 1 adult and 1 juvenile female) captive yellow-breasted capuchin monkeys (*C. xanthosternos*) living in a single group. The group inhabited a small island (approximately 10 x 7m) within an artificial lake in a zoo setting (Parque Ecológico Municipal, Americana city, São Paulo state, Brazil). All individuals were wild born and reared in captivity. They came from a capture from IBAMA (Brazilian Institute for the Environment and Renewable Natural Resources). They had used tools before in previous experiments (unpublished data).

Observational phase

In this stage, the manipulative activities of the individuals were recorded through instantaneous scan sampling (Altmann, 1974). The manipulative events were registered at 1-minute intervals in sessions lasting 45 minutes each, along 19 days, with 8 sessions per day. The order of sampling the animals in the intervals was fixed for each day and varied between days in a pseudo-random fashion. Observations were conducted from 7:00 to 17:30. A total of 6120 data points were obtained due to the loss of some data points.

A behavioral category 'manipulative event' was operationally defined as any behavior that involved modification of an external object through two or more coordinated motor activities. For example, digging the soil as a necessary step to obtain a buried food item (e.g. grass roots) was classified as a manipulative event, but picking a food piece directly from the ground was not. In our category we included object-object manipulation instances, but only if the event could *not* be categorized as tool use (for example, if the animal hit a hard fruit against other substrates this was considered a manipulative event). Tool use, for the purposes of our work, was defined as any action of a deployed external object upon other object(s) that resulted in a modified state of the later, which was then subsequently used by the animal, e.g. hitting a stone on a nut and eating the edible parts inside it (similar to Beck, 1980). In terms of Parker and Gibson's (1977) scheme, our 'manipulative event' class encompasses the following categories: simple object manipulation (e.g. opening a closed leaf to reach a larvae inside it), object substrate manipulation (e.g. rubbing a twig on a hard surface) and complex object manipulation, but excluding tool use (e.g. banging two branches against each other).

Our behavioral category 'manipulative event' was defined in order to include those motor patterns sharing formal characteristics with the ones involved in tool use, which by necessity involves at least two manipulative events. Stereotyped or automatically-performed behaviors (auto grooming for example) were not included. 'Manipulative event' is a specific behavioral category, and does not function as a general activity index. At the end of the observational series, it was calculated the percentage of the total number of data points that each animal was recorded as performing manipulative events, which was used as a proxy for their manipulative propensities.

Experimental phase

After the end of the observational stage, the subjects were tested in a tool-using task. The apparatus consisted of a transparent 9 mm Plexiglas box with a transparent 3 mm Plexiglas lid and a couple of different sized quartzite stones to serve as potential hammers to break the lid. The Plexiglas box with a visible piece of food (maize) inside it was then secured to the ground, and two quartzite stones of different sizes were placed alongside. The only way for the animals to obtain the food was by breaking the lid.

Each subject was tested in a relative isolation from others. We prevented close approach with a small physical barrier and our presence. However, visual contact with the remainder of the group could not be prevented.

Each individual was given 10 different trials in succession. A trial began when a subject attempted to get the food inside the box, that is, when the subject approached and touched the box for the first time, in a clear movement to reach for the food placed within it. The trial ended when the lid was broken or if the box was abandoned for more than 5 minutes. In either case the box was replaced and a new trial began. Mere manipulation of the stones was not considered as a criterion for the beginning of the trial. Only trials that involved use of stones to break the lid were considered here (14 in total), in order to exclude from start the possibility of

breaking the lid with their teeth or hands only (something that happened before in experiments with another *Cebus* species). Every time a monkey attempted to break the lid with a stone, he/she was successful. Trials were conducted before the two regular feeding times of the animals (morning and at the end of the day) so as to keep their motivation high.

We then tested, through Spearman rank correlation test, if the percentage of the time the monkeys were involved with manipulative activities (in the observational phase) was correlated both to the number of tool use trials by them and to average trial duration, which can be considered a measure of the animal's efficiency in the use of tool.

Results

During the observational phase, all individuals performed a great number of manipulative behaviors, and were involved for a considerable amount of time on such activities, although there are marked individual differences (Table 1). When presented with the transparent box, all individuals, except one (Catriona), used the stones to break the lid (Table 2).

Table 1
Summary of the manipulative activities of the study group

Subjects	Number of manipulative events	Percentage of time spent in manipulative activities
Ahab (♂)	297	4.85
Ben Gunn (♂)	418	6.83
Moonstone (♂)	506	8.27
Smollet (♂)	473	7.73
Parthepone (♀)	424	6.93
Catriona (♀)	364	5.95
Average	413.67	6.76

Table 2
Summary of tool using activities of the study group

Subjects	Number of tool using events	Duration (s)
Ahab (♂)	3	270.51
Ben Gunn (♂)	5	163.59
Moonstone (♂)	3	273.22
Smollet (♂)	2	157.76
Parthepone (♀)	1	153.23
Catriona (♀)	-	-
Total	14	1018.31

The Spearman rank correlation between the frequency of time each animal performed manipulative events and the number of tool-using behaviors displayed by them (excluding Catriona, which did not use tools) proved to be non-significant: Spearman rank correlation coefficient $R = -0.31$; $t = -0.56$; $p > 0.6$ (Figure 1).

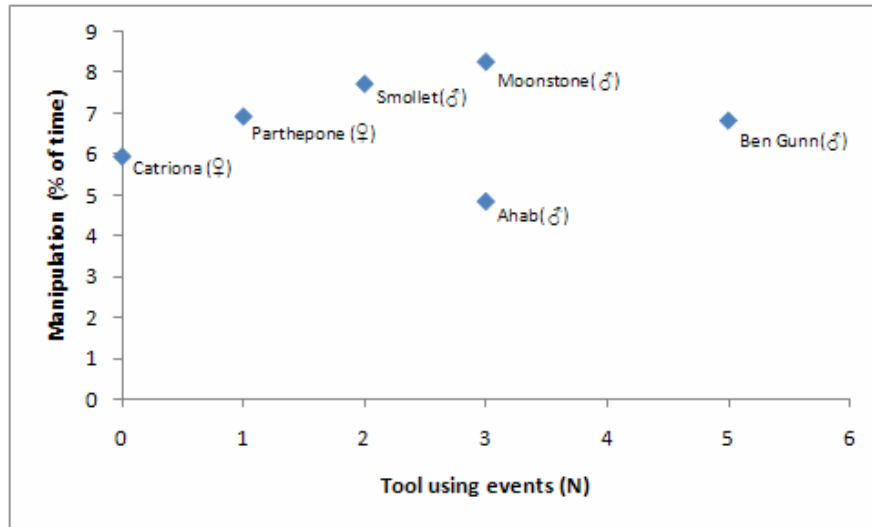


Figure 1. Relation between the time spent in manipulative activities and number of tool using events.

Since males were more manipulative than females (Goodness-of-fit test for different proportions $\chi^2 = 60.68$; $p < 0.001$), only one female used tools, and at a single occasion, we conducted another test, but excluding such female. The results were still non-significant: Spearman rank correlation coefficient $R = -0.37$; $t = -0.47$; $p > 0.68$.

The correlation with average trial duration was also non-significant: Spearman rank correlation coefficient $R = -0.1$; $p = 0.87$.

Discussion

The results show that the overall manipulative propensity of the monkeys is not correlated to the disposition and efficiency to use tools or in any way could be used to predict tool use. Thus, the present work does not support the contention that tool use in *C. xanthosternos* is a product of both overall manipulative propensities and tendency to use objects displayed by these animals, at least in an individual level.

Note that although Fragaszy and Visalberghi (1989) found out a correlation between exploratory behavior and tool use, exploration in their case involved the same tool and apparatus used in the experiments, and was not a measure of overall manipulative tendencies, such as the one used here. Thus, it may also be the case that manipulation/exploration with the tool itself or part of it may be necessary or a pre-requisite for correct tool use, as suggested by Visalberghi (1987; see also Fragaszy, Visalberghi, & Fedigan, 2004, p. 185) and also found by Fragaszy and Visalberghi (1989). Such result, however,

bears more significance to explain the ontogeny or emergence of tool-using behaviors in *Cebus*, than to its evolution.

A similar criticism might be raised in our case, that is, that the results at the individual level cannot be extrapolated to the evolutionary level. Nonetheless, note that the connection here is given by the common ground linking manipulative and tool use behaviors. If such factor has been selected for and is subject to individual variation, then we might expect to see such variation equally reflected in its consequences, both manipulation and tool use. It remains to be studied however, the alternative proposed by Byrne and Suomi (1996) that “individuals that are more diverse in their manipulative styles are more successful at tool use problems”. A similar proposal has been advanced by Frigaszy and Adams-Curtis (1991), who defend that differences in tool use between species are correlated to characteristics of generativity in manipulative behaviors, including the variety of acts towards a given object. Diversity, and other aspects of generativity, was not captured by our study design. If these aspects are found to be good predictors of tool using abilities, both at an individual and a species level, then they may provide a good foundation for hypothesis attempting to explain the origin of tool using capacities in the genus *Cebus*.

Thus, we are by no means stating that manipulation is completely uncorrelated to the emergence of tool use in evolutionary terms. What we are arguing is that one aspect of manipulation, namely, the tendency to perform the behavior, does not seem to be correlated to the tendency to perform tool using behaviors. The conclusions are by necessity preliminary, given the scope of the experiment and the limited group size. Nonetheless, we believe that it deserves attention, not only because it can lead to a more careful consideration of future studies (see below), but also because it replicates findings of another species of the genus (Jalles-Filho, 1995).

A possible confounding factor in our analysis is the potential influence of social factors in the access to the apparatus. Social influences have been argued before to influence manipulation (Byrne & Suomi, 1996) and possibilities for social learning (Fragaszy & Visalberghi, 1989). However, note that (based on *ad libitum* observations) Ahab was clearly the dominant male, but this did not prevent other males from using the tool at comparable frequencies. Nonetheless, one might still argue that social factors may play a role both in determining the fact that females almost did not use tools, and also in the anxiety of subordinate individuals when manipulating the apparatus or close to it. Thus, further studying the influence of social factors on tool use and performance, especially by testing the individuals separately, is a promising field of enquiry.

Having considered this potential caveat, our results are in line with other findings, all of which point to the need to expand and detail even further the test of the hypothesis that the evolution of tool use is somehow connected to manipulative tendencies. These tests should encompass other species of the genus, other measures of manipulation, and other kinds of tool use, in order to check which aspects of manipulation, if any, are correlated to tool use abilities. Then, we may be in a better position to propose and test theories about the evolution of tool use capacities in the genus that take into account ecological and/or social factors.

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A Novel Behavioral Test Battery to Assess Global Drug Effects Using the Zebrafish

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The zebrafish (*Danio rerio*) has been at the forefront of neurobiological research and is steadily gaining favor as a model for behavioral applications. The ease of handling, high yield of progeny, and efficient mode of drug delivery make this species a particularly useful model for behavior. Here, we append to the growing body of literature on zebrafish behavior by introducing a novel behavioral battery of tests aimed at identifying drug induced alterations in social and motoric behaviors. In a series of experiments, zebrafish were exposed to MK-801 (0, 2 μ M, 20 μ M), SKF 38393 (0, 10 μ M, 100 μ M), and ethanol (0, 0.5%, 1.0%) for one hour and overt locomotor behaviors were scored. Following a one-hour treatment exposure, circling behavior (a thigmotaxic display typical of dysregulated glutamate function) was scored from videotape at specific time points over a 37-minute session. In a separate experiment the zebrafish's natural tendency to shoal (social display) was analyzed using a novel open-field paradigm that examined fish distribution over quadrants. Most notably, MK-801 (20 μ M) significantly increased circling behavior compared to controls. However, shoaling displays were disrupted when zebrafish were exposed to both MK-801 and SKF 38393 (20 μ M and 100 μ M respectively). Our results, in part, complement existing knowledge about zebrafish behavior following acute drug exposure. Additionally, our novel approach to assessing shoaling behavior, reported here, introduces an alternative view of social/group behavior in the zebrafish that is sensitive to both NMDA and dopaminergic manipulation.

Viable animal models (i.e. rodent and primate) have enabled researchers to infer about the fundamental features of human behavior and physiology. Since the zebrafish's introduction as a model for neural development by Streisinger in the 1960's (Grunwald & Eisen, 2002), its promise as a genetic tool for biological research has nearly been realized (Beis & Stainier, 2006; Driever et al., 1996; Guo, 2004; Haffter et al., 1996). Recent years, however, have seen a steady increase in the use of this species in behavioral applications (Miklósi & Andrew, 2006; Spence, Gerlach, Lawrence, & Smith, 2008). Many features of the zebrafish make it a particularly attractive candidate for inferring higher-level vertebrate behavior. The anatomical similarities to other vertebrates and nervous system structure, albeit in a less complex form, of this particular species of fish have allowed researchers to draw conclusions regarding the function of the human nervous system. Zebrafish central nervous system development closely resembles that of other vertebrates and has been the focus of most research thus far (for a review see Blader & Strähle, 2000). Recently, commercial resources (e.g. Zebrafish Information Resource Center, ZIRC) and the availability of selective genetic progeny (Sprague, Doerry, Douglas, & Westerfield, 2001) make research on the zebrafish an efficient and inexpensive addition to behavioral inquiries.

There have been an increasing number of research investigations highlighting the behavioral spectrum of the zebrafish and drug challenges. Because the zebrafish model affords an alternative and efficient mode of drug delivery via the gills, submersion has been the primary method used (Gerlai, Lee, & Blaser, 2006; Levin, Bencan, & Cerutti, 2007; Lockwood, Bjerke,

Kobayashi, & Guo, 2004). Exogenous compounds, such as ethanol, have been shown to rapidly enter systemic circulation following introduction into the tank environment (Dlugos & Rabin, 2003). Acute ethanol exposure elicited a variety of behavioral effects in adult zebrafish on locomotor activity and shoal cohesion (Gerlai, Lahav, Guo, & Rosenthal, 2000). The reported effects on motoric function follow a characteristic inverted U-shaped function with intermediate doses of ethanol increasing locomotor activity and higher doses suppressing it. Interestingly, the effects of ethanol exposure on shoaling tendency are less clear. Fish treated with lower doses of acute ethanol and tested individually spent significantly more time in the vicinity of their reflection indicating possible shoaling tendencies, according to the authors (Gerlai, 2003). However, subsequent studies of group behavior revealed reduced shoal cohesion and increased scattering throughout the testing environment (Gerlai et al., 2000). The former may be due to aggressive tendencies elicited by acute ethanol exposure, as the author states. Decreased predator avoidance has been demonstrated following ethanol exposure leading researchers to draw conclusions regarding anxiolysis in the zebrafish previously seen in the rodent model for anxiety (Gerlai et al., 2006). The N-methyl-D-aspartate (NMDA) receptor antagonist MK-801 increased circling behavior when measured in 13-cm diameter round glass dishes (Swain, Sigstad, & Scalzo, 2004). Exposure to MK-801 also induced behavioral changes on measurements such as locomotor activity and swim location.

The zebrafish shares more similar features to humans than other genetically homologous models, such as the *Drosophila* (Guo, 2004). Likewise, it is also postulated that the anatomical similarities of neurotransmitter pathways, like dopamine, may indicate comparable neural functionality. Dopaminergic modulation has been shown to produce overt changes in swim behavior that included disorientation and marked decreases in movement in the zebrafish (Anichtchik, Kaslin, Peitsaro, Scheinin, & Panula, 2004). Dopamine agonists (e.g. SKF 38393) elicited a variety of behavioral responses in the rodent model including hyperactivity and increased locomotion (Sobrian, Jones, Varghese, & Holson, 2003). Taken with the above evidence, it is highly plausible that zebrafish would exhibit a similar behavioral repertoire to pharmacological exposure. To date, little focus has been given to a comparative approach aimed at comparing and contrasting the behavioral changes that result from pharmacological intervention.

The current sets of experiments were designed to extend on the existing research regarding zebrafish behavioral assessment. We sought to investigate the effects of three established pharmacological agents (ethanol, MK-801, and SKF 38393) on previously reported behaviors seen in the *Danio* model (Engeszer, Da Barbiano, Ryan, & Parichy, 2007; Gerlai et al., 2000; Gerlai et al., 2006; Speedie & Gerlai, 2008). The drugs chosen were based on neurotransmitter systems effected and their potential roles in locomotion and shoaling displays. Furthermore, MK-801 (Chartoff, Heusner, & Palmiter, 2005), SKF 38393 (Rosengarten, Bartoszyk, Quartermain, & Lin, 2006), and ethanol (Colombo et al., 1998) have been shown to effect locomotor activity and produce stereotypic behavior in several animal models on a variety of behavioral paradigms. First, we examined the effect of each drug on individual behaviors previously shown to be sensitive to water-soluble compounds. The effect of drug exposure on shoaling displays was then explored in a separate

series of experiments using a novel open-field paradigm that assessed fish distribution over time. The results of the current study append to the growing body of literature on zebrafish behavior and introduce a novel approach to studying drug induced changes in overt locomotive behavior and shoaling tendency.

Method

Subjects

Adult zebrafish (*Danio rerio*) were housed in a community tank system at a temperature of approximately 27°C. The community system consisted of two 10-gallon tanks (holding approx. 30 fish per tank) and four 5-gallon tanks (holding approx. 10 fish each) connected to a main reservoir. Additionally, a separate (autonomous) 10-gallon tank was used to hold approximately 30 fish. Fish were kept on a 14 h on and 10 h off light cycle and were fed flake food twice daily (TetraMin, Blacksburg, VA). Tank system contained aeration and filtration units with de-chlorinated H₂O. Drug exposure and testing took place during the light cycle between 8:00 a.m. and 5:00 p.m. Both male and female fish were used in the experiments.

General procedure

In these series of reported investigations, subjects were videotaped and later observed to assess the effects of ethanol, MK-801, and SKF 38393 on behaviors that reflect typical motor and schooling output as well as the assessment of side effects of drug exposure in zebrafish. As part of this three-tiered test battery, fish were individually exposed to treatment and tested both during exposure (overall mobility in a 250ml beaker) and for 37 minutes after exposure (circling behavior). In the final phase (open-field/schooling) we quantified the shoaling displays of zebrafish during drug exposure and tested subjects by using a group setting design that allowed for the display of social behaviors.

In the first study, subjects (n=30) were acutely exposed to the NMDA antagonist MK-801 at concentrations of 0.0 μM, 2 μM, and 20 μM (based on the dose range used by Swain et al., 2004). In the second study, a separate group of subjects (n=30) were immersed in micromolar concentrations (0.0 μM, 10 μM, 100 μM) of the dopamine D₁ subtype agonist SKF 38393. Doses of SKF 38393 were chosen to reflect minimum and maximal (but non-lethal) ends of a proposed dose range. The last study exposed a final group of fish (n=30) to three concentrations of ethanol: 0.0%, 0.5%, and 1.0% (v/v) to evaluate the aforementioned behavioral effects, where the dose range was acquired from numerous investigations of ethanol and zebrafish behavior (Dlugos & Rabin, 2003; Gerlai et al., 2006; Lockwood et al., 2004). Ethanol (95%, 190 proof) was diluted and prepared prior to fish exposure. All drugs were mixed with dechlorinated tank water at a temperature of approximately 27°C. In all three studies subjects were randomly placed into one treatment group for each of the drugs in the investigation (fish were only tested once). Drug doses were calculated using the weights of the salts with the exception of ethanol, which was calculated as a percentage concentration (volume ethanol/volume pre-conditioned tank water).

Drug exposure behavior

Solutions were mixed in a 250 mL beaker containing 200 mL of de-chlorinated tank water. Each subject was randomly assigned to a treatment group and immersed in a solution containing the drug for one-hour. During this time, all behavior was videotaped using a Sony 8 mm camcorder positioned perpendicular to the 250 mL beakers for later assessment and scored by observers blind to treatments conditions. Scoring was done at set time intervals for thirty seconds at specific time points (5, 10, 20, 30, 45, and 55 min.) based on increments outlined by Swain and colleagues (2004). Observers scored for three behaviors when subsequently viewing videotape: *immobility time*, *erratic swimming*, and *top time*. With the exception of fin movement, immobility time was defined as the time a subject spent without movement in any direction. Erratic swimming was observed as the amount of time a subject spent swimming in an irregular and jostling fashion and included darting motions and rapid looping movements around the beaker. Finally, top time was the amount of time spent occupying the top half of the exposure

beaker. To minimize the potential influence of nearby subjects, opaque barriers were placed between the testing beakers to obscure the view of neighboring fish.

Circling behavior

The tendency for zebrafish to engage in repetitive circling behavior around the testing environment following acute exposure to the NMDA antagonist MK-801 has been reported previously (Swain et al., 2004). We sought to replicate, in part, these findings and to compare the known effects with two of the other agents under investigation in the current study (ethanol and SKF 38393). In this study, circling behavior was analyzed following a one-hour exposure to a treatment condition; this is in contrast to Swain and colleagues (2004), they measured drug-induced circling behavior in zebrafish during exposure time. Zebrafish were placed in a 6.5 x 13 cm Pyrex dish filled with 550 mL de-chlorinated tank water after being exposed for one hour to one of the following treatment conditions (in a separate 250 mL beaker): ethanol (0, 0.5%, 1.0%), SKF 38393 (0.0 μ M, 10 μ M, 100 μ M), or MK-801 (0.0 μ M, 2 μ M, 20 μ M). A camcorder was placed aurally above the dishes to record circling behavior exhibited by the fish. Following one-hour exposure at one of the aforementioned treatment conditions, subjects were placed in the dish of de-chlorinated tank water for 37 minutes while their behavior was recorded and subsequently scored by raters blind to treatment conditions. Observers would later score these videos and record behavior based on the number of laps (complete 360 degrees around test dish) swam in 30 seconds at five different time points (5, 10, 15, 20, 25 min.) as outlined by Swain et al. (2004).

Open-field – schooling/shoaling displays

Zebrafish are naturally a schooling fish (Gleason, Weber, & Weber, 1977). We tested their tendency to shoal in a separate series of experiments investigating the effects of the highest doses of the treatment regimens used, as defined by the results of the first two series of experiments. We used a novel open-field paradigm to investigate the distribution of fish in a set of quadrants over a given time frame. Two methods were used to examine the shoaling display of zebrafish when exposed to the highest dose of ethanol (1.0%), SKF 38393 (100 μ M), MK-801 (20 μ M), or control (de-chlorinated tank water). Nonparametric analyses were first applied to evaluate if the distribution of fish at a particular time point was significantly different from a known probability distribution. In the first method, the thirty-minute session time was broken into six five-minute time blocks (0-5, 5-10, 10-15, 15-20, 20-25, 25-30 minutes) to demonstrate change in schooling habits during drug exposure as a function of time. For each time block, the average frequency of fish (tabulated every 10 seconds) was calculated per quadrant (Cartesian system). The frequencies were then analyzed with the exact multinomial test to compare to a known frequency distribution. Secondly, multinomial tests were run at every ten-second interval for each five-minute time block to account for variations in independent measurements. An average p-value (cumulative probability) was then computed and graphed to illustrate significant shoaling on the part of the treatment and control groups. This was also done to complement quadrant statistical analysis. Groups consisted of either 5000 mL of tank water (control), 1% ethanol solution in 5000 mL of tank water, SKF38393 (100 μ M in 5000 mL of tank water), or MK-801 (20 μ M in 5000 mL of tank water). Fish (n=10 for each group) were placed in a plastic tub (13 cm x 18 cm x 29 cm) filled with 5000 mL of regular de-chlorinated or drug-treated de-chlorinated tank water for 30 minutes. Each tub was divided into four equal quadrants with markings on the back wall of the tank so that the observers' views would not be hindered by the quadrant marking and fish would remain visible at all times. Schooling behavior was recorded via video and later scored in ten second intervals throughout the 30-minute session. At each interval, the number of fish in each quadrant was tabulated and recorded. In the case where a fish was isolated against a quadrant marking, the head of the fish was counted as the midpoint of the body axis and the corresponding quadrant was recorded (this procedure was used to represent swim path on the part of the fish). Quadrants were labeled using the Cartesian coordinate system and labeled in a counterclockwise fashion.

Statistical analyses: drug exposure, circling behavior, shoaling activity

Individual behaviors (immobility, erratic swimming, top time, and circling behavior) were analyzed using a mixed model ANOVA to assess significance for both within (time) and between (treatment) subjects effects. Post hoc analysis (Fisher's LSD) was used for multiple

comparisons of significant effects where found. Open-field/shoaling data were analyzed using the exact multinomial test to assess the frequency distribution of fish over the quadrants. Each 30-minute session was broken into six five-minute time blocks to evaluate schooling behavior change over time. The frequency of fish was averaged across quadrants for each time block and analyzed using the multinomial test. Frequencies were truncated to the .10 decimal place and rounded to the nearest integer before multinomial analysis. Independence of measures was accounted for by calculating cumulative probabilities at each ten-second interval using the multinomial test. Exact probabilities (p-values) were then averaged for each time block for subsequent illustration.

Results

Drug exposure behavior

The effects of ethanol, SKF 38393, and MK-801 were evaluated during a one-hour exposure time in an effort to characterize the behavioral effects of three drugs previously reported to be biologically active in zebrafish (Gerlai et al., 2000; Gerlai, Lee, & Blaser, 2006; Miller & Gerlai, 2007; Speedie & Gerlai, 2008). The most compelling effect was seen with the NMDA antagonist MK-801. Exposure to this drug resulted in increased time spent by each subject closer to the surface of the water. There was a significant effect of time [$F(2.52,62.93) = 3.21, p < .05$] and a significant interaction [$F(5.03,62.93) = 5.09, p < .01$] in the highest dose group (20 μM) for top time behavior. Figure 1 illustrates the effects of MK-801 on top time during an acute one-hour exposure. Post hoc testing (Fisher's LSD) revealed that at 45 and 55 minutes into the exposure session, fish in the highest dose group (20 μM) spent significantly ($p < .05$) more time in the top half of the exposure beaker than fish in the other treatment groups. No significant effects of the dopamine agonist SKF 38393 or ethanol were seen on any of the behaviors measured during acute drug exposure.

Circling behavior

Prior exposure to MK-801 (for 1 hour) and testing immediately afterward in "clean" tank water revealed a significant increase in circling behavior when compared to control [$F(2,21) = 3.77, p < .05$]. Post hoc analysis (Fisher's LSD) revealed that the 20.0 μM dose significantly increased circling activity over the two other treatment groups of 2.0 μM and 0 μM ($p < .05$; Fig. 2) for time measurements at five and ten minutes. SKF 38393 and Ethanol did not elicit any significant effects on circling behavior at the doses used in the current study.

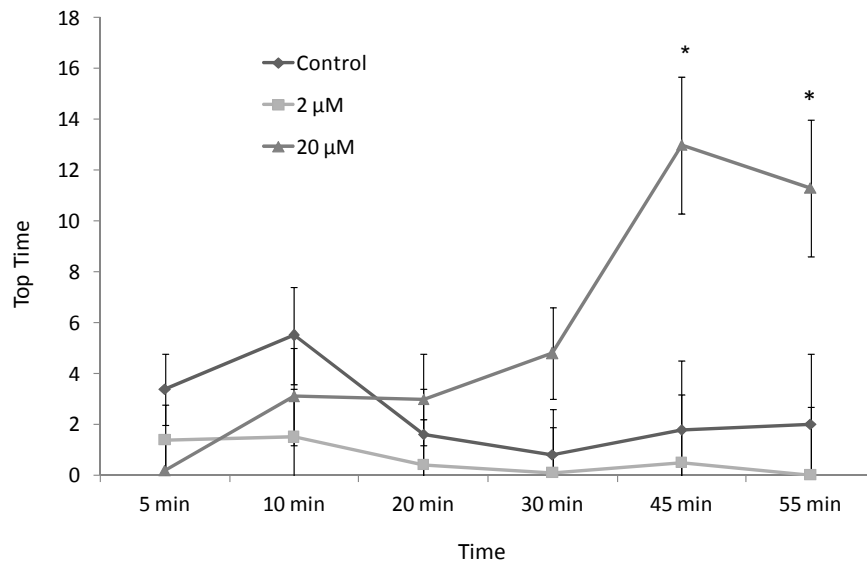


Figure 1. Effects of MK-801 on top time in adult zebrafish. There was a significant effect of session time and a significant interaction effect during acute exposure to the NMDA antagonist. As the session progressed, zebrafish increased the amount of time spent in the top half of the exposure beaker at the highest dose (20 μM; $p < .05$). Mean differences were analyzed using a mixed model ANOVA and Fisher's LSD post-hoc tests were used to indicate direction of effects where significant. Mean (\pm SEM) time (seconds) for each condition is shown, * $p < .05$.

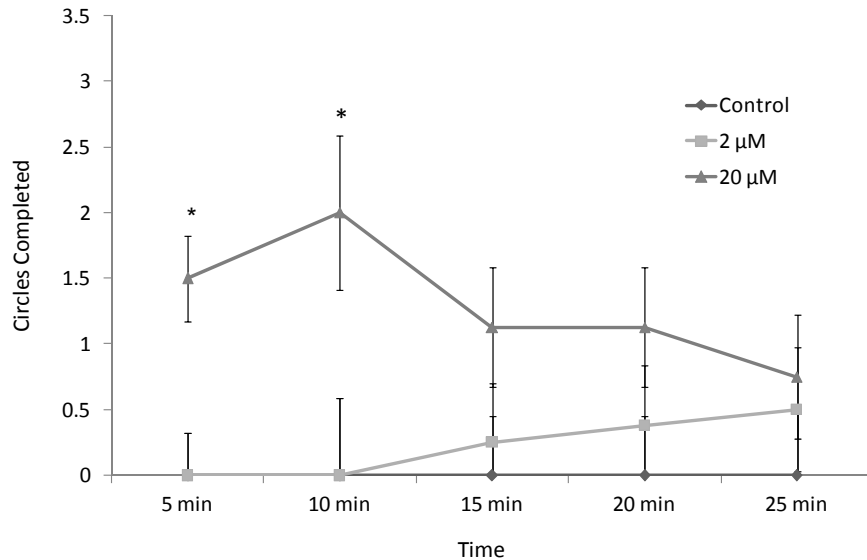
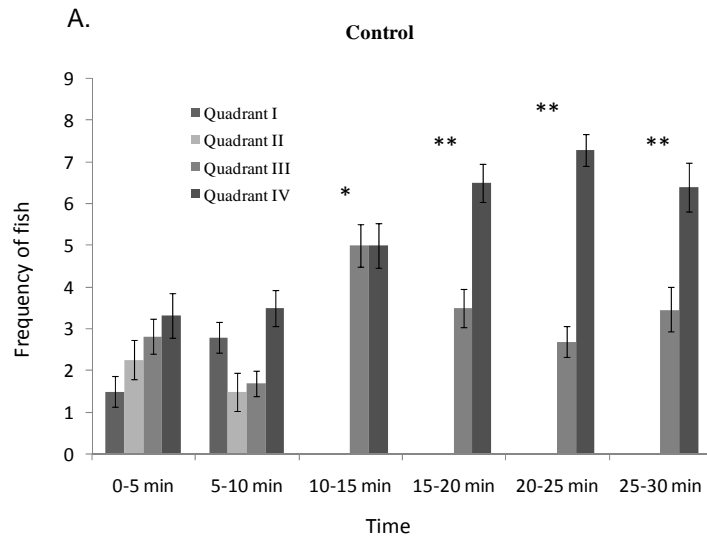


Figure 2. Effects of circling behavior following acute treatment with MK-801. The effects of MK-801 on circling behavior show the largest dose (20 μM) significantly increased circling behavior over the 2 μM dose and controls ($p < .05$; $n = 24$). Mean differences were analyzed using a mixed model ANOVA and Fisher's LSD post-hoc tests were used to indicate direction of effects where significant. Mean (\pm SEM) circles completed during 30 sec for each time point for each dose are shown.

Shoaling and schooling behavior

Shoaling behavior was notably affected by two of the three drugs under investigation in the current study. During these experiments subjects were tested in tanks containing either drug infused water or “clean” tank water (control). Results of the multinomial analysis for MK-801 (20 μ M in 5000 mL of tank water) and shoaling displays can be seen in Figures 3a,b and 4. Analysis of the control group revealed an initial disorganization for the first ten minutes of testing evidenced by non-significant multinomial probabilities for the 0-5 and 5-10 minute time blocks (p 's>.05). The distribution of control fish of each time block for the last twenty minutes was found to be statistically significant (p 's<.05) indicating an acclimation period in which fish eventually began to shoal as the session progressed. Results of the MK-801 group indicated a markedly different distribution over time. The distribution (average frequency) of fish was, in the first time block (0-5 min), found to be briefly significantly grouped (p =.03). The remaining distributions for the session were not significantly different from chance (p 's>.05). Figure 4 illustrates the average exact probability (p-value) for each time block during MK-801 treatment and largely agrees with quadrant analyses. Although the graphical representation of p-values shows increasing and decreasing slopes, the interpretation of the data is strictly dichotomous. Average p-values found above the indicated significance level should, in theory, represent non-significant results in regards to the behavior of interest (lack of shoaling).



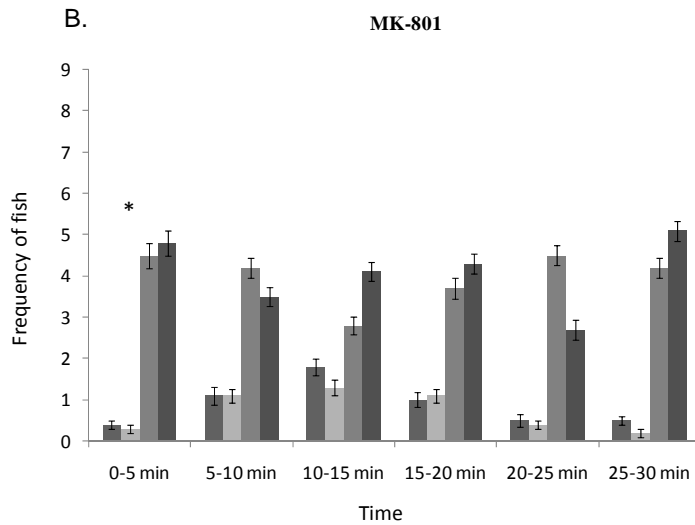


Figure 3a,b. Effects of the NMDA antagonist MK-801 (20 μ M) on shoaling displays. (a) The control group (n=10) initially shows a lack of shoaling behavior before becoming acclimated to their surroundings; fish began shoaling around 10 minutes into the session as shown by significant multinomial probabilities across the remaining time blocks (p 's<.05). (b) Fish exposed to MK-801 (n=10) exhibited greater disruption of shoaling behavior than controls. Shoaling behavior was briefly displayed (p =.03) but as the session progressed, fish began to display disorganization that proceeded until the end of testing. Multinomial statistical analyses were used to calculate p-values from mean frequencies of each quadrant. Mean (\pm SEM) frequencies are shown, * p <.05 ** p <.001.

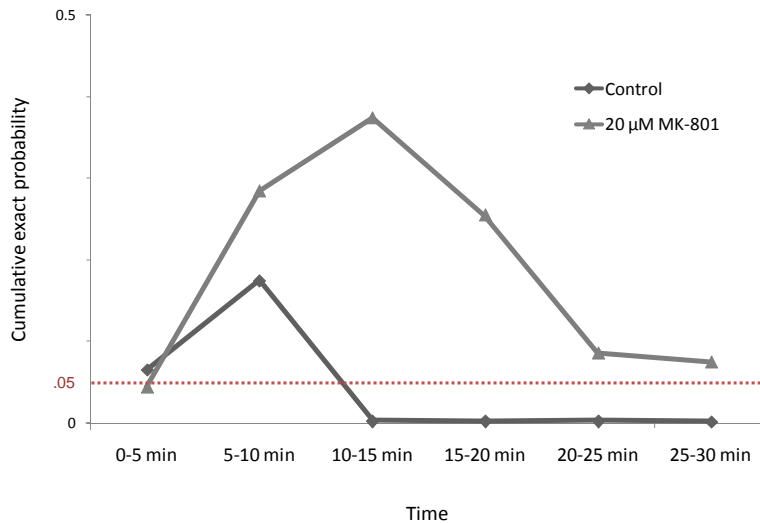
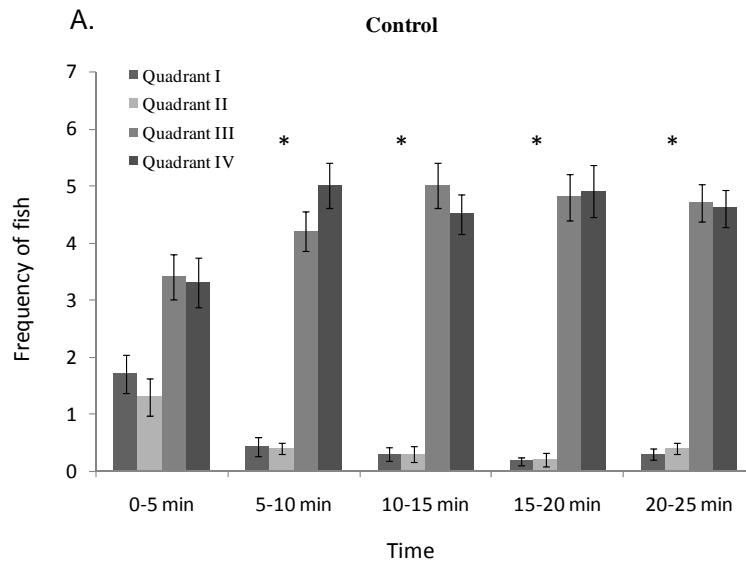


Figure 4. Distribution of cumulative probabilities reflecting the effects of MK-801 (20 μ M) on shoaling behavior. Data points represent average p-values of each time block for control and drug groups. Interpretation of data indicates a dichotomous relationship with respect to significance. Cumulative probabilities show similar trends to quadrant analyses. The first ten minutes reveals non-significant p-values for control fish after which they become and remain significant throughout the session. Fish exposed to MK-801 briefly shoal (p =.03; 0-5 min) the group then becomes disrupted and disorganized for the remainder of the session (p 's>.05). Multinomial tests were used to calculate exact probabilities at 10 second intervals during open-field testing. Significance is indicated by the dashed line labeled at .05.

The results of exposure to SKF 38393(100µM in 5000 mL of tank water) also indicated a deviation in shoaling distribution over time as compared to controls. Figure 5a,b illustrates the results of multinomial analysis of quadrant frequency during SKF 38393 exposure. Similar to control fish during MK-801 testing, subjects not exposed to the dopamine agonist showed an initial (0-5 min. time block) acclimation period for five minutes followed by shoaling display for the remainder of the session. Multinomial tests were significant for each quadrant for the remainder of the session (p 's<.05). Exposure to SKF 38393 initially mirrored that of controls with a non-significant p-value during the first five minutes ($p=0.86$) followed by brief shoaling in the 5-10 minute time block ($p<.05$). Results of statistical analyses revealed non-significant p-values for the remaining time blocks in the session; cessation of shoaling (p 's>.10). A graphical representation of the cumulative probabilities for SKF 38393 can be seen in Figure 6. Again, fish exposed to the dopamine agonist initially follow the same results as control fish until the 10-15 minute time block where the average multinomial probability becomes non-significant and shoaling behavior is disrupted.



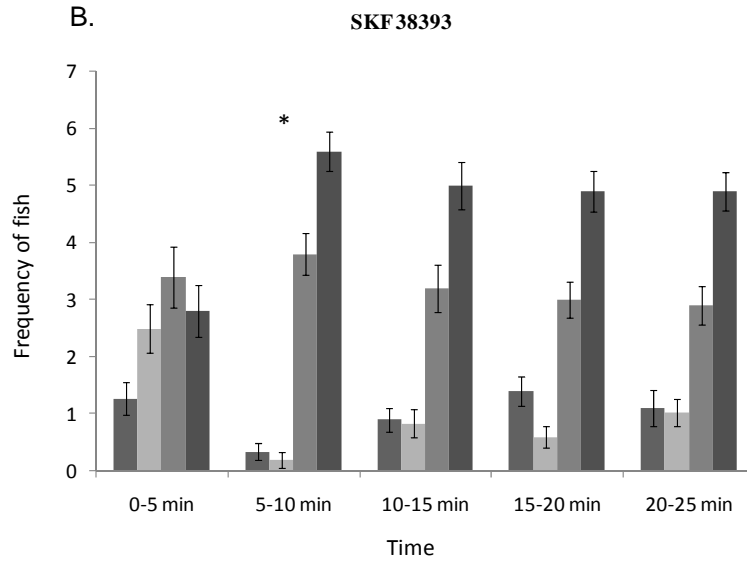


Figure 5a,b. Effects of the dopamine D₁ subtype agonist SKF 38393 (100 μ M) on shoaling displays. (a) The control group (n=10) after 5 minutes of acclimation display shoaling for the remainder of the session as shown by significant multinomial probabilities across the remaining time blocks (p 's<.05). (b) Fish in the SKF 38393 group followed the same trend as the control group in which probabilities were not significant for the first 5 minutes (p =.86) and significant during the 5-10 minute time block (p =.03). The remainder of the session witnessed a disruption in shoaling behavior with non-significant multinomial probabilities (p 's>.05). Multinomial statistical analyses were used to calculate p-values from mean frequencies of each quadrant. Mean (\pm SEM) frequencies are shown, * p <.05.

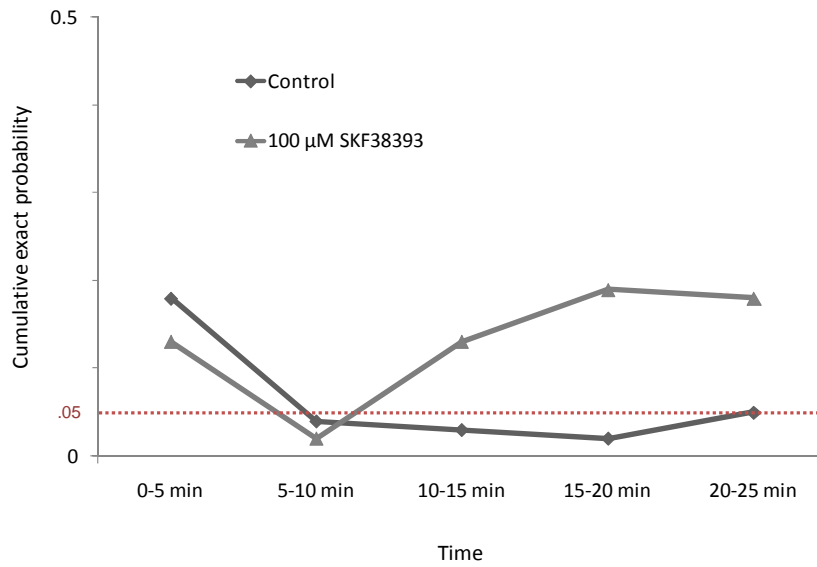


Figure 6. Distribution of cumulative probabilities reflecting the effects of the dopamine agonist SKF 38393 (100 μ M) on shoaling behavior. Data points represent average p-values of each time block for control and drug groups. Multinomial tests were used to calculate exact probabilities at 10 second intervals during open-field testing. Significance is indicated by the dashed line labeled at .05.

The non- effect of ethanol (1% ethanol solution in 5000 of mL of tank water) on shoaling behavior can be seen in Figure 7a,b. Multinomial probabilities throughout the entire session were found to be statistically significant for each time block (p 's<.01). A distribution of the cumulative probabilities calculated from each ten-second interval can be seen in Figure 8. Like quadrant analyses, multinomial probabilities were found to be significant for both control fish and fish exposed to 1% ethanol. Therefore, 1% ethanol yielded no disruption of shoaling display when compared to controls.

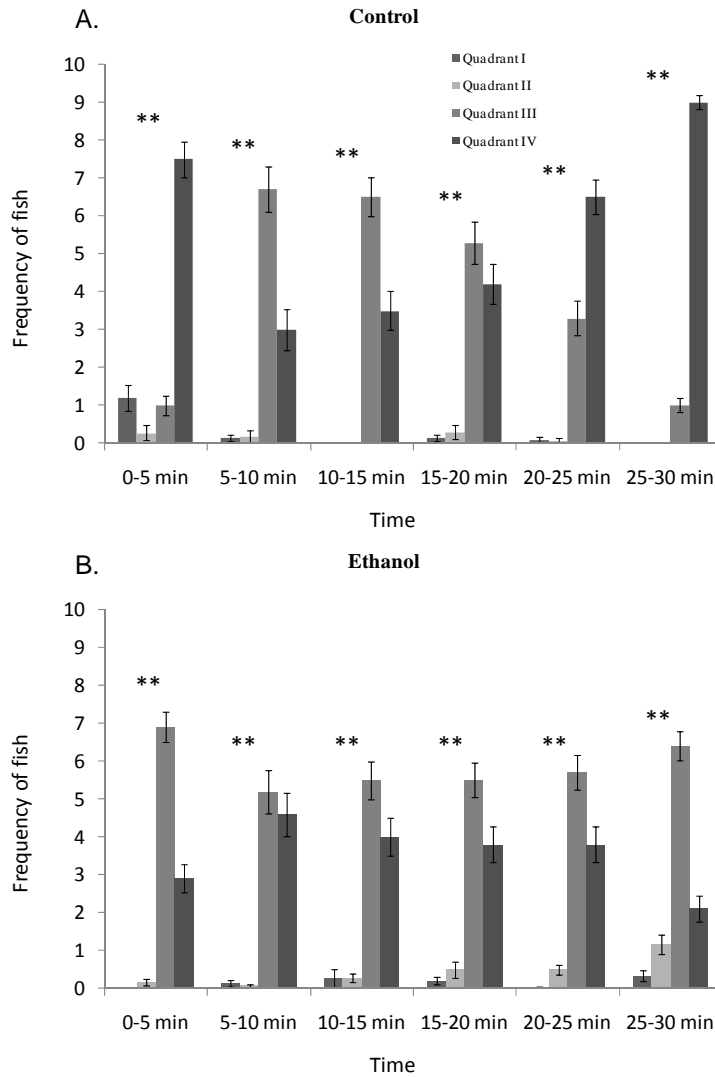


Figure 7a,b. Effects of 1% ethanol on shoaling displays. (a) Multinomial probabilities were found to be statistically significant throughout the shoaling session for the control group (p 's<.01). (b) Fish exposed to 1% ethanol displayed similar shoaling behavior to controls with significant probabilities for the entire exposure session (p 's<.01). Multinomial statistical analyses were used to calculate p-values from mean frequencies of each quadrant. Mean (\pm SEM) frequencies are shown, $**p < .001$.

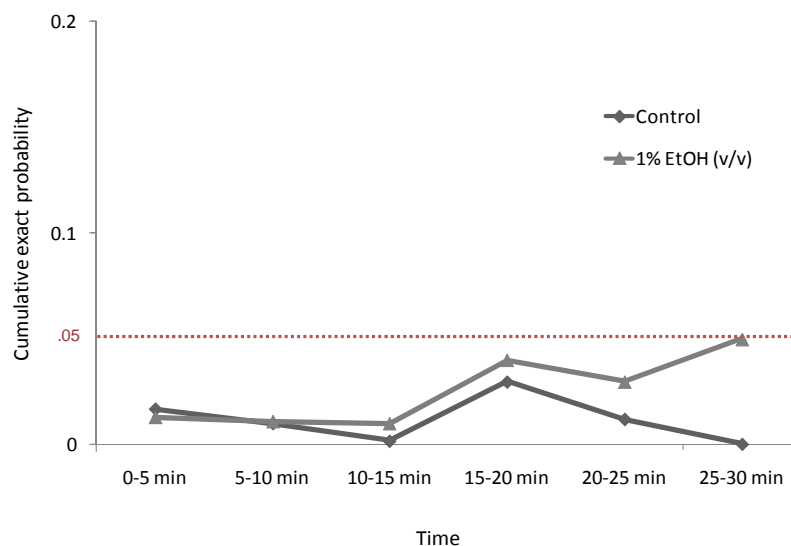


Figure 8. Distribution of cumulative probabilities reflecting the non-effect of 1% ethanol on shoaling behavior in zebrafish. Data points represent average p-values of each time block for control and drug groups. Multinomial tests were used to calculate exact probabilities at 10 second intervals during open-field testing. No difference in average cumulative probability was found at each time block for ethanol and control. Significance is indicated by the dashed line labeled at .05.

Discussion

The current studies reported here introduce a novel behavioral test battery aimed at identifying drug-induced alterations in social and motoric behaviors. The drugs used in the current study (ethanol, MK-801, and SKF 38393) were chosen for their observed effects on both rat and human performance. Previous reports of induced alterations in swim behavior following treatment with ethanol (Dlugos & Rabin, 2003; Gerlai, 2000) and MK-801 (Swain et al., 2004) also provided an impetus and rationale for the current investigations. In the studies reported here, both individual and group behaviors were significantly affected by exposure to one or more of the agents under investigation. Exposure to the NMDA antagonist MK-801 (0.0 μ M, 2 μ M, 20 μ M) led to an increase in the amount of time spent at the water surface during drug exposure and an increase in circling behavior following a one-hour exposure, but only with the largest dose used in this study. Measures on these two tests (drug exposure and circling behavior) yielded no difference from control groups when fish were exposed to SKF 38393 (0.0 μ M, 10 μ M, 100 μ M) or ethanol (0%, 0.5%, 1.0%). Most notably, when employing the use of a novel open-field paradigm designed to measure shoaling displays, subsequent analyses revealed distinctive differences between drug and control groups for MK-801 (20 μ M) and SKF 38393 (100 μ M). Both of these drug treatment groups exhibited a period where shoaling activity was displayed briefly, during early stages of the observation session. Eventually, fish began to disperse, and shoaling ceased as the sessions progressed (approximately 5 and 10 minutes for MK-801 and SKF 38393, respectively). This behavior can be plausibly

correlated to the duration of time needed for drug absorption via the gills, and time needed for the drug to systemically circulate and arrive at the receptor. Exposure to ethanol (1%) had no significant effect on shoaling behavior as compared to control fish. Control groups for all three investigations (MK-801, SKF 38393, and ethanol) exhibited stable and reliable baselines of the species-specific behavior of interest, shoaling.

Our results make an interesting complement to previous behavioral assessments of MK-801 (e.g., Swain et al., 2004). The current study observed circling behavior *following* a one-hour exposure to MK-801, in contrast to the aforementioned study that assessed behavior *during* drug exposure. This deviation from previously reported methods enabled the illustration of a time course for circling behavior and likely, the drug's effect at the receptor site. Our results showed a distinct decrease in circles as a function of time for the group exposed to the 20 μ M dose. One possible explanation could be that the outward behavioral exhibition of circling is a function of MK-801's effects at the receptor level. The fact that circling behavior decreased across all subjects as the session progressed further supports this idea. Using the post-exposure method in the current study might also explain the discrepancies found between the current results and those reported by Swain et al. (2004) regarding the significance of the lower dose (2 μ M) and mean number of circles completed during a session. Our findings on top time behavior *during* MK-801 exposure are consistent with the zebrafish literature. Previous reports of increased surface time have been hypothesized to be the result of a possible disruption in swim bladder functioning (Swain et al., 2004), which is often coupled with a fish's inability to remain upright. However we did not observe this behavior. An alternative, and potentially more plausible, explanation might be the increasing need for an oxygen-rich environment due to an overall global impairment resulting from exposure to the highest dose, 20 μ M (Fig. 1). This behavior has been previously demonstrated in the zebrafish exposed to environmental toxins and was coupled with increased respiration and darkened pigmentation (Bretaud, Lee, & Guo, 2004).

The novel open-field paradigm used in these studies can reliably gauge shoaling behavior in the zebrafish and this behavior appears to be sensitive to both NMDA and dopamine receptor manipulation. This is particularly evident for the drugs MK-801 and SKF 38393, by which the overall distribution of fish increased as time progressed resulting in a decrease in shoaling behavior. Changes in shoaling activity during MK-801 exposure (20 μ M) may be associated with increased disorientation, as seen in other animal models, or a change in overall activity level. Decreased shoaling behavior, in this case, is probably not due to a decrease in motor activity. 20 μ M MK-801 as reported produced an increase in circling behavior, which is indicative of hyperactivity. In rodents, thigmotaxic displays have previously been reported as an increase in behavioral responding, likely do to anxiety (Lukoyanov & Paula-Barbosa, 2000; Prut & Belzung, 2003). The supposition that increased locomotor activity may account for decreased shoaling behavior is further supported by findings that high doses (20 μ M and 50 μ M) of MK-801 have been shown to increase swim speed and turning angle in the zebrafish (Panula et al., 2006).

The lack of effect for ethanol on this three-tiered test battery (exposure, circling behavior, shoaling displays) reported here might seem at first glance, to be inconsistent with previous reports of acute exposure in the zebrafish. The

most notable of these discrepancies is the purported disruption of shoaling behavior resulting from ethanol exposure using concentrations of 0.25%, 0.50%, and 1.0% reported elsewhere (Dlugos & Rabin, 2003; Gerlai, 2003; Gerlai et al., 2000). Consider that our shoaling paradigm is a novel design that has the essential element of assessing the frequency distribution of group behavior over a set of quadrants. This is in contrast to previous shoaling analyses where inter-individual distances were the dependent measure (Dlugos & Rabin, 2003) and another study which assessed a zebrafish's reaction to its own reflection (Gerlai, 2003). Results obtained by Gerlai found that conspecific preference (time spent in proximity to stimulus fish) was reduced in the presence of ethanol and the effect was dose dependent. This novel open field paradigm assesses the overall behavior of a group, instead of looking at individual differences. Future directions dictate that a wider dose range for ethanol be tested, as shoaling display is sensitive to changes induced by NMDA and dopaminergic manipulation (evidenced by exposure to MK-801 and SKF 38393).

The results presented here append to the growing body of literature utilizing the zebrafish as a behavioral model of investigation. We present for the first time, a novel paradigm that measures shoaling displays based on a modified open field design. This task in conjunction with the drug exposure and circling behavior tasks form a comprehensive test battery that can profile drug-induced alterations in social and motoric behaviors. Although only our first report this novel task is sensitive to NMDA and dopamine receptor manipulation. While both manipulations disrupted shoaling displays only the NMDA manipulation also yielded higher than normal circular swimming displays and increased surface swimming across the entire test battery. That is, MK-801 (20 μ M) disrupted group-shoaling behavior when tested on the novel task. This same dose when administered to fish individually (during two different experiments) caused exposed fish to exhibit circular swimming patterns more frequently than controls and during drug exposure caused individual fish to spend significantly more time in the top half of the testing apparatus. This pattern of impairment suggests that MK-801 (20 μ M) increases motoric output and may also cause the zebrafish to require a more oxygen-rich environment due to an overall global impairment. Compare this to the application of SKF 38393 (100 μ M), where dopaminergic manipulation resulted in no behavior change during individual drug exposure or assessment on the circular swimming task. However, group-shoaling displays were significantly impaired when fish were exposed to SKF 38393 (100 μ M).

The use of nonparametric analyses to date has not been utilized when assessing shoaling displays in the zebrafish. Our findings suggest that the design is appropriate for analysis of fish distribution as it relates to shoaling behavior. The fact that quadrant analysis and average probability trends compliment and agree with one another further supports the reliability of nonparametric analysis use when assessing these behaviors. Furthermore, consistent significant grouping behavior for control fish indicates uniformity among independent variables. Taken together, these tasks form a comprehensive battery of tests aimed at unambiguously identifying drug-induced alterations in social and motoric behaviors in the zebrafish. Lastly, we have presented additional evidence that supports the utility of this species in behavioral applications.

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Vigilance in Female Bottlenose Dolphins (*Tursiops* sp.) Before and After Calving

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Previous research has indicated that bottlenose dolphins alternate activity levels between hemispheres while at rest. This rest strategy allows dolphins to maintain continuous vigilance of their external environment. Dolphins in the care of humans exhibit different behaviors while presumably at rest, including floating at the surface, lying at the bottom, and swimming at very slow speeds in stereotyped patterns. Dolphin mothers in the care of humans have been reported to "not rest" and swim continuously for extended periods of time (weeks or even months) when their calves are first born. The current study examined the night-time rest patterns and vigilance of five female bottlenose dolphins before and after parturition. By differentiating between two types of resting behaviors (floating and slow swimming), we found that mothers altered their rest strategy depending on the parturition state. Floating was only observed at high levels pre-parturition. In contrast, mothers primarily exhibited active swims (a vigilant state) the first two weeks, post-parturition. The remaining six weeks were characterized by a steady increase in slow swimming (a resting, vigilance state). This change in swim behavior may be associated with neonatal development and may allow mothers to sustain high levels of vigilance for extended periods of time. The results of a behavioral test of vigilance indicated that the mothers also increased their vigilance level post-parturition. Mothers sustained their increased response rate over the eight-week post-parturition period, demonstrating that dolphin mothers maintain high levels of vigilance for an extended period of time.

Vigilance is defined as an awareness of one's immediate environment for stimuli significant to the survival of the individual and/or its offspring. Significant stimuli can include the appearance of an obstacle (e.g., nets, boats), perceived threats (e.g., predators, other animals), or a needed resource (e.g., food). The level of vigilance displayed by an individual will vary depending on a number of factors, including group size, predator threat, foraging need, available energy resources, the presence of young, current physiological capability, immediate spatial location, type of habitat, and species (Lima, Rattenborg, Lesku, & Amlaner, 2005).

Many group living primates, elephants, meerkats, and migrating birds alternate vigilance duties among individuals (Beauchamp, 1998, 2003; Clutton-

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Brock et al., 1999; Elgar, 1989; Horrocks & Hunte, 1986; Kenward, 1978; Moran, 1984; Munoz-Delgado et al., 2004; Roberts, 1996; Treves, 1998). Some species (e.g., meerkats) have social roles in which specific individuals act as sentinels for the group. Other species (e.g., flocking birds) rely on individuals located at the group's periphery to maintain the greatest levels of vigilance until they are replaced by other animals. The presence of sentinels allows the majority of the social group to rest and forage without having to monitor all other aspects of their environment. The resting or foraging members must simply maintain a level of vigilance that would allow them to respond to an alarm call or behavior of the sentinel. When sentinels are absent or unavailable, individuals must monitor their environment with higher levels of vigilance when they are both awake and resting (Lima et al., 2005).

Cetaceans also monitor their environment for group member activities and location, prey, predators, and environmental barriers such as sand bars and reefs in their natural habitat or pool walls and bottoms when in the care of humans (as reviewed in Connor, et al., 2000). Several lines of evidence suggest that cetaceans in the care of humans use various strategies to monitor their environment. For example, while rest swimming with one eye open and one eye closed (unilateral eye closure), a pair of Pacific white-sided dolphins (*Lagenorhynchus obliquidens*) monitored each other's position with their open eye rather than the external environment (Goley, 1999). Moreover, when these dolphins switched positions, the open eye also switched sides. A group of bottlenose dolphins also displayed similar behavior in which the group members tended to monitor each other's activities during unilateral eye closure (*Tursiops* sp.; Gnone, Benoldi, Bonsignori, & Fognani, 2001).

Also like many terrestrial animals, dolphins do not always maintain vigilance. For example, a group of resting dolphins in their natural habitat apparently remained unaware of a juvenile white shark swimming near them and did not disperse until the shark was among them (Connor & Heithaus, 1996). Although it is known that small cetaceans can vary the intensity of their responses to sharks (Wood, Caldwell, & Caldwell, 1970), the above anecdote suggested a lack of environmental awareness or vigilance by the dolphins. Whether dolphins are in a large social group or swimming independently, some level of vigilance must be maintained. Dolphins that are travelling, foraging independently, or have young calves are particularly vulnerable and must maintain heightened levels of vigilance for extended periods.

While the presence of other group members can facilitate environmental monitoring, the need for vigilance is magnified by the presence of a calf. Cetacean mothers are the primary, and usually, sole caregivers to their calves. Allomaternal care has been observed in some cetaceans (e.g., dolphins, sperm whales, and killer whales) in which non-related or related juveniles and adults monitor calves' activities while mothers forage or dive (as reviewed in Whitehead & Mann, 2000). However, the bulk of the care of calves is the mothers' responsibility. Bottlenose dolphin neonates (*Tursiops truncatus*) swim and rise to breathe on their own, although not very proficiently (Cockcroft & Ross, 1990; Mann & Smuts, 1999; McBride & Kritzler, 1951; Miles & Herzing, 2003; Reid, Mann, Weiner, & Hecker, 1995; Tavalga & Essapian, 1957). Dolphin mothers must constantly observe their newborn calves' actions so that they may assist their struggling neonate to the surface for a breath or redirect their erratically swimming offspring away from

potential threats (e.g., dangerous conspecifics, environmental threats such as sharks or pool walls). Constant vigilance on the part of a mother with a neonate is critical whether the mother-calf pair is in their natural habitat or in human care.

As with terrestrial animals, the need for constant vigilance for cetaceans is in direct conflict with their need for sleep or rest as mammals. A period of rest or sleep replenishes depleted energy resources, repairs damage experienced by the body throughout the day, and consolidates memories (Lilly, 1964; Lima et al., 2005; Siegel, 2003). In order to counter the many different constraints species experience as a result of their habitats and survival needs, a number of sleep and vigilance strategies have evolved. One strategy displayed by cetaceans is their capacity for unihemispheric slow-wave sleep (SWS, Lyamin, Mukhametov, & Siegel, 2004; Mukhametov, 1984; Ridgway, 2002; Ridgway et al., 2006). Recordings of brain activity of dolphins during various activity states have indicated that slow-wave sleep (SWS) patterns occur and alternate between each hemisphere (unihemispheric SWS) while the dolphins are at rest, be it a motionless or moving rest behavior (Lyamin et al., 2004; Mukhametov, 1984; Mukhametov, Supin, & Polyakova, 1977; Ridgway, 2002). Occasional bihemispheric SWS for short periods between breaths has also been observed in dolphins (Lyamin et al., 2004; Mukhametov 1984; Mukhametov et al., 1977; Ridgway, 2002, Lyamin, Manger, Ridgway, Mukhametov, & Siegel, 2008).

The ability to alternate which hemisphere is “sleeping” is a particularly adaptive behavior for animals that must sustain vigilance for extended periods of time. Free-swimming dolphins without calves have been shown to maintain vigilance with few errors for extended periods of time on a variety of behavioral tasks (Hoffman-Kuhnt, 2003; Ridgway et al., 2006). Clearly, this capability would be advantageous to a mother who must be continuously vigilant to ensure her neonate’s survival.

Previous reports have shown that dolphins without calves in the care of humans often float, or remain relatively motionless, for extended periods of time (e.g., surface floats or quiescent hanging behavior, Flanigan, 1974; Gnone et al., 2001; McCormick, 1969; Mukhametov, 1984; Ridgway, 2002). These periods of motionlessness are presumably rest periods during which vigilance may be low or moderate. One eye or both eyes may be shut and floating is often uninterrupted except for periodic breathing (Lyamin et al., 2004; Mukhametov, 1984; Mukhametov et al., 1977; Ridgway, 2002; Ridgway et al., 2006).

Dolphins also rest as they swim. Dolphins in human care have consistently been observed swimming in slow, stereotyped circular swim patterns periodically throughout the 24-hour day, especially during the nighttime hours when human attendants are absent (Cockcroft & Ross, 1990; Flanigan, 1974; Goley, 1999; Gnone et al., 2001; Gnone, Moriconi, & Gambini, 2006; Gubbins, McCowan, Lynn, Hooper, & Reiss, 1999; McBride, & Kritzler, 1951; McCormick, 1969; Mukhametov, 1984; Ridgway, 2002; Sekiguchi, Arai, & Kohshima, 2006). Similar slow-moving, group-synchronized resting behaviors have also been observed in many species of dolphins in their natural habitat (Connor & Heithaus, 1996; Norris & Dohl, 1980; Wursig & Wursig, 1980). Wild dolphins swim into shallow, well-protected bays where they may also float or engage in synchronized slow-

paced swims (Scott, Wells, & Irvine, 1990; Shane, 1990). Surface floating is considered a reduced vigilance state (Connor & Heithaus, 1996; Lyamin, Pryaslova, Lance, & Siegel, 2005; Norris & Dahl, 1981;) while slow swimming and active swimming are described as states with increasing levels of vigilance (Goley, 1999; Gnone et al., 2001).

Although data on wild dolphins mothers is lacking, research with dolphins in human care has suggested that mothers do not stop to rest or float for extended periods of time after their calves are born (Lyamin et al., 2005). Rather, they appear to swim continuously until their neonates become more proficient swimmers at around two months of age (Fellner, Stamper, Losch, Dahood, & Bauer, 2005; Lyamin et al., 2005). These observations have two implications: (1) mothers of neonates may experience few periods of SWS (i.e., sleep) as indicated by the lack of corresponding eye closures, which have been found to be associated reliably with SWS by some studies (Lyamin et al., 2004; but see Supin et al., 1978), and (2) mothers of neonates should have heightened levels of vigilance during this critical survival period.

The current study investigated the rest and swim patterns of bottlenose dolphin mothers in the care of humans the month before and two months after parturition. Based upon previous research with animals in the care of humans, the following hypotheses were tested:

1. As anticipated from previous research examining mother-calf swim patterns, shortly after birth, female dolphins should alter their night-time resting patterns pre-parturition to post-parturition. Specifically, females will surface float more, pre-parturition, and engage in more active swim patterns, post-parturition (Flanigan, 1974; Goley, 1999; Gnone et al., 2001; Gnone et al., 2006; Lyamin et al., 2005; McCormick, 1969; Mukhametov, 1984; Ridgway, 2002; Sekiguchi et al., 2006).
2. More specifically, mothers will not exhibit substantial surface floating, post-parturition, but rather should increase the frequency of slow circular swims, post-parturition (Cockcroft & Ross, 1990; Gubbins et al., 1999; Gnone et al., 2001; Gnone et al., 2006; McBride & Kritzler, 1951; Sekiguchi et al., 2006).
3. Parity (whether this was the mother's first calf or the mother had experienced births in the past) was expected to be related to post-parturition resting activities.

We also investigated the level of vigilance displayed by our female dolphins pre- and post-parturition, at night. The following hypotheses were tested:

4. Female dolphins should respond more often to the vigilance stimulus when engaged in an active swim or a slow swim than when surface floating (Connor & Heithaus, 1996; Goley, 1999; Gnone et al., 2001; Lyamin et al., 2005; Norris & Dahl, 1981).
5. Female dolphins should respond more frequently (i.e., be more

vigilant) post-parturition as compared to pre-parturition, when given a behavioral test of vigilance. This hypothesis was derived from observations of unilateral eye closures of mothers swimming with their calves and their reported responses to changes in their environment, including avoidance of other swimming mother-calf pairs or dolphins (Cockcroft & Ross, 1990; Gnone et al., 2001; Gubbins et al., 1999).

6. If swimming occurred continuously for two weeks or more, mothers were expected to decrease their levels of vigilance due to physical fatigue (in part from Lyamin et al., 2005).
7. Parity was hypothesized to be related to level of vigilance, although it was unclear how they might be related due to the lack of available information.
8. Mothers were hypothesized to demonstrate laterality in their responses to a behavioral vigilance task due to their capacity for unihemispheric SWS and previous studies demonstrating behavioral laterality (Lyamin et al., 2004; Mukhametov, 1984; Ridgway, 2002; Ridgway et al., 2006).

Method

Subjects

Six pregnant bottlenose dolphins (*Tursiops* sp.) located at the Navy Marine Mammal Program (MMP) in San Diego, California were observed during the summer of 2002. Within a period of two and a half weeks, five of the six dolphins gave birth to healthy calves. The remaining dolphin did not give birth until December 2002. However, she (dolphin C) remained in the study and served as a pregnant control. A listing of the dolphins, their parity, and the birth dates of their calves may be found in Table 1.

Table 1
Summary of Descriptive Information for Each Dolphin

Mother	Age of Mother	Parity	Calf	Birth Date	Total Pre-Parturition Samples	Total Post-Parturition Samples
O	27	M	M ^a	5/31/2002	127	434
S	22	M	H ^b	6/6/2002	167	421
B	37	P	I ^b	6/10/2002	208	400
K	16	P	T ^b	6/16/2002	261	370
A	16	M	D ^a	6/18/2002	266	371
C	15	--	----- --	-----	608	-----

Note. M = Multiparous; P = Primiparous.
^a Male. ^b Female.

All female dolphins were housed in the same 18m x 18m enclosures prior to parturition. The MMP facility is located in San Diego Bay and is constructed as a series of floating enclosures that rise and fall with the tidal range. All the enclosures are connected by gates which allow for a number of different social groupings (schematic shown in Figure 1).

During the four-month study, all six subjects were initially housed together during the night-time hours. As the birth date approached, determined by ultrasound examination and behavior, the pregnant dolphin and one other companion female, the next dolphin due, were placed into an adjacent birthing enclosure each night until parturition occurred. The other pregnant females were housed overnight with one another. After parturition, the mother and neonate were in the birthing enclosure until the veterinarian and training staff had determined that the pair had bonded well and nursing was regular. Most mothers and their calves were re-located to a nursery enclosure and grouped with a pregnant female or mother-calf pair within four days of the calf's birth. As all calves were born within a two and half week period, the social groupings were fairly consistent with the exception of an animal being removed every couple of days due to the next calf delivery. After the last calf was born, all five mother-calf pairs and the pregnant female were housed together at night by the end of the study. Mother-calf pairs had access to all enclosures identified as nursery enclosures in Figure 1.

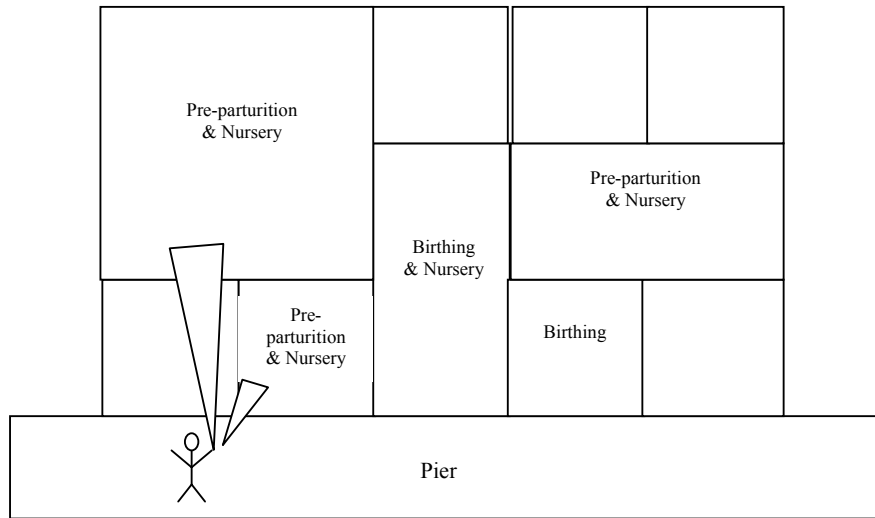


Figure 1. Spatial layout of housing enclosures and vigilance testing procedure for study dolphins. Schematic is not drawn to scale. A trained observer stood on a pier, approximately three to six meters, above the free-floating enclosures. Baseline behaviors were determined for both behavioral and vigilance aspects of the study for each female using a scan sampling procedure. To administer the vigilance test, the target female was identified, her baseline behavior was recorded, and the flashlight beam was directed near the eye that was facing the observer. The light was shined near each eye (within a third of a meter above the eye) and never directly into any animal's eye. If any other animal was in close proximity (including the calf), the observer did not administer the test until the target animal or the confounding animal had cleared the area. In the case of the calf, the observer administered the test with the greatest precision possible to avoid stimulating the calf. Animals were presumed vigilant if they responded to the light.

Behavioral Data

Procedure. This study was conducted between May 2002 and August 2002. Instantaneous behavioral samples were collected with an observation data sheet using a scan sampling procedure (Altmann, 1974/1996; Mann, et al., 2000; Martin & Bateson, 1993). That is, once a female was identified and her initial behavior recorded, the next visible female was sampled, and so on until all females had been sampled or five minutes had passed. Sample points were taken every hour during the night between 2100 and 0600 by a single observer, before the training staff came to work and began the day's activities. As the facility was a working one, observations were made outside of the normal training day to avoid the influence of human activities on the dolphins' behaviors. The females were identified by large zinc oxide markings placed in pre-determined locations by trainers during the last feeding session of the day. These marks were long-lasting and were re-applied when necessary throughout the study.

Prior to the onset of the study, a pilot study was performed to test the efficacy of the above procedure with several adult males and females in a night-time housing set-up similar to the pregnant females. Zinc oxide marks were easy to see with the ambient lighting from the pier, and the observer's appearance on the pier never disturbed the animals' night-time behavior. These potential confounds did not appear during the pilot study nor during data collection for the final study. Furthermore, all adult and juvenile animals at this facility were habituated to the appearance of individuals on the pier as night security guards patrolled the area at regular intervals.

Five of the six pregnant females were observed for a month prior to the birth of the first calf. The control dolphin was also observed during this time. Table 1 presents the number of sample points available for each mother observed. The sample points increased across mothers due to their longer pre-parturition observation periods, ranging between one month to one and half months. The control female produced three months of data as her calf was not born during the course of the study.

The same data collection procedure was used once the calves were born. Observations of the night-time behaviors of the mothers and calves were made every night for the first two months of calf life. The total number of post-parturition sample points (summed across two months of data) for each mother are presented in Table 1¹.

Dolphin behaviors were coded into one of four categories for the current study: floating, slow swimming, active swimming, and other. Floating was defined as maintaining a stationary position at the surface for at least five seconds; this behavior was considered a resting behavior. Slow swimming involved a slow (less than 1 m/s), stereotyped, circular swim, which is also a resting behavior. Active swimming was defined as any swim greater than 1 m/s in which dolphins were clearly alert and continuously altering their swim patterns. Finally, other behaviors were defined by any other non-swim behaviors observed, including but not limited to bows, dives, spy hops, or breaches. Definitions were adapted from Goley (1999), Gnone et al. (2001), and Mann and Smuts (1999).

Tests of Vigilance

In addition to the data collection of the dolphins' night-time behaviors, behavioral tests of vigilance were also conducted during those times.

Apparatus for Vigilance Testing. A Mag-lite 2 D cell flashlight with a well defined beam was used to administer the vigilance tests. During pilot testing with adult male and female dolphins, the flashlight was initially set at low intensity. The pilot subjects responded to this low level light immediately whether they were resting or awake. Thus, the intensity of the light beam was ultimately filtered by placing two plastic, semi-opaque filters inside the lens cover in front of the beam. The final light intensity was sufficient to travel 10m (approximately the farthest distance to be tested) and still be detected by an animal that was awake. Thus, the light was not detectable by floating dolphins, who were presumed to be sleeping. It did, however, elicit an observable reaction in an alert animal.

Behaviors of Interest. As in the behavioral part of the study, floating, slow swimming, active swimming, and other behaviors were of interest for the behavioral tests of vigilance. However, this portion of the study focused on the responses of each animal to the vigilance stimulations. A response to the vigilance stimulation was defined as a change in behavior from the initial behavior recorded. Thus, if the animal performed a behavior (e.g., active swim) different from the baseline behavior (e.g., surface float) then the animal was considered to be vigilant. If a change in behavior did not occur, the animal was considered not vigilant (e.g., baseline behavior: surface float, post-stimulation behavior: surface float).

Procedure

Pre-parturition. Figure 1 presents a diagram and an explanation of the procedure used to administer the behavioral tests of vigilance. Prior to the birth of the first calf, all females received three vigilance tests per night, 4 to 5 times a week for the month of May. The frequency of the tests was chosen for two reasons: (1) to determine if the animals would become habituated or sensitized to the stimulations, and (2) to have a number of stimulations that would be

¹ As with the pre-parturition data, the total number of sample points fluctuated some depending on the arrival date of the calves. Other sources of differences included animals not being visible during the allotted sampling time frame (5 minutes) or missed observation times by the observer.

comparable to those completed once the calves were born and observed for eight weeks. The females did not appear to habituate or become sensitized to the multiple stimulations either across the night or across the pre-parturition period².

Three time frames were chosen to randomly administer the stimulations across the night: (1) 2100-2359, (2) 2400-0259, and (3) 0300-0600. Thus, stimulations were given during one of the three time frames at previously determined hours. For example during the pre-parturition period, each of the six females was individually tested within the 2300 hour, the 0100 hour, and then the 0500 hour. These time frames were chosen to spread the stimulations across the night so that animals never experienced more than one “back-to-back” stimulation (i.e., a stimulation at 2300 and another one at 2400) in the course of the night. Most importantly, all animals had a minimum of an hour to return back to their normal activity level before the next observation was made.

Although it was possible that the females could have influenced the behavior of each other during the pre-parturition period in which multiple stimulations occurred during a given time frame, we followed a rigorous protocol to minimize these concerns. First, a habituation period occurred prior to all stimulations. This procedure was to ensure that the animals were not responding to the presence of the observer³. Second, the behaviors of all females were recorded before administering the stimulations (i.e., were they surface floating, slow swimming, active swimming, or engaged in another behavior?). Third, after a stimulation was conducted, the observer re-assessed the remaining females to be tested. An animal that had not changed her behavior from the previously recorded behavior was selected for the next stimulation test. This re-assessment was used only during the pre-parturition study to ensure that the previous stimulation had not alerted the other animals, as multiple females were tested during the same hour. This process was continued until all six females had been tested. If the testing protocol lasted most of the hour, a minimum of an hour was observed before a new round of tests was performed.⁴

Post-parturition. Using the same protocol as described above, each mother was initially tested two to three days after her calf was born and bonding had occurred. After the first night of vigilance testing, each mother was tested on her calf’s weekly birthday for the first two weeks and then again during weeks five through eight. The number of vigilance tests conducted during weeks three and four was increased for each mother. This change in the testing protocol reflected our concern that stimulation sessions conducted once a week would not give us sufficient data to address our fatigue hypothesis sufficiently.

As described above, the mothers and their calves were initially housed independently. As additional calves were born, mother-calf pairs were housed in a common nursery. Thus, the social groupings remained fairly consistent over the course of several nights as calves were born. Once all the calves were born, all mother-calf pairs were given access to the nursery. The nursery was a combination of multiple enclosures, which encompassed a very large area. Mothers were able to control their proximity to one another (Figure 1). Moreover, in an effort to minimize the potential influence of other animals during vigilance testing, only one mother was targeted during each testing interval. All testing involved three stimulations a night, each during one of the three previously described time intervals. Again, a minimum of an hour had to pass before the next test was administered to control for the potential carryover effect of the vigilance tests.

As in the pre-parturition period, no evidence was found that baseline behaviors were related to the administration of the vigilance tests in the post-parturition period. Furthermore, no evidence was found that the frequent appearance of the observer on the pier during this part of the study reliably disturbed or altered the behavior of the study animals or of non-study animals

² Chi square tests of independence indicated that responses to the light stimulus were independent of the order in which they were given, both across the night and the pre-parturition period. Thus, the dolphins did not respond any more or any less to the stimulations over the course of the night or before the calves were born.

³ By all behavioral indications, the presence of the observer never affected the animals’ behavior. No changes in behavior occurred between the time the observer appeared on the pier and the baseline behavior was recorded.

⁴ A visual inspection of the data indicated that the females did not appear to influence each other’s behaviors during the pre-parturition period in which stimulations were given. That is, baseline behaviors remained the same for the remaining animals before and after stimulations were given to each target animal. Sessions in which multiple light stimulations were administered generally lasted less than 15 minutes.

in adjacent enclosures. Although we were unable to control any influence the calf's behavior may have had in alerting the mother to a change in her environment, we attempted to minimize this confound as well. If the mother or calf had responded to the actions of the observer, then the test was not conducted at that time.

Finally, we were also interested in whether or not laterality of vigilance could be ascertained. In order to assess vigilance laterality, when possible, a second stimulation was given to the other non-stimulated eye after determining a baseline behavior. We felt that it was particularly important to assess the vigilance of the contralateral side without additional delay. If stimulations to both eyes were possible, the observer administered both stimulations at approximately equal distances, to control for changes in light intensity due to distance from the animal. The ambient lighting at the facility was not sufficient to consistently determine if a subject's eye was open or closed, and these data were not collected.

Results

Behavioral Data

All behavioral data were examined using Wilcoxon Matched-Pairs Signed-Ranks Tests for pre- and post-parturition analyses and Friedman Analysis of Variance by Ranks Tests for changes in maternal behaviors over time. Additionally, a Mann-Whitney U test was conducted to assess the influence of parity on the mothers' behaviors. Behaviors are reported as mean percentages and mean ranks when appropriate for group comparisons. These procedures were chosen due to the repeated measures design and ordinal dependent measure (Gibbons, 1993; Haslom & McGarty, 2003).

Activity Patterns Pre- and Post-Parturition. Results of a series of Wilcoxon matched-pairs signed-ranks test supported the hypothesis that mothers would engage in different night-time activities between pre- and post-parturition. Specifically, the five mothers were significantly more likely to rest by floating ($M = 56.9\%$, $SD = 26.5\%$) before parturition than after parturition ($M = 3.9\%$, $SD = 3.1\%$), $z = -28.8$, $N = 2,043$, $p < .001$, two-tailed, Point biserial $r = -.50$. Comparably, mothers were significantly more likely to slow swim ($M = 57.9\%$, $SD = 4.8\%$) and active swim ($M = 38.0\%$, $SD = 4.7\%$) after parturition than before parturition (slow swim: $M = 23.7\%$, $SD = 18.6\%$, $z = -23.3$, $N = 2,043$, $p < .001$, two-tailed, Point biserial $r = .39$; active swim: $M = 20.6\%$, $SD = 10.4\%$, $z = -15.4$, $N = 2,043$, $p < .001$, two-tailed, Point biserial $r = .25$). There was no difference for the other behavior category before and after parturition. Table 2 presents the positive and negative ranks for each behavior category.

Results of a series of Friedman tests supported the hypothesis regarding the time course and frequency of surface floating and slow circular swims, post-parturition. Figure 2 presents a graphical representation of the numerical data presented in Table 3. The data represent the average percentage of each behavioral category, calculated using the relative percentage of the four categories for each mother. These data are presented by week to demonstrate the chronological trends of the two resting behaviors. As a group, mothers did not return to their baseline floating levels by the end of the eight week study, supporting our hypothesis. They engaged in significantly more floating pre-parturition than they did across the eight week, post-parturition period, χ^2 ($df = 8$, $N = 5$) = 19.80, $p < .049$, $W = .50$. The mothers also significantly increased their slow swimming rest behavior over the course of the study, again supporting our hypothesis, χ^2 ($df = 8$, $N = 5$) = 31.21, $p < .001$, $W = .80$.

Specifically, mothers engaged in the least slow swimming prior to and the first week following parturition. The percentage of slow swims steadily increased with each week with three quarters of the night-time activities being slow swims the last few weeks of the study.

Table 2
Results from Wilcoxon Matched Pairs Signed Ranks Test for Pre- and Post-Parturition Behaviors

		<i>N</i>	Rank Sums
Float	Negative Ranks	937	457256.00
	Positive Ranks	38	18544.00
	Ties	1068	
Slow swim	Negative Ranks	162	88938.00
	Positive Ranks	935	513315.00
	Ties	946	
Active swim	Negative Ranks	191	78787.50
	Positive Ranks	633	261112.50
	Ties	1219	
Other	Negative Ranks	27	634.50
	Positive Ranks	19	446.50
	Ties	1997	

Note. All calculations were performed by subtracting the pre-parturition behaviors from the post-parturition behaviors.

Although not specifically hypothesized, our mothers also significantly altered the frequency with which they actively swam over the eight week post-parturition period, χ^2 ($df = 8$, $N = 5$) = 31.89, $p < .001$, $W = .78$. As demonstrated in Figure 2 and Table 3, mothers almost quadrupled the frequency with which they actively swam during the first week post-parturition as compared to their active swims pre-parturition. Mothers engaged in their greatest levels of active swims the first two weeks post-parturition. Then as the calves matured, mothers gradually decreased their active swims while steadily increasing their slow swims. The category including other behaviors did not significantly change over the study's duration.

Pregnant control. This study also provided an opportunity to observe the night-time activities of a pregnant female (Dolphin C) who was housed with the mother-calf pairs across the duration of the study. Although not specified as a particular hypothesis, we expected that the activities of the pregnant control would be similar to the mothers' pre-parturition behaviors. Results of a Chi Square Goodness of Fit test indicated that she engaged in a pattern similar to the mothers' averaged pre-parturition behaviors. The pregnant control was significantly more likely to float (58.9%, $N = 359$) than active swim (21.7%, $N = 132$) and slow swim (17.6%, $N = 107$) and least likely to engage in other behaviors (1.8%, $N = 11$) χ^2 ($df = 3$) = 427.95, $p < .001$. Interestingly, she displayed significant variation in her activities across the four months, Pearson Chi Square Test of Independence: χ^2 ($df = 9$) = 41.44, $p < .001$, $V = .15$. As seen in Table 4, surface floating accounted for close to three quarters of all her activities during May (before calves) and August (after all the calves were two months of age), significantly more than during June and

July. During the month of June when the calves were the youngest, C continued to primarily float at night but was significantly more likely to engage in slow swims. Surface floating continued to be her primary behavior in July, however she was significantly more likely to swim actively. She returned to her baseline levels by August.

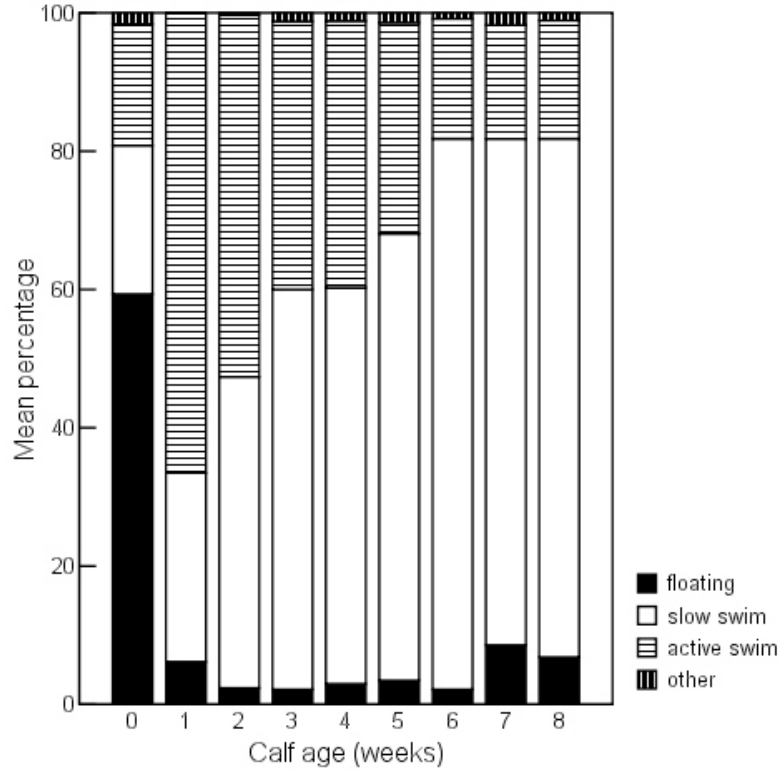


Figure 2. Average percentage of night-time behaviors by mothers pre- and post-parturition. *Note.* The calf age, zero weeks, indicates pre-parturition.

Parity. A Mann-Whitney U test was conducted to investigate the relationship between parity and post-parturition behaviors. The test indicated that primiparous mothers were generally similar to multiparous mothers in their overall activity level across the two months, post-parturition. Thus, there were no differences in the type of rest behavior exhibited by mothers with different degrees of calf rearing experience, which failed to support our hypothesis

Tests of Vigilance

Analyses of the vigilance data were primarily conducted using Chi Square Tests of Independence. Although data were collected from the same subjects multiple times, we assumed that the sample points were independent of one another as they were obtained at distinct intervals and independently of each other (Gibbons, 1993; Haslom & McGarty, 2003). Laterality was also assessed using Chi Square Tests of Independence.

In general, a Binomial test indicated that as a group, the five mothers were significantly more likely to respond (0.71, $N = 330$) than not respond

(0.29, $N = 132$) during initial vigilance tests, $p < .001$. The pregnant control also exhibited this trend by always responding to her vigilance tests (1.0, $N = 41$, $p < .001$).

Table 3
Mean Percentage of Night-Time Behaviors Pre- and Post-Parturition ($n=5$).

	Calf Age (weeks)								
	0	1	2	3	4	5	6	7	8
Float*									
<i>M</i>	59.4	6.2	2.4	2.2	3.0	3.5	2.2	8.6	6.8
(<i>SD</i>)	(26.6)	(8.6)	(2.2)	(2.1)	(3.3)	(3.0)	(1.2)	(7.3)	(7.5)
<i>n</i>	615	15	7	7	9	9	5	17	7
Mean rank	9.0	5.5	2.7	3.4	3.7	4.2	4.3	6.2	6.0
Slow swim**									
<i>M</i>	22.3	27.3	45.0	57.8	57.3	64.6	79.6	73.2	75.0
(<i>SD</i>)	(18.5)	(6.0)	(8.3)	(13.1)	(13.8)	(8.3)	(9.8)	(10.6)	(14.9)
<i>n</i>	232	71	133	180	171	181	166	149	100
Mean rank	1.4	1.8	3.8	4.8	4.2	6.0	8.0	7.4	7.6
Active swim**									
<i>M</i>	16.7	66.6	52.3	38.8	38.6	30.5	17.3	16.5	17.1
(<i>SD</i>)	(9.2)	(13.0)	(7.1)	(15.6)	(16.9)	(8.0)	(8.1)	(7.7)	(17.4)
<i>n</i>	164	172	1556	114	118	87	40	34	30
Mean rank	2.6	8.8	7.4	6.4	6.8	4.8	3.0	2.5	2.7
Other									
<i>M</i>	1.6	0.0	1.6	1.3	1.2	1.4	.9	1.8	1.1
(<i>SD</i>)	(0.7)	(0.0)	(0.0)	(2.9)	(2.6)	(1.3)	(1.3)	(1.9)	(2.5)
<i>N</i>	16	--	1	4	3	3	2	4	2

Note. The calf age, zero weeks, indicates pre-parturition.
* $p < .01$, Friedman test. ** $p < .001$, Friedman test.

Table 4
Percentage of Night-time Behaviors for Pregnant Control by Month

	May	June	July	August
Float				
%	73.8	49.4	57.7	74.5
<i>n</i>	79	115	123	41
Slow swim				
%	15.0	26.6	11.7	7.3
<i>n</i>	16	62	25	4
Active swim				
%	10.3	21.9	28.2	18.2
<i>n</i>	11	51	60	10
Other				
%	0.9	2.1	2.3	0
<i>n</i>	1	5	5	--

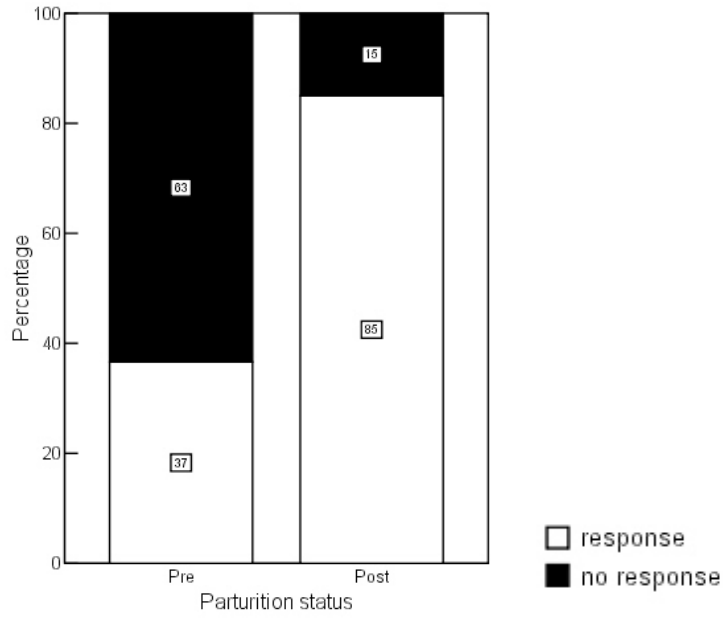
Current Behavioral Activity and Vigilance Test Outcome. A Chi Square Test of Independence was conducted to test our hypothesis that more active behaviors were linked with more vigilant states and less active behaviors were linked to less vigilant states. The results partially supported our hypothesis as the outcome of the vigilance tests were significantly related to the behavior recorded immediately before administration of all vigilance tests, $\chi^2 (df = 3) = 26.56, p < .001, V = .25$. Mothers were more likely to fail to respond while floating, $N = 62$ (47.7%) and were more likely to respond while slow swimming, $N = 130$ (45.0%).

Pre-Parturition versus Post-Parturition Vigilance. The results of a Chi Square Test of Independence supported the hypothesis that mothers should exhibit greater vigilance post-parturition as compared to pre-parturition, $\chi^2 (df = 1) = 35.63, p < .001, V = .29$. Specifically, mothers were significantly less likely to respond to initial vigilance tests, pre-parturition, $N = 106$ (42.6%), and significantly more likely to respond to initial vigilance tests, post-parturition, $N = 146$ (84.9%). Figure 3a displays these relationships.

The distribution of vigilance responses of the contralateral eye in follow-up vigilance tests was also examined for its relationship to parturition status. A Spearman's rank correlation indicated that a significant moderate, positive correlation ($r_s = .44, N = 421, p < .001$) existed between the responses of an initial vigilance test and an immediate follow-up vigilance test of the contralateral eye. That is, if mothers responded to the first test they would also respond to the second test, $N = 31$ (31.0%), and if mothers failed to respond to the first test, they also failed to respond to the second test, $N = 41$ (40.6%). Interestingly, unlike the results of the initial vigilance tests, mothers were just as likely to respond (0.42, $N = 42$) as they were not to respond (0.58, $N = 58$) to the follow-up vigilance tests of the contralateral eye (Binomial test: $p = .13$). Also similar to the results of the initial vigilance tests, the distribution of responses to the follow-up vigilance tests was significantly related to the parturition status, $\chi^2 (df = 1) = 19.92, p < .001, V = .44$. That is, mothers were significantly less likely to respond to the follow-up vigilance tests, pre-parturition, $N = 42$ (71.2%), and significantly more likely to respond to the follow-up vigilance tests, post-parturition, $N = 31$ (73.8%). Figure 3b displays these relationships.

Influence of Possible Fatigue on Vigilance Testing. In order to assess our hypothesis that mothers might become fatigued by weeks three and four due to their constant swimming activities since the birth of their calves, we collapsed the outcomes of initial vigilance tests for individual weeks into two week intervals so that we could minimize the number of cells with expected counts of less than 5. The results of the Chi Square Test of Independence failed to support our hypothesis that mothers would become less vigilant over the course of the eight weeks. We would like to point out though that the mothers failed to respond the most often between three and four weeks, post-parturition, $N = 16$, (9.5%), compared to the next highest frequency of no responses during weeks five and six, $N = 4$, (2.4%).

a. Initial Vigilance Tests



b. Follow-up Vigilance Tests

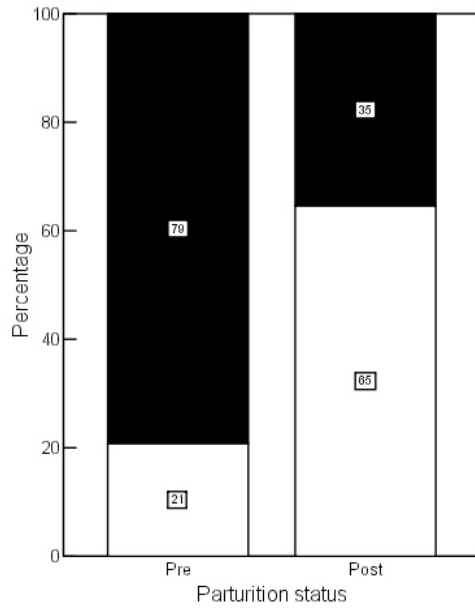


Figure 3. Results from vigilance tests conducted pre- and post-parturition.

Parity. Chi Square Tests of Independence were conducted to examine the relationship between parity and vigilance. Parity was found to be independent of vigilance for both the initial and the follow-up vigilance tests.

Laterality. Mothers received a total of 101 vigilance tests pre- to post-parturition in which both sides were tested sequentially— an initial test and a follow-up test. Of these tests, $N = 28$ (28.0%) indicated laterality or unihemispheric sleep.

Pre-parturition. There were a total of 54 instances in which mothers sequentially received two tests of vigilance. A Chi Square Test of Independence failed to fully support our hypothesis that dolphins would show laterality of vigilance as the test approached significance, $\chi^2 (df = 1) = 3.38, p = .066, V = .25$. Five (9.3%) test combinations resulted in responses for both eyes, 35 (64.8%) test combinations resulted in no response for either eye, and 14 (26.0%) test combinations resulted in a response for one of the two eyes. Additionally, a Chi Square Test of Independence indicated that the side to which the stimulus was presented was not related to the test's outcome.

Post-parturition. There were a total of 47 instances in which mothers received an initial test of vigilance and a follow-up test. A Chi Square Test of Independence partially supported our hypothesis of laterality in vigilance, $\chi^2 (df = 1) = 4.48, p = .034, V = .31$. Specifically, mothers were more likely to respond to a follow-up test if a response had occurred during the first test, $N = 27$ (57.4%) and were more likely to fail to respond to a follow-up test if they had done so during the first test, $N = 6$ (12.8%). However, they were also likely to respond with one side and not the other (i.e., laterality) although significantly less often than expected, $N = 14$ (29.8%). Finally, a Chi Square Test of Independence indicated that the side to which the stimulus was presented was not related to the test's outcome.

Discussion

The night-time activities and levels of vigilance for pregnant female dolphins in the care of humans were examined one month prior to parturition and two months after parturition. A number of hypotheses were tested. The first series of hypotheses involved night-time activities with an emphasis on changes in types of resting behaviors over the course of the study. The second series of hypotheses tested various aspects of vigilance in female dolphins, including differences between parturition states and laterality.

Behavior

Pre-Parturition and Post-Parturition Night-Time Activities. As expected from previous research with non-pregnant dolphins in the care of humans, pregnant female dolphins, located at a working and research facility, in natural ocean water with a regular tidal exchange, were generally not very active at night (Flanigan, 1974; Goley, 1999; Gnone et al., 2001; Gnone et al., 2006; Lyamin et al., 2005; McCormick, 1969; Mukhametov, 1984; Ridgway, 2002; Sekiguchi et al., 2006). Our females displayed two types of resting patterns during night-time activities: surface floats and very slow, stereotyped circular swims. On average, 60% of their nightly activities involved surface floating and 20% involved slow swims. The remaining 20% of their activities included active, alert swims and other kinds of activities such as bows, breaches, and interactions.

As anticipated, the arrival of the calves altered the females' night-time activity levels. Surface floating immediately dropped to minimal levels (< 10% of the activities) and was replaced by active swims with the calves, during the first two weeks of life. Despite the variability in social groupings, mothers followed similar trends whether they were alone with their calves or if multiple mother-calf pairs were present. These observations complement previous observations that bottlenose dolphin and killer whale mothers rarely float post-parturition (Lyamin et al., 2005) and observations of a captive dolphin calf who displayed no surface floating until 15 weeks (Gnone et al., 2006). Our results also correspond with the high level of calf dependence on maternal vigilance during the first few weeks of life when the calves have the greatest need for continuous monitoring in terms of swimming proficiency and susceptibility to danger (Cockcroft & Ross, 1990; Fellner et al., 2005; Mann & Smuts, 1999; McBride & Kritzler, 1951; Miles & Herzing, 2003; Reid et al., 1995; Tavolga & Essapian, 1957). However, the increase in active swimming may also be related to the hydrodynamic benefits received by calves when swimming in close proximity to their mothers, such as slipstreaming while in an echelon or infant position (Fellner, Bauer, & Harley, 2006; Gubbins et al., 1999; Noren & Edwards, 2007; Weihs, 2004). These benefits include decreased energy expenditure, navigation and respiration assistance, and thermoregulation opportunities for the calf.

By the third and fourth weeks, the mothers had significantly reduced their active swimming and were more likely to swim slowly in a stereotyped circular pattern (almost 60% of their night-time activities). This change in behavior may have occurred for several reasons: (1) mothers may have begun to tire and therefore engaged in less active swimming and more slow swimming, (2) the mothers may have altered their behaviors as their calves no longer needed to be monitored as constantly because they now had greater control over their swim and respiration patterns, (3) calves were growing, becoming more efficient at nursing and consuming more milk or (4) a combination of these reasons. Whatever the reason, these data suggest that the presence of calves dramatically changed the mothers' night-time activities.

It is unclear how much rest dolphin mothers are able to obtain while caring for a neonate. Lyamin et al. (2005) suggested that dolphin mothers could not have engaged in extended periods of sleep until sometime after the first month. They drew this conclusion for two reasons: (1) their mothers did not display a significant amount of surface floating during the first month, and (2) the mothers' monitoring activities would have constantly interrupted any sustained sleep. Our mothers also did not return to their baseline floating levels by the end of two months. However, they did engage in a significant proportion of slow swimming beginning at two weeks, a rest behavior not specifically measured by Lyamin et al. (2005). We suggest that these periods of slow swimming could have been periods in which the mothers slept for substantive periods of time, via unihemispheric sleep (Flanigan, 1974; Mukhametov, 1984; Ridgway, 2002).

As noted earlier, slow swimming (i.e., additional resting opportunities) steadily increased over the course of the second month while active swimming decreased. By the second month, calves are much better at maintaining their echelon swim position and slipstream advantage (Fellner et al., 2006; Gubbins et al., 1999; Noren & Edwards, 2007; Weihs, 2004). The increase in slow

swimming may also provide the mothers opportunities to restore her own energy levels. Slow swimming is a slower paced, less active behavior that involves relatively long periods of gliding. Hypothetically, this swim pattern may allow the mothers to expend less energy, and thus restore their energy reserves from the exertions required during the first month of their calf's life and provide for the neonate's increasing demands for nursing. Slow swimming, rather than surface floating, may also be beneficial to the calves as they may still require additional assistance in thermoregulation, respiration maintenance, and navigation of their environment during this second month of life (Cockcroft & Ross, 1990; Fellner et al., 2005; Lyamin et al., 2005; Mann & Smuts, 1999; McBride & Kritzler, 1951; Miles & Herzing, 2003; Reid et al. 1995; Tavalga & Essapian, 1957).

Pregnant control. When the activities of Dolphin C were examined, we found that they differed dramatically from the mothers' activities. Unlike the mothers, C continued to engage in surface floats for at least half of her night-time activities once the calves were present. This trend persisted across a variety of social groupings. However, she was not immune to the presence of the calves and their subsequent effect on night-time activities.

During the month of June when the calves were young, the pregnant control engaged in a greater number of slow swims while resting (27%) as compared to the previous month when no calves were present (15%). This change in behavior may have reflected a disruption in the night-time activity routine with the increased swimming levels of the mother-calf pairs. For example, surface floating may have been more difficult to maintain with the increased number of mother-calf pairs present. Another explanation may simply have involved a social facilitation effect, in which the control dolphin swam more because more animals were swimming.

Surprisingly, July brought a greater increase in activity. Although C was still likely to rest while floating, she did increase her active swims. It is unclear why C became more active at a time when most of the mothers and calves were beginning to engage in slow swims. However, by the end of the study, she had returned to her pre-calf activities, which included resting by surface floats.

Vigilance. We also examined the vigilant state of each female before and after parturition. Overall, our female dolphins maintained high levels of vigilance with and without calves present, supporting previous research on sustained vigilance in dolphins (Hoffman-Kuhnt 2003; Ridgway et al. 2006). Moreover, the outcome of the vigilance tests was correlated with the type of behavior exhibited just prior to the administration of an initial vigilance test. Previously, floating had been associated with low levels of vigilance (Connor & Heithaus, 1996; Lyamin et al., 2005; Norris & Dahl, 1981) while slow swimming and active swimming were associated with increasingly higher levels of vigilance (Goley, 1999; Gnone et al., 2001). The results corroborated these previous observations as our females were more likely not to respond while surface floating and more likely to respond while slow swimming.

Vigilance Pre-Parturition and Post-Parturition. As expected, mothers were more vigilant post-parturition than pre-parturition. They were more likely to respond to vigilance tests when their calf was present, and they were more likely to not respond when their calf was absent. When immediate follow-up tests of vigilance were conducted for the contralateral eye, mothers tended to

respond if they had responded on the first test. In fact, mothers and the pregnant control were generally vigilant for both tests.

There were occasions though in which the females were not vigilant for either test suggesting that both hemispheres were “sleeping.” These behavioral data support previous empirical evidence for occasional bihemispheric sleep in dolphins (McCormick, 1969; Ridgway, 2002; Ridgway et al., 2006). The majority (85%, $N = 35$) of these proposed bihemispheric periods occurred pre-parturition. However, six instances did occur post-parturition. Clearly, mothers altered their vigilance efforts when calves were present, corroborating the behavioral changes observed in their night-time activities. Overall, they were much more vigilant across the eight week period compared to the month before parturition.

Like their terrestrial counterparts, a state of sustained sleep comes at a cost for dolphins, particularly those with very young offspring (Lima et al., 2005; Lyamin et al., 2005). Failures to respond to stimuli, in this case, a light stimulus, may be the result of occasional bihemispheric sleep patterns, fatigue, divided attention from monitoring their calves’ activities and the activities of other animals, or simply a failure to perceive the stimuli. Clearly, having alternative vigilance strategies, such as resting as part of a group with periphery members having vigilance duties, having sentinels present, or sleeping in a protected place is advantageous to the survival of a species (as reviewed in Lima et al., 2005). In group settings, such as in the current study, mothers could possibly decrease their levels of awareness for brief periods, and “sleep” because the care of their calves is taken over by some member of the group. However, this vigilance strategy did not seem to be supported by the night-time behavioral data and responses to the vigilance tasks. Mothers did not rely on other mothers to care for their calves during the night-time hours as calves were observed only swimming with their mothers during the observations. Similarly, the mothers were highly responsive to the behavioral tests of vigilance. Alternatively, the mothers may have relied more heavily upon a different vigilance strategy, induction of unihemispheric SWS (Lilly, 1964; Mukhametov, 1984; Mukhametov et al., 1977; Ridgway, 2002, Lyman et al., 2008), as indicated by their slow swimming behavior. This strategy would enable the mothers to maintain vigilance over their calves and rest simultaneously. Additional research should examine rest patterns of mothers and their calves during the day as well to determine if mothers use different vigilance strategies depending on the context and social groupings.

Influence of Fatigue on Vigilance. Despite these increases in rest opportunities over the two month period, the first two weeks were characterized by active swimming. Two weeks of continuous swimming and nursing neonates were expected to tire the mothers and result in a decrease of vigilance. Our results did not support this hypothesis as mothers did not significantly change their level of vigilance (i.e., more response failures) over time. Lyamin et al. (2005) had previously ruled out the possibility that the ability to swim continuously was not facilitated by parturition status or hormonal state as there were no differences in stress hormones pre- to post-parturition. It is possible that the mothers became sensitized to the vigilance tests as the frequency of the tests during weeks three and four did increase. However, this explanation does not appear to be adequate as they consistently performed on the vigilance task over the eight weeks and they did fail to

respond more often during the third and fourth weeks than they did at any other point in the study, post-parturition. Perhaps the best explanation is that with the increase in slow swims after the second week of parturition, mothers were able to rest enough to sustain high levels of vigilance. This transition to slow swims may have also been facilitated by the establishment of regular nursing and slipstreaming.

Laterality. Although we were unable to directly measure the mothers' brain activity to determine the presence of unihemispheric SWS, the vigilance tests provided some indirect evidence. Our females showed laterality in their responses to approximately 30% of the dual vigilance tests given pre- or post-parturition. Laterality was indicated when females responded with one side or the other, but not both, to two successive light stimuli. Lyamin et al. (2004) showed that eye closure corresponded to unihemispheric SWS in the brain hemisphere opposite the closed eye in about 75 % of their observations. Opposite eye closure is to be expected because the dolphin optic nerves cross completely at the optic chiasm and directly supply only the opposite brain hemisphere (Tarpley, Gelderd, Bauserman, & Ridgway, 1994). These data support previous research indicating that dolphins are capable of unihemispheric sleep and constant vigilance (Lyamin et al., 2004; Mukhametov, 1984; Mukhametov et al., 1977; Ridgway, 2002; Ridgway et al., 2006, Lyamin et al., 2008).

Parity. In the current study, parity was not related to the mothers' resting activities or to their performances on the vigilance tests. These outcomes were not surprising considering the biological importance of maintaining high levels of vigilance around very young offspring. Calf survival is the ultimate goal for both experienced and inexperienced mothers. Thus, parity should not influence level of vigilance. However, it is possible that differences in activity and/or vigilance may exist between individual mothers. Recent research has suggested that dolphins have unique and stable characteristics (Highfill & Kuczaj, 2007) and exhibit different degrees of maternal control (Hill, Greer, Solangi, & Kuczaj, 2007). Thus, mothers may differ in their activity level and selected rest strategies. Future research should continue to examine the role of maternal experience in combination with individual differences when examining vigilance and calf outcomes.

Conclusion

In summary, the current study specifically examined the night-time activities of females before and after parturition, unlike previous studies in which the swim activities and positions of mothers and their calves were only generally described (Cockcroft & Ross, 1990; Gnone et al., 2001; Gubbins et al., 1999; Mann & Smuts, 1999; Miles & Herzing, 2003; Reid et al., 1995). By differentiating between two types of resting activities, floating and slow swimming, we documented an important change in the mothers' nightly rest strategies that may be associated with neonatal development and enable mothers to sustain high levels of vigilance for extended periods of time. Lyamin et al. (2005) observed that dolphin mothers swam almost continuously, which led them to propose that dolphins may engage in little sleep for extended

periods of time. In contrast, we suggest that the slow circular swimming we observed is likely a form of unihemispheric sleep, which may enable the mothers to sleep as they maintain vigilance over their calves. As demonstrated in continuously swimming porpoises two and half decades ago, unihemispheric slow wave activity alternated between the hemispheres (Mukhametov & Poliakova, 1981). Therefore, stopping to rest or becoming immobile is not an absolute requirement for sleep.

We suggest that future research must also examine alternative rest strategies (i.e., slow swimming). Similar to the different vigilance strategies adapted by various group living terrestrial animals, cetaceans may have evolved a vigilance strategy appropriate to their aquatic environment and their specific biological capabilities. Namely, mothers may incorporate one of their sleep strategies (slow swimming) to a greater degree when they are no longer able to float for long periods of time immediately after birth due to their calves' limitations. Through neonatal slipstreaming during slow swims, mothers are able to sleep or conserve energy for extended periods of time while continuing to monitor their environment and provide the neonate with nursing opportunities, thermoregulation, and navigational assistance. This alternative swim and vigilance strategy may not deprive mothers of sleep as long as Lyamin et al. (2005) suggested. As we did not continuously record behaviors, we cannot provide an estimate of the average time mothers engaged in floating, slow swimming, or active swimming. Future studies in which these behaviors are measured continuously throughout the day would elucidate the importance of slow swimming to maternal sleep and vigilance. This knowledge would be especially important for dolphin mothers in their natural habitat, since floating is not a frequently observed resting strategy.

The current study also provided the first empirical evidence for high levels of vigilance in bottlenose dolphin mothers over an extended period of time. These findings augment those of Ridgway et al. (2006) in which two bottlenose dolphins independently demonstrated continuous auditory vigilance for five days. Finally, our findings suggested that mothers showed laterality in their vigilance both before and after their calves were born. These results imply that mothers may engage in a unihemispheric sleep strategy at times to remain continuously vigilant while resting even though they continue to swim slowly or glide with the calf at their side. Future studies should follow mothers' resting activities beyond the first two months of their calf's life as well as include observations during the daylight hours. These studies would enable researchers and managers of dolphins in human care to ascertain when mothers return to pre-parturition activities. Knowledge of the developmental course of the night-time rest and vigilance activities could facilitate better care of mothers and their calves, including the timing of mother-calf separations and weaning and management of group composition and habitat. Studies should also continue to examine the calves' activities. Lyamin et al. (2005) indicated that their killer whale calves engaged in less floating than their mothers. This behavioral trend was replicated by a captive study examining the resting activities of a bottlenose dolphin calf over the course of the first year of life (Gnone et al., 2006). Although much of the calves' behaviors are regulated by the mothers, it is also important to examine the rest patterns of calves as they develop to begin to assess the influence calves have on their mothers' activities.

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