

PHYSIOLOGICAL AND BEHAVIOURAL DYNAMICS OF INDO-PACIFIC BOTTLENOSE DOLPHINS DURING THE PROCESS OF TRANSFER, ADAPTATION, AND ENVIRONMENTAL ENRICHMENT IN A NEW *EX-SITU* HABITAT

Dinamika Fisiologis dan Perilaku Lumba-Lumba Hidung Botol Indo Pasifik Selama Proses Pemindahan, Adaptasi, dan Pengayaan di Habitat *Ex-situ* Baru

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Abstract

This study aimed to evaluate the effects of *ex-situ* habitat transfer on physiological and behavioral stress responses in Indo-Pacific bottlenose dolphins (*Tursiops aduncus*) and to assess the effectiveness of environmental enrichment in mitigating stress and enhancing welfare. Seven dolphins were monitored across four periods: before transfer, immediately upon arrival at the new facility, after 15 days of adaptation, and following 30 days of environmental enrichment using interactive toys. Parameters measured included neutrophil-to-lymphocyte (N/L) ratio, respiratory rate, frequency of stereotypic behaviors, food intake, social interactions, and compliance with trainer commands. The results demonstrated that habitat transfer induced significant increases in N/L ratio and respiratory rate. A 15-day adaptation period was insufficient to restore physiological and behavioral parameters to baseline levels. However, after 30 days of environmental enrichment, stress indicators improved markedly,

with decreased N/L ratios, normalized respiratory rates, reduced stereotypic behaviors, and increased food intake, social interactions, and compliance. These findings underscore the importance of integrating structured environmental enrichment strategies into *ex-situ* conservation and management programs to support optimal welfare in captive dolphins.

Keywords: animal welfare, environmental enrichment, Indo-Pacific bottlenose dolphin, physiological stress, stereotypic behavior

Abstrak

Penelitian ini bertujuan untuk mengevaluasi dampak pemindahan habitat *ex-situ* terhadap respons stres fisiologis dan perilaku pada lumba-lumba hidung botol Indo-Pasifik (*Tursiops aduncus*), serta menilai efektivitas pengayaan lingkungan dalam menurunkan tingkat stres dan meningkatkan kesejahteraan satwa. Sebanyak tujuh individu lumba-lumba diamati dalam empat periode, yaitu sebelum dipindahkan, sesaat setelah tiba di fasilitas baru, setelah 15 hari masa adaptasi, dan setelah 30 hari penerapan pengayaan lingkungan berupa mainan interaktif. Parameter yang diukur meliputi rasio neutrofil terhadap limfosit (N/L), frekuensi pernapasan, frekuensi perilaku stereotipi, jumlah asupan pakan, frekuensi interaksi sosial, dan tingkat kepatuhan terhadap perintah pelatih. Hasil penelitian menunjukkan bahwa pemindahan habitat memicu peningkatan signifikan pada rasio N/L dan frekuensi pernapasan. Masa adaptasi selama 15 hari belum cukup untuk mengembalikan kondisi fisiologis dan perilaku ke tingkat normal. Namun, setelah 30 hari pengayaan lingkungan, indikator stres mengalami perbaikan yang nyata, ditandai dengan penurunan rasio N/L, normalisasi frekuensi pernapasan, berkurangnya perilaku stereotipi, serta peningkatan asupan pakan, interaksi sosial, dan kepatuhan terhadap pelatih. Temuan ini menegaskan pentingnya integrasi strategi pengayaan lingkungan yang terstruktur dalam program konservasi dan manajemen *ex-situ* untuk mendukung kesejahteraan optimal lumba-lumba di habitat buatan.

Kata kunci: kesejahteraan satwa, lumba-lumba hidung botol Indo-Pasifik, perilaku stereotipi, pengayaan lingkungan, stres fisiologis

INTRODUCTION

The Indo-Pacific bottlenose dolphin (*Tursiops aduncus*) is a highly intelligent and social marine mammal species distributed widely across tropical and subtropical regions (Shirihai and Jarrett, 2006). Its cognitive abilities, social complexity, and behavioral adaptability have enabled this species to adjust to various environmental conditions, including artificial (*ex-situ*) habitats established for conservation, research, and educational purposes (Johnson, 2010). In Indonesia, *ex-situ* conservation programs for dolphins are conducted in both open-water enclosures such as floating sea pens and land-based pools constructed to comply with national animal welfare and conservation standards (Ministry of Forestry, 2014).

In Indonesia, the transfer of marine mammals between facilities represents a standard yet delicate procedure in animal management (Ministry of Forestry, 2013). Although necessary for various operational and conservation objectives, this process can act as a significant stressor, particularly for highly intelligent and socially complex species such as dolphins. Previous studies have shown that stress in marine mammals can be induced by environmental changes, social disruption, transport procedures, and the challenges of adapting to unfamiliar environments (Alava *et al.*, 2020; Clegg *et al.*, 2017; Collier *et al.*, 2022; Noda *et al.*, 2007; Waples and Gales, 2002). Physiological indicators of stress typically include alterations in hematological parameters, such as an increased neutrophil-to-lymphocyte (N/L) ratio, and elevated respiratory rates (Melo & da Silva, 2019; Noda *et al.*, 2007). Behavioral responses to

stress can manifest as reduced food intake, emergence of stereotypic behaviors, alterations in social interactions, and diminished responsiveness to trainers (Waples and Gales, 2002).

To mitigate stress and promote animal welfare in *ex-situ* settings, environmental enrichment has become a widely recommended strategy. Enrichment activities such as the introduction of interactive toys, varying environmental features, and social opportunities are designed to stimulate natural behaviors, improve psychological well-being, and support the acclimatization process in captive animals (Clegg & Delfour, 2023; Clegg *et al.*, 2023; Colbert *et al.*, 2009).

On April 27, 2021, seven Indo-Pacific bottlenose dolphins were transferred from the PT-Piayu Conservation Institution (CI) in Sanur to the PT-Taman Benoa Eksotik (TBE) CI in Benoa, Bali. The dolphins, previously maintained in floating sea pens, were relocated to a land-based pool facility constructed according to the national guidelines for dolphin care and display (Ministry of Forestry, 2014). Although the transport duration was relatively short (approximately 40 minutes), the capture, handling, and transfer procedures extended the overall process, potentially contributing to acute stress responses. This is a unique opportunity to study and to evaluate the physiological and behavioral conditions of those seven dolphins before, during, and after the transfer process, as well as during a 15-day adaptation phase and a subsequent 30-day period following the introduction of environmental enrichment. Records made by the TBE CI medical team in the form of N/L ratio, respiratory rate, appetite, stereotypic behaviors, social interactions, and trainer responsiveness were evaluated as important parameters in this study. The findings are intended to inform best practices for managing marine mammal transfers and acclimatization in *ex-situ* environments, contributing to the development of evidence-based welfare guidelines for dolphin conservation management in Indonesia and similar settings.

RESEARCH METHODS

Study Period and Location

This study was conducted from late May to early June 2025 at the TBE *ex-situ* Wildlife Conservation Facility, Bali. Data collection and tabulation were carried out from May 29 to June 10, 2025, using daily records spanning a 45-day period. This timeframe included the pre-transfer period (hereafter referred to as Period 0 or P0), immediately post-transfer (P1), a 15-day adaptation phase (P2), and a 30-day environmental enrichment phase (P3) (Fair *et al.*, 2006ab; Barratclough *et al.*, 2019).

Study Materials

The study materials consisted of secondary data from seven (7) Indo-Pacific bottlenose dolphins, comprising six males and one female, with no available age data. Initially, the dolphins were maintained in a floating sea pen measuring approximately 20 x 10 meters, then relocated to a land-based pool using a speedboat and truck following animal welfare procedures (Colbert *et al.*, 2009). Environmental enrichment included plastic toys such as floating balls, rings, and suspended balls in various shapes, colors, and sizes (Clark *et al.*, 2013ab).

Study Design and Methods

This research employed an observational case study with a pre-post intervention design (transfer, adaptation, and enrichment phases). Physiological parameters measured included neutrophil-to-lymphocyte (N/L) ratios from caudal venous blood samples and respiratory rate (breaths per minute) (Fair *et al.*, 2006a). Behavioral parameters observed included appetite, stereotypic behaviors, social interactions, and trainer responsiveness, recorded using continuous focal animal sampling methods (Altmann, 1974; Hill *et al.*, 2016).

Data Collection Procedures

Blood Sampling: Approximately 3 mL of blood was collected from the caudal vein using a sterile syringe, placed into EDTA tubes, and analyzed at a commercial laboratory. Sampling was conducted between 08:00 and 09:00 to maintain consistency (Fair *et al.*, 2006a).

Behavioral Observations: Behavioral observations were conducted for 15 minutes per individual, three times daily (morning, midday, and afternoon) from observation points around the pool without disrupting the animals' activities. Recorded parameters included feed intake, frequency of stereotypic behaviors, social interactions, and trainer response to commands (scored as 0 = no response, 1 = slow, 2 = prompt) (Altmann, 1974; Hill *et al.*, 2016).

Data Analysis

The N/L ratio and respiratory rate data were analyzed descriptively and statistically tested using the Friedman test. If significant differences were found, post-hoc analysis was performed using the Wilcoxon Signed Rank Test (Ghasemi & Zahediasl, 2012). Behavioral data were analyzed based on frequency and percentage, then tested across periods using the Wilcoxon Signed Rank Test (Field, 2013).

RESULTS AND DISCUSSION

Results

In general, the results of this study indicate that the relocation of animals between *ex-situ* habitats has the potential to trigger physiological and behavioral stress in Indo-Pacific bottlenose dolphins. This condition was identified through an increase in the Neutrophil-to-Lymphocyte (N/L) ratio and respiratory rate. A 15-day adaptation period was insufficient to fully restore the animals' physiological condition to baseline. However, the provision of environmental enrichment in the form of interactive toys proved to have a positive impact by reducing stereotypic behaviors, increasing feed intake, improving the frequency of social interactions, and enhancing trainer command responsiveness. This indicates a decrease in stress levels and an improvement in the welfare of the dolphins in their new habitat.

Neutrophil-to-Lymphocyte (N/L) Ratio

Analysis of the N/L ratio in seven *T. aduncus* individuals showed dynamic changes throughout the observation period (Figure 1). The mean N/L ratio prior to transfer was recorded at 3.49 ± 0.31 , increasing slightly to 3.53 ± 0.40 immediately after arrival, then rising significantly to 4.85 ± 0.95 at 15 days post-adaptation, and decreasing to 4.38 ± 1.88 after 30 days of environmental enrichment. The Friedman test produced a Chi-Square value (χ^2) = 8.314, df = 3, p = 0.040 (Table 2), indicating a significant difference between observation phases. Further analysis using the Wilcoxon Signed Ranks Test confirmed a significant difference between the pre-transfer phase and the adaptation phase (p = 0.018), but no significant difference between the pre-transfer and post-enrichment phases (p = 1.000) (Table 3). Data distribution revealed outliers in the adaptation and enrichment phases due to persistently high N/L ratios in individuals D4 and D6 during both phases (Figure 2).

Respiratory rate

The pattern of changes in *T. aduncus* respiratory rate showed a slightly different trend compared to the N/L ratio. The mean respiratory rate increased from 3.7 ± 0.1 breaths per minute before transfer to 6.2 ± 0.2 breaths per minute immediately after arrival at the new location. During the 15-day adaptation phase, this value decreased to 4.3 ± 0.1 breaths per minute and further returned to near baseline at 3.4 ± 0.1 breaths per minute after the enrichment phase (Figure 3). Analysis using the Friedman test indicated a significant difference between

phases ($p = 0.018$) (Table 4). The Wilcoxon Signed Ranks Test showed significant changes between the pre-transfer and immediate post-arrival phases ($p = 0.018$), and between the pre-transfer and adaptation phases ($p = 0.018$) (Table 5). Like the N/L ratio data, the respiratory rate of *T. aduncus* increased during P1 and P2 and declined at P3, except in individuals D4 and D6 (Figure 4).

Food intake

The mean feed intake increased significantly from 2.12 ± 0.30 kg during the adaptation phase to 3.54 ± 0.78 kg after enrichment (Figure 5). The Wilcoxon test showed a significant difference ($p = 0.017$) (Table 6). Individual D4 showed no significant increase in feed intake (Figure 6).

Stereotypic Behavior Frequency

The mean frequency of stereotypic behaviors drastically decreased from 12.14 ± 3.29 times per 15 minutes during the adaptation phase to 6.00 ± 3.96 times per 15 minutes after enrichment (Figure 7). The Wilcoxon test showed a significant difference ($p = 0.018$) (Table 7). Although all individuals showed a reduction, the frequency of stereotypic behaviors in D4 and D6 remained within the mean range of the adaptation phase (Figure 8).

Social Interaction Frequency

The mean frequency of social interactions increased from 5.57 ± 0.98 times per 15 minutes during adaptation to 8.00 ± 2.45 times per 15 minutes after enrichment (Figure 9). The Wilcoxon test showed a significant difference ($p = 0.044$) (Table 8). An insignificant increase was observed in D4 and D6 (Figure 10), causing the lower quartile of the enrichment phase to remain low.

Trainer command responsiveness

The frequency of dolphin compliance to trainer commands increased from 1.14 ± 0.69 times per 15 minutes during adaptation to 1.71 ± 0.49 times per 15 minutes after enrichment (Figure 11). The Wilcoxon test showed a significant difference ($p = 0.046$) (Table 9). Individuals D4 and D6 still exhibited low compliance levels after enrichment (Figure 12).

Discussion

The transfer of dolphins between *ex-situ* habitats is a high-risk management procedure that can trigger both physiological and behavioural stress. Previous studies have demonstrated that sudden environmental changes, human manipulation, and social disruptions can provoke significant stress responses in marine mammals, including dolphins (Thomson & Geraci, 1986; St Aubin *et al.*, 2007; Fair *et al.*, 2014; Atkinson & Dierauf, 2018; Cory *et al.*, 2018; Reid *et al.*, 2019; Wilson *et al.*, 2019; Díaz López & Shirakihara, 2020; Bearzi & Pace, 2021).

Physiological Stress Responses to Relocation

One of the physiological indicators used in this study was the neutrophil-to-lymphocyte ratio (N/L ratio). Physiologically, an increase in the N/L ratio occurs due to the influence of stress hormones such as cortisol, which stimulates neutrophil elevation and lymphocyte suppression in blood circulation. In this study, the mean N/L ratio increased from 3.49 ± 0.31 before relocation to 3.53 ± 0.40 immediately after arrival at the new site. Although not statistically significant, this trend indicates activation of an acute stress response.

A significant increase in N/L ratio was observed during the adaptation phase (mean 4.85 ± 0.95). This finding aligns with the mechanism of hypothalamic-pituitary-adrenal (HPA) axis activation, in which cortisol release peaks several hours following exposure to a stressor

(Atkinson & Dierauf, 2018). In the early phase of acute stress, the dominant response is driven by the sympathetic nervous system (catecholamines), while the effects of cortisol on leukocytes require a longer time to manifest significantly (Clegg *et al.*, 2017).

In addition to haematological parameters, the increase in respiratory rate from 3.7 ± 0.1 to 6.2 ± 0.2 breaths per minute immediately after relocation also served as an indicator of acute stress. Activation of the sympathetic nervous system and the release of epinephrine and norepinephrine accelerate respiration to meet the elevated oxygen demand caused by stress-induced metabolic surges (Fair *et al.*, 2014; Serres & Delfour, 2019). Moreover, cortisol enhances the sensitivity of respiratory receptors to changes in blood O₂ and CO₂ levels (Atkinson & Dierauf, 2018).

Impact of Stress on Feed Intake and Behaviours

Relocation-induced stress also affected feed intake, resulting in reduced consumption. Sympathoadrenal activation suppresses digestive functions and reallocates energy to vital systems (Atkinson & Dierauf, 2018). Sustained elevation of cortisol further influences appetite-regulating hormones such as leptin and ghrelin, and diminishes hypothalamic sensitivity to hunger signals (Fair *et al.*, 2014). Additionally, psychological disturbances caused by environmental changes, unfamiliar sounds, and physical discomfort may reduce cetacean' feeding motivation (Curry *et al.*, 1995; Clegg *et al.*, 2017; Marino *et al.*, 2020).

Beyond physiological parameters, behavioral changes also serve as important indicators. An increase in stereotypic behaviors, such as circular swimming, tail-slapping, and aimless pacing, reflects stress responses to environmental restrictions, loss of control, and limited mental stimulation (Delfour & Beyer, 2012; Clark *et al.*, 2013a,b; Marino *et al.*, 2020). Elevated cortisol levels are directly associated with impaired adaptive behavioural control, resulting in the emergence of repetitive behaviours (Fair *et al.*, 2014).

A decline in social interactions was also observed during the adaptation phase. Under stressful conditions, dolphins tend to reduce social contact as an adaptive strategy to minimize the risk of conflict (Waples & Gales, 2002; Marino *et al.*, 2020). This behaviour is supported by sympathetic nervous system activation and increased cortisol levels, which prioritize vital functions while lowering the priority of social activities (Fair *et al.*, 2014; Clegg *et al.*, 2017a,b).

Relocation-related stress also affected the dolphins' responsiveness to trainer commands. Psychological disturbances can impair cognitive functions, attention, and working memory, thereby reducing responsiveness to trainer cues (Fair *et al.*, 2014). Moreover, chronic stress negatively impacts the trust relationship between dolphins and trainers, which is fundamental in reward-based training systems (Delfour & Beyer, 2012; Clegg *et al.*, 2017a,b).

Effectiveness of Environmental Enrichment

The implementation of environmental enrichment in the form of interactive toys for 30 days proved effective in reducing both physiological and behavioural stress indicators. The N/L ratio decreased to 4.38 ± 1.88 , respiratory rates returned near normal levels (3.4 ± 0.1 breaths per minute), stereotypic behaviours significantly diminished, feed intake increased, and frequencies of social interaction and trainer responsiveness improved. These results are consistent with previous studies reporting that environmental enrichment enhances the welfare of marine mammals in captive facilities (Clark *et al.*, 2013a,b; Fair *et al.*, 2014; Lauderdale & Miller, 2023; Serres & Delfour, 2023; Mate *et al.*, 2024).

Adaptation Failure in Two Individuals

Despite the overall positive response to the intervention, two dolphins died within two months post-relocation. Individual responses to environmental stressors are highly variable and influenced by factors such as age, sex, health status, prior experiences, and social position within the group (Fair *et al.*, 2014; Clegg *et al.*, 2017; Clegg & Delfour, 2023). It is likely that these individuals had low stress thresholds or undetected subclinical conditions prior to relocation.

Previous studies have indicated that pre-transfer health status critically determines an individual's adaptive capacity after relocation (Atkins & Derauf, 2018). Relocation inevitably introduces intense stressors, ranging from temperature shifts and unfamiliar sounds to new social structures, which can accelerate physiological decompensation in individuals with limited adaptive reserves (Clegg *et al.*, 2017).

Another contributing factor is suspected to be unhealthy social interactions, such as social isolation, excessive dominance, or group conflict, which exacerbate stress and reduce physiological resilience (Delfour & Beyer, 2012). Additionally, the rate of adaptive response to environmental enrichment varies among individuals (Clark *et al.*, 2013a,b). These two individuals likely failed to reach the recovery phase before their physiological condition deteriorated.

CONCLUSION AND SUGGESTIONS

Conclusion

In conclusion, the relocation of Indo-Pacific bottlenose dolphins (*Tursiops aduncus*) between *ex-situ* habitats triggers significant physiological and behavioral stress responses. This stress is evidenced by a marked increase in the neutrophil-to-lymphocyte (N/L) ratio and a sharp rise in respiratory frequency during the initial adaptation period. However, the implementation of environmental enrichment proved effective in mitigating these effects and improving overall welfare; this was demonstrated by the gradual normalization of physiological parameters and a significant reduction in stereotypic behaviors. Furthermore, the enrichment led to enhanced feed intake, more frequent social interactions, and increased responsiveness to trainer commands. Despite these general improvements, individual responses varied, with individuals D4 and D6 exhibiting more persistent stress characterized by consistently elevated N/L ratios and respiratory rates, as well as lower levels of social and trainer engagement compared to the rest of the group.

Suggestions

Based on the findings of this study, it is recommended that the relocation of *ex-situ* animals be managed through a structured adaptation phase complemented by intensive monitoring of physiological and behavioral parameters for a minimum of 45 days post-transfer. Following the initial adaptation, environmental enrichment should be implemented immediately, ensuring that the type, frequency, and variety of enrichment media are specifically tailored to the unique characteristics and responses of each individual. For animals exhibiting persistent stress such as individuals D4 and D6 specialized attention is required, including supplemental enrichment programs, targeted behavioral interventions, and comprehensive health evaluations. Furthermore, future research should involve larger sample sizes and extended monitoring periods to better identify the underlying factors of inter-individual response variations and to evaluate the long-term efficacy of diverse enrichment strategies.

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REFERENCES

- Alava, J. J., Calle, P., Tirapé, A., Biedenbach, G., Alvarado, O., Maruya, K., Wenjian, L., Aguirre, W., Jiménez, P. J., Dominguez, G. A., Bossart, G. D., & Fair, P. A. (2020). Persistent organic pollutants and mercury in genetically identified inner estuary bottlenose dolphin (*Tursiops truncatus*) residents of the Guayaquil Gulf, Ecuador: Ecotoxicological science in support of pollutant management and cetacean conservation. *Frontiers in Marine Science*, 7(122), 1–24. <https://doi.org/10.3389/fmars.2020.00122>
- Altmann, J. (1974). Observational study of behaviour: Sampling methods. *Behaviour*, 49(3–4), 227–267. <https://doi.org/10.1163/156853974X00534>
- Atkinson, S., & Dierauf, L. A. (2018). Stress and marine mammals. Dalam F. Gulland, L. A. Dierauf, & K. A. Whitman (Eds.), *CRC handbook of marine mammal medicine: Anatomy and physiology* (hlm. 153–168). CRC Press.
- Barratclough, A., Wells, R. S., Schwacke, L. H., Rowles, T. K., Gomez, F. M., Fauquier, D. A., Sweeney, J. C., Townsend, F. I., Hansen, L. J., Zolman, E. S., Balmer, B. C., & Smith, C. R. (2019). Health assessments of common bottlenose dolphins (*Tursiops truncatus*): Past, present, and potential conservation applications. *Frontiers in Veterinary Science*, 6, Pasal 444. <https://doi.org/10.3389/fvets.2019.00444>
- Bearzi, G., & Pace, D. S. (2021). Captivity effects on dolphins: Environmental deprivation and consequences on behaviour and health. *Journal of Marine Mammal Science*, 37(3), 1254–1273.
- Champagne, C. D., Kellar, N. M., Trego, M. L., Delehanty, B., Boonstra, R., Wasser, S. K., Booth, R. K., Crocker, D. E., & Houser, D. S. (2018). Comprehensive endocrine response to acute stress in the bottlenose dolphin from serum, blubber, and faeces. *General and Comparative Endocrinology*, 266, 178–193. <https://doi.org/10.1016/j.ygcen.2018.05.015>
- Clark, F. E., Davies, S. L., Madigan, A. W., Warner, A. J., & Kuczaj, S. A. (2013a). Cognitive enrichment for bottlenose dolphins: Evaluation of effectiveness and welfare impact. *Animal Welfare*, 22(3), 347–357. <https://doi.org/10.7120/09627286.22.3.347>
- Clark, F. E., Fitzpatrick, M., Hartley, A., King, A. J., Lee, T., Routh, A., & Shepherdson, D. J. (2013b). Relationship between behaviour, adrenal activity, and environment in captive bottlenose dolphins (*Tursiops truncatus*). *Zoo Biology*, 32(1), 1–13. <https://doi.org/10.1002/zoo.21033>
- Clegg, I. L. K., & Delfour, F. (2023). Environmental enrichment factors associated with the activity level of bottlenose dolphins under professional care. *Journal of Zoological and Botanical Gardens*, 3(1), 4. <https://doi.org/10.3390/jzbg3010004>
- Clegg, I. L. K., Domingues, M., Ström, E., & Berggren, L. (2023). Cognitive foraging enrichment (but not non-cognitive enrichment) improved several longer-term welfare indicators in bottlenose dolphins. *Animals*, 13(2), 238. <https://doi.org/10.3390/ani13020238>
- Clegg, I. L. K., Rödel, H. G., Boivin, X., Delfour, F., & Hausberger, M. (2017). Looking forward to interacting with their caretakers: Dolphins’ anticipatory behaviour indicates positive

anticipation. *Applied Animal Behaviour Science*, 202, 85–93.
<https://doi.org/10.1016/j.applanim.2018.02.005>

Colbert, D. E., Delfour, F., & Young, R. J. (2009). The behavioural effects of environmental enrichment on captive bottlenose dolphins (*Tursiops truncatus*). *Journal of Applied Animal Welfare Science*, 12(3), 200–212. <https://doi.org/10.1080/10888700902955952>

Collier, M. A., Mann, J., Ali, S., & Bansal, S. (2022). Impacts of human disturbance in marine mammals: Do behavioral changes translate to disease consequences? Dalam B. Würsig & G. N. Di Sciara (Eds.), *Marine mammals: The evolving human factor* (hlm. 277–305). Springer. https://doi.org/10.1007/978-3-030-98100-6_9

Curry, B. E., et al. (1995). Physiological and behavioral responses of bottlenose dolphins to exposure to oil and oil-dispersant mixtures. *Aquatic Toxicology*, 33(2–3), 191–206.

Delfour, F., & Beyer, H. (2012). Assessing the effectiveness of environmental enrichment in bottlenose dolphins (*Tursiops truncatus*). *Zoo Biology*, 31(2), 137–150.

Díaz López, B., & Shirakihara, M. (2020). Behavioral changes in captive bottlenose dolphins: A case study of welfare indicators. *Zoo Biology*, 39(2), 115–124.

Fair, P. A., Schaefer, A. M., Romano, T. A., Bossart, G. D., Lamb, S. V., & Reif, J. S. (2006a). Haematology and serum chemistry reference ranges for wild bottlenose dolphins (*Tursiops truncatus*) from the Indian River Lagoon, Florida. *Journal of Wildlife Diseases*, 42(3), 681–691. <https://doi.org/10.7589/0090-3558-42.3.681>

Fair, P. A., Schaefer, A. M., Romano, T. A., Bossart, G. D., Lamb, S. V., & Reif, J. S. (2006b). Stress response of wild bottlenose dolphins (*Tursiops truncatus*) during capture–release health assessment studies. *General and Comparative Endocrinology*, 148(2), 282–289. <https://doi.org/10.1016/j.ygcen.2006.03.002>

Fair, P. A., Schaefer, A. M., Romano, T. A., Bossart, G. D., Lamb, S. V., & Reif, J. S. (2014). Stress response of wild bottlenose dolphins (*Tursiops truncatus*) during capture-release health assessment studies. *General and Comparative Endocrinology*, 206, 203–212. <https://doi.org/10.1016/j.ygcen.2014.07.002>

Field, A. (2013). *Discovering statistics using IBM SPSS statistics* (Edisi ke-4). Sage.

Ghasemi, A., & Zahediasl, S. (2012). Normality tests for statistical analysis: A guide for non-statisticians. *International Journal of Endocrinology and Metabolism*, 10(2), 486–489. <https://doi.org/10.5812/ijem.3505>

Hill, H. M., Ramirez, D., Ridgway, S., & Green, M. (2016). Behavioural responses to environmental enrichment in bottlenose dolphins (*Tursiops truncatus*). *International Journal of Comparative Psychology*, 29. <https://escholarship.org/uc/item/1hj2n8h7>

Johnson, C. M. (2010). Observing cognitive complexity in primates and cetaceans. *International Journal of Comparative Psychology*, 23, 587–624. <https://doi.org/10.46867/ijcp.2010.23.04.12>

Lauderdale, L. K., & Miller, L. J. (2023). Efficacy of an interactive apparatus as environmental enrichment for common bottlenose dolphins (*Tursiops truncatus*). *Animal Welfare*, 32(1), 1–9. <https://doi.org/10.1017/awf.2022.34>

Marino, L., Naomi, A. R., Ingrid, N. V., Heather, R., Hope, F., & Veronica, S. (2020). The harmful effects of captivity and chronic stress on the well-being of orcas (*Orcinus orca*). *Journal of Veterinary Behavior*, 35, 69–82.

Máté, E., Ng, A. K. W., Chan, M. M. H., & Pongrácz, P. (2024). Experiments with a set of cooperative enrichment devices used by groups of Indo-Pacific bottlenose dolphins. *Biologia Futura*, 75(1), 45–56. <https://doi.org/10.1007/s42977-024-00218-2>

Melo, D. M. D., & da Silva, V. M. F. (2019). Hematologic profile of Amazon river dolphins *Inia geoffrensis* and its variation during acute capture stress. *PLoS ONE*, 14(12), Artikel e0226955. <https://doi.org/10.1371/journal.pone.0226955>

Ministry of Forestry. (2013). *Regulation of the Minister of Forestry of the Republic of Indonesia Number P.63/Menhut-II/2013 concerning Procedures for Obtaining Specimens of Plants and Wildlife for Conservation Institutions*.

Ministry of Forestry. (2014). *Regulation of the Director General of Forest Protection and Nature Conservation Number P.16/IV-SET/2014 concerning Dolphin Demonstration Guidelines*. Director General of Forest Protection and Nature Conservation.

Noda, K., Akiyoshi, H., Aoki, M., Shimada, T., & Ohasi, F. (2007). Relationship between transportation stress and polymorphonuclear cell functions of bottlenose dolphins, *Tursiops truncatus*. *Journal of Veterinary Medical Science*, 69(4), 379–383. <https://doi.org/10.1292/jvms.69.379>

Reid, K. R., Smith, A. M., McCoy, K. D., & Fair, P. A. (2019). Environment, endocrinology, and biochemistry influence expression of stress proteins in bottlenose dolphins (*Tursiops truncatus*). *Comparative Biochemistry and Physiology Part D: Genomics and Proteomics*, 32, 100613.

Serres, A., & Delfour, F. (2019). Social behaviors modulate bottlenose dolphins (*Tursiops truncatus*) breathing rate. *Animal Behavior and Cognition*, 6(2), 127–140. <https://doi.org/10.26451/abc.06.02.04.2019>

Serres, A., & Delfour, F. (2023). Benefit of cognitive environmental enrichments on social tolerance and play behaviour in bottlenose dolphins (*Tursiops truncatus*). *Applied Animal Behaviour Science*, 261, 105776. <https://doi.org/10.1016/j.applanim.2023.105776>

Shirihai, H., & Jarrett, B. (2006). *Whales, dolphins and other marine mammals of the world*. Princeton University Press.

St. Aubin, D. J., Fair, P. A., Bossart, G. D., & Romano, T. A. (2007). Relationship between transportation stress and polymorphonuclear cell functions of bottlenose dolphins, *Tursiops truncatus*. *Journal of Comparative Physiology*. <https://doi.org/10.1093/icb/icn011>

Thomson, C. A., & Geraci, J. R. (1986). Cortisol, aldosterone, and leucocytes in the stress response of bottlenose dolphins, *Tursiops truncatus*. *Canadian Journal of Fisheries and Aquatic Sciences*, 43, 1010–1016. <https://doi.org/10.1139/f86-125>

Waples, K. A., & Gales, N. J. (2002). Evaluating and minimizing social stress in the care of captive bottlenose dolphins (*Tursiops aduncus*). *Zoo Biology*, 21(1), 5–26.

Wilson, A. E., Fair, P. A., Carlson, R. I., Houde, M., Cattet, M., Bossart, G. D., Houser, D. S., & Janz, D. M. (2019). Environment, endocrinology, and biochemistry influence expression of stress proteins in bottlenose dolphins. *Comparative Biochemistry and Physiology Part D: Genomics and Proteomics*, 32, 100613. <https://doi.org/10.1016/j.cbd.2019.100613>

Tables

Table 1. Matrix of observational parameters for Indo-Pacific Bottlenose Dolphins (*Tursiops aduncus*) at the present study.

| Parameters | Prior to transfer from the sea pen (P0) | Shortly after arrival at the new facility (P1) | 15 days after adaptation (P2) | 30 days after enrichment (P3) |
|--------------------------------------|---|--|-------------------------------|-------------------------------|
| Neutrophil-to-Lymphocyte ratio (N/L) | Day 0 | Day 0 | Day 15th | Day 45th |
| Respiration rate | Day 0 | Day 0 | Daily | Daily |
| Total feed intake | - | - | Daily | Daily |
| Stereotype behaviors | - | - | Daily | Daily |
| Social interaction | - | - | Daily | Daily |
| Response to trainer's command | - | - | Daily | Daily |

(-) indicates no data

Table 2. N/L ratio and Friedman Test results of *T. aduncus* during the pre-transfer period (P0), immediately after transfer (P1), post-adaptation (P2), and post-enrichment (P3). An asterisk (*) indicates a significant difference (P<0.05).

| Period | n | Mean | SD | Minimum | Maximum | Mean Rank | c ² | df | p-value |
|--------|---|------|------|---------|---------|-----------|----------------|----|---------|
| P0 | 7 | 3,49 | 0,31 | 3,02 | 4,00 | 2,00 | 8,314 | 3 | 0,040* |
| P1 | 7 | 3,53 | 0,40 | 3,12 | 4,17 | 2,14 | | | |
| P2 | 7 | 4,85 | 0,95 | 4,17 | 6,87 | 3,71 | | | |
| P3 | 7 | 4,38 | 1,88 | 3,13 | 7,36 | 2,14 | | | |

Table 3. Wilcoxon Signed Ranks Test of N/L ratio in *T. aduncus* during the pre-transfer period (P0), immediately after transfer (P1), post-adaptation (P2), and post-enrichment (P3). An asterisk (*) indicates a significant change, while (ns) indicates no significant change

| Period | P0 | P1 | P2 | P3 |
|--------|-----------------------|-----------------------|-----------------------|-----------------------|
| P0 | | p=0.735 ^{ns} | p=0.018* | p=1.000 ^{ns} |
| P1 | p=0.735 ^{ns} | | p=0.018* | p=1.000 ^{ns} |
| P2 | p=0.018* | p=0.018* | | p=0.310 ^{ns} |
| P3 | p=1.000 ^{ns} | p=1.000 ^{ns} | p=0.310 ^{ns} | |

Table 4. Summary of respiratory frequency data and Friedman Test results for *T. aduncus* during the pre-relocation period (P0), immediately after relocation (P1), post-adaptation (P2), and post-enrichment (P3). An asterisk (*) indicates a significant difference.

| Period | n | Mean | SD | Minimum | Maximum | Mean | c ² | df | p-value |
|--------|---|------|------|---------|---------|------|----------------|----|---------|
| P0 | 7 | 2,37 | 0,18 | 2,10 | 2,60 | 1,43 | 10,791 | 3 | 0,013* |
| P1 | 7 | 3,17 | 0,29 | 2,70 | 3,50 | 2,93 | | | |
| P2 | 7 | 3,34 | 0,15 | 3,10 | 3,50 | 3,50 | | | |
| P3 | 7 | 2,81 | 0,89 | 2,20 | 4,50 | 2,14 | | | |

Table 5. Wilcoxon Signed-Ranks Test of respiratory frequency in *T. aduncus* during the period before relocation (P0), immediately after relocation (P1), post-adaptation (P2), and post-enrichment (P3). An asterisk (*) indicates a significant change, while “ns” indicates no significant change.

| Period | P0 | P1 | P2 | P3 |
|--------|-----------------------|-----------------------|-----------------------|-----------------------|
| P0 | | p=0.018* | p=0.018* | p=0.175 ^{ns} |
| P1 | p=0.018* | | p=0.068 ^{ns} | p=0.270 ^{ns} |
| P2 | p=0.018* | p=0.068 ^{ns} | | p=0.176 ^{ns} |
| P3 | p=0.175 ^{ns} | p=0.270 ^{ns} | p=0.176 ^{ns} | |

Table 6. Summary of feed intake data and Wilcoxon Signed-Ranks Test results for Indo-Pacific Bottlenose Dolphins during the adaptation period (P2) and enrichment period (P3). An asterisk (*) indicates a significant difference.

| Period | n | Mean | SD | Minimum | Maximum | Asymptotic Significance |
|--------|---|------|------|---------|---------|-------------------------|
| P2 | 7 | 2,12 | 0,30 | 1,47 | 2,35 | 0,017* |
| P3 | 7 | 3,54 | 0,78 | 1,80 | 4,00 | |

Table 7. Summary of stereotypic behavior data and Wilcoxon Signed-Ranks Test results for *T. aduncus* during the adaptation period (P2) and enrichment period (P3). An asterisk (*) indicates a significant difference.

| Period | n | Mean | SD | Minimum | Maximum | Asymptotic Significance |
|--------|---|-------|------|---------|---------|-------------------------|
| P2 | 7 | 12,14 | 3,29 | 9 | 19 | 0,018* |
| P3 | 7 | 6,00 | 3,96 | 2 | 12 | |

Table 8. Summary of social interaction data and Wilcoxon Signed-Ranks Test results for *T. aduncus* during the adaptation period (P2) and enrichment period (P3). An asterisk (*) indicates a significant difference.

| Period | n | Mean | SD | Minimum | Maximum | Asymptotic Significance |
|--------|---|------|------|---------|---------|-------------------------|
| P2 | 7 | 5,57 | 0,98 | 4 | 7 | 0,018* |
| P3 | 7 | 8,00 | 2,45 | 4 | 10 | |

Table 9. Summary of Trainer Compliance Category Data and Wilcoxon Signed-Ranks Test Results. This section summarizes the data for *T. aduncus*' compliance categories with trainers and presents the Wilcoxon Signed-Ranks Test results for the adaptation and enrichment periods. An asterisk (*) indicates a significant difference.

| Period | n | Mean | SD | Min | Max | Asymptotic Significance |
|--------|---|------|------|-----|-----|-------------------------|
| P2 | 7 | 1,14 | 0,69 | 0 | 2 | 0,046* |
| P3 | 7 | 1,71 | 0,49 | 1 | 2 | |

Figures

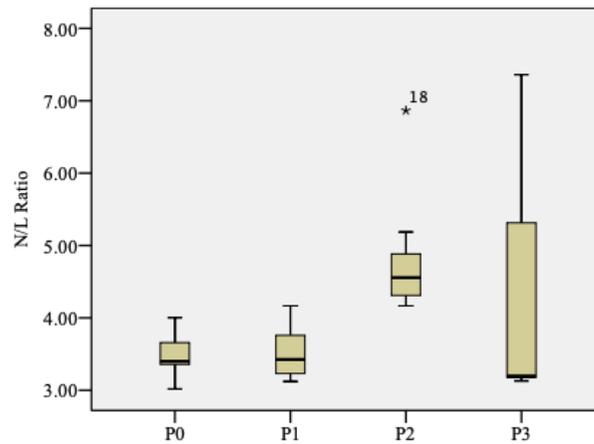


Figure 1. Box and Whisker Plot diagram of the N/L ratio data distribution of *T. aduncus* in each period. There is a very high outlier during the adaptation period that persists into the enrichment phase, resulting in a very wide upper quartile distribution in the enrichment period (extending upwards).

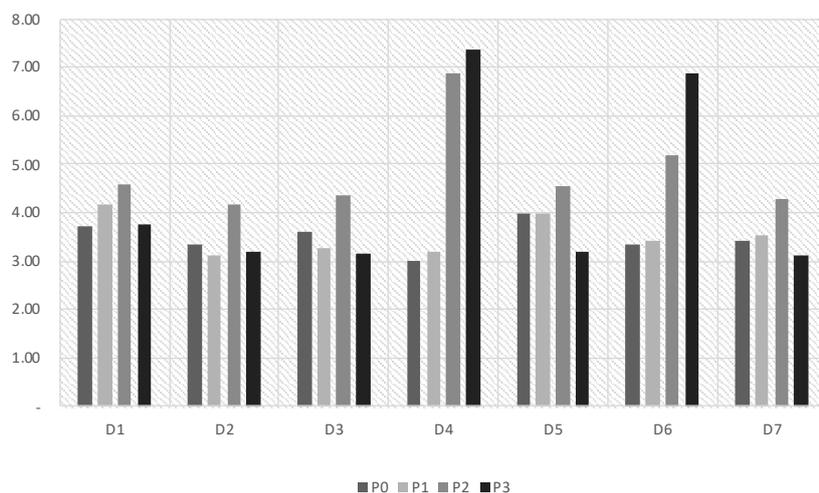


Figure 2. Bar chart of the individual N/L ratio of *T. aduncus* measured at the period just before relocation (P0), immediately upon arrival at the new location (P1), 15 days post-adaptation (P2), and 30 days post-enrichment (P3). The N/L ratio of *T. aduncus* D4 and D6 appeared to continuously increase while at the new location and was relatively very high compared to other individuals during the adaptation and enrichment periods.

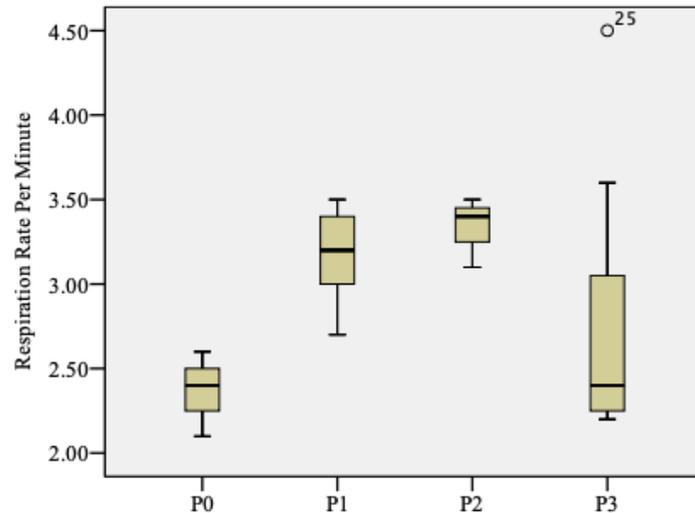


Figure 3. Box and Whisker Plot diagram of *T. aduncus* respiration frequency data distribution in each period. There is a very high outlier during the enrichment period, causing the upper quartile distribution for this phase to become very wide (upwards).

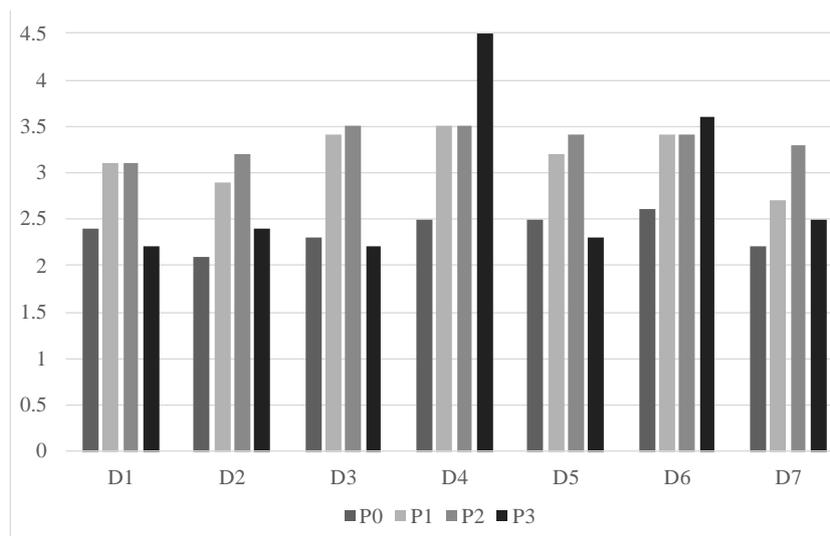


Figure 4. Bar chart of individual *T. aduncus* respiratory frequency measured at the period just before relocation (P0), immediately upon arrival at the new location (P1), 15 days post-adaptation (P2), and 30 days post-enrichment (P3). The respiratory frequency of *T. aduncus* increased at P1 and P2, but decreased at P3, except for D4 and D6, which continued to increase even through the enrichment period.

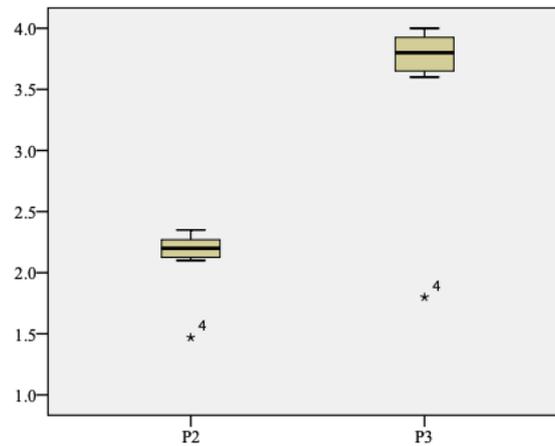


Figure 5. Box and Whisker Plot diagram showing the distribution of feed intake data (kg). There's a very low outlier during both the adaptation and enrichment periods, which upon investigation was identified as dolphin D4.

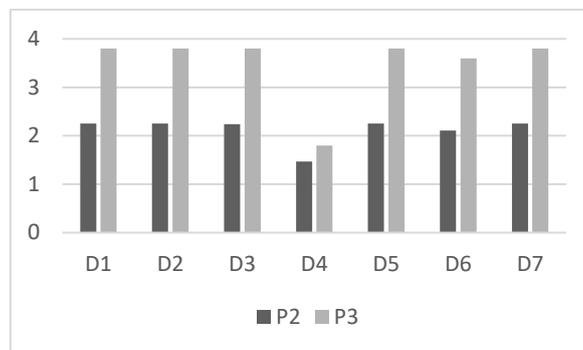


Figure 6. Bar chart of individual *T. aduncus* feed intake (kg) calculated at 15 days post-adaptation (P2) and 30 days post-enrichment (P3). Except for D4, *T. aduncus*' feed intake sharply increased post-enrichment (P3)

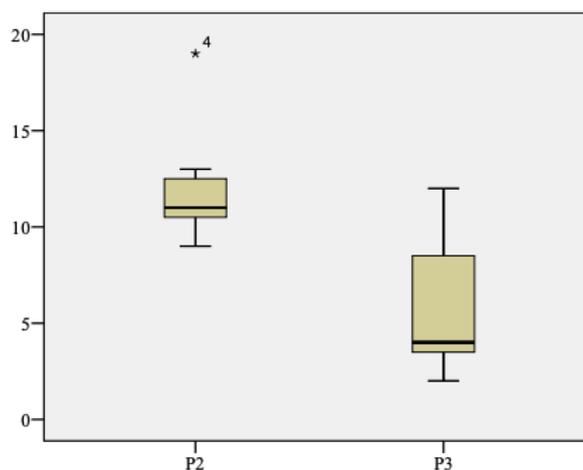


Figure 7. Box and Whisker Plot diagram showing the distribution of stereotypic behavior data. There's a very high outlier during the adaptation period, which upon investigation was identified as dolphin D4.

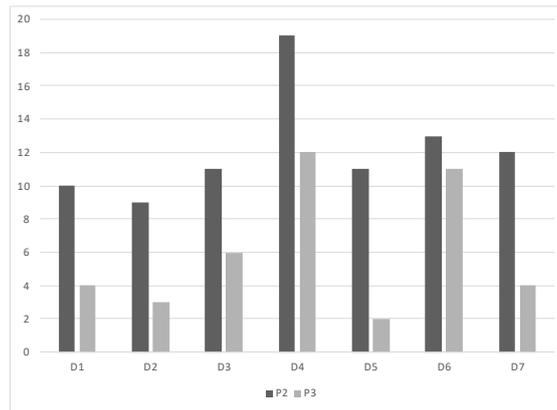


Figure 8. Bar chart of individual *T. aduncus* stereotypic behavior frequency calculated at 15 days post-adaptation (P2) and 30 days post-enrichment (P3). Except for D4 and D6, *T. aduncus*' stereotypic behavior sharply decreased post-enrichment (P3).

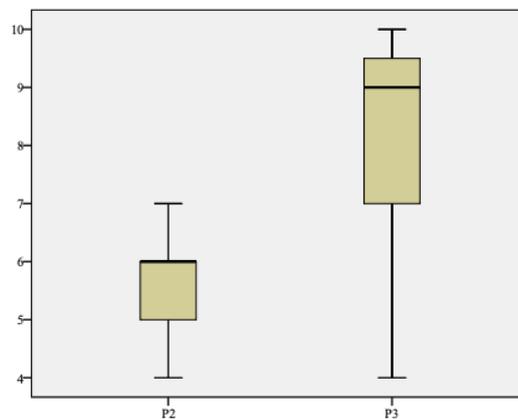


Figure 9. Box and Whisker Plot diagram showing the distribution of social interaction data for *T. aduncus*. The lower quartile for both phases is relatively similar, which indicates that some individual dolphins did not change their social interaction behavior.

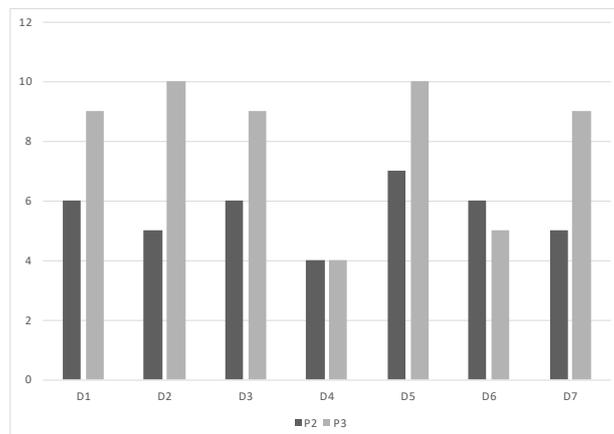


Figure 10. Bar Chart of Individual *T. aduncus* Social Interaction Frequency This bar chart displays the frequency of social interactions for individual *T. aduncus*, calculated at 15 days post-adaptation (P2) and 30 days post-enrichment (P3). Except for D4 and D6, the frequency of social interaction behavior of *T. aduncus* sharply increased post-enrichment (P3).

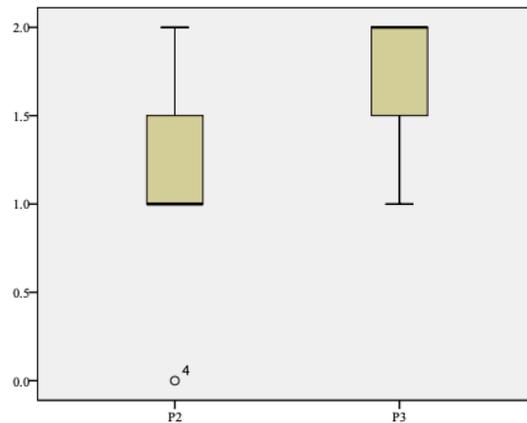


Figure 11. Box and Whisker Plot of *T. aduncus* Compliance with Trainer Instructions. This Box and Whisker Plot illustrates the distribution of data regarding *T. aduncus*' compliance with trainer instructions. There's a very low outlier during the adaptation phase, and a long lower quartile in the enrichment period.

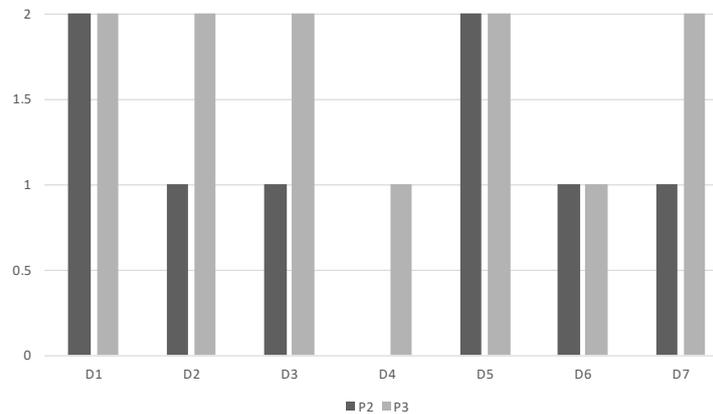


Figure 12. Bar Chart of Individual *T. aduncus* Compliance Categories. This bar chart shows the compliance categories for individual *T. aduncus*, observed at 15 days post-adaptation (P2) and 30 days post-enrichment (P3). Except for D4 and D6, the compliance category of *T. aduncus* sharply increased or stable post-enrichment (P3).