ISSN 1984-4689 (online)

www.scielo.br/zool

SHORT COMMUNICATION

Humpback whale *Megaptera novaeangliae* (Cetartiodactyla: Balaenopteridae) group sizes in line transect ship surveys: An evaluation of observer errors

Guilherme A. Bortolotto^{1,2}, Daniel Danilewicz^{2,3}, Artur Andriolo⁴ & Alexandre N. Zerbini⁵

¹Sea Mammal Research Unit, Scottish Oceans Institute, University of St Andrews. St Andrews, Fife KY16 8LB, Scotland, UK. Corresponding author. E-mail: gabdo@st-andrews.ac.uk

²Programa de Pós-graduação em Zoologia, Universidade Estadual de Santa Cruz. Avenida Ilhéus-Itabuna, 45662-900 Ilhéus, BA, Brazil.

³Grupo de Estudos de Mamíferos Aquáticos do Rio Grande do Sul. Avenida Tramandaí 976, 95625-000 Imbé, RS, Brazil. ⁴Laboratório de Ecologia Comportamental e Bioacústica, Departamento de Zoologia, ICB, Universidade Federal de Juiz de Fora. Rua José Lourenço Kelmer, 36036-900 Juiz de Fora, MG, Brazil.

⁵Alaska Fisheries Science Center, NOAA Fisheries. 7600 Sand Point Way NE, Seattle, WA, 98115, USA.

ABSTRACT. Accurate estimates of group sizes through line transect sampling methods are important to correctly ascertain the abundance of animals that occur in groups. Since the average observed group size is a component of the distance sampling formula, bias in these data leads to biased abundance estimates. This study aimed to evaluate the potential errors in group size estimation during line transect ship surveys to estimate abundances of the humpback whale, Megaptera novaeangliae (Borowski 1781). In a research cruise along the Brazilian coast, an experiment to collect group size information was conducted from two different sighting platforms on the same vessel. Group sizes were recorded by primary observers at first sighting (PO1) and, in some cases, after some time (PO2). A tracker (T) was located on a higher platform to estimate the sizes of groups detected by the primary observers, but tracked one group at a time until it passed abeam. Thus, the dedicated effort to obtain multiple group counts (i.e. higher platform, more time and no responsibility for detecting new groups) was expected to provide more accurate numbers. PO2 estimates were compared with PO1 estimates, and T estimates were compared with both PO1 and PO2. Additionally, ratios between T and both PO2 (R1) and PO1 (R2), and between PO2 and PO1 (R3) were calculated. To investigate a possible improvement in abundance estimates, a correction factor (CF) was computed from the ratio of T and PO2 means. Primary observer self-correction (= 1.60, CV% = 70.3) was statistically similar to the correction for the tracker (= 1.62, CV% = 84.1). CF resulted in 1 and would not improve abundance estimates. This study supports that observers conducting line transect surveys on large whales have the potential to provide group size information that is as adequate as the correction procedure adopted.

KEY WORDS. Abundance, bias, conservation, distance sampling, Mysticetus.

Density (D) and abundance (N) are the essential parameters in the conservation of biological populations (HAMMOND 2010). When it comes to threatened species, they are fundamental for understanding past and future trends, and for planning conservation and management efforts. In the case of cetaceans, density and abundance are often estimated using distance sampling methods through line transect designs (BUCKLAND et al. 2001, THOMAS et al. 2002).

tor

ournal

International

Line transect distance sampling methods have been used to estimate abundances of the humpback whale, *Megaptera novaeangliae* (Borowski 1781), around the world (CALAMBOKIDIS & BARLOW 2004, ZERBINI et al. 2004, ANDRIOLO et al. 2006, 2010, SECCHI et al. 2011, JOHNSTON et al. 2012). Humpback whales are found in all major oceans and typically migrate each year between feeding grounds in high latitude cold waters, and breeding grounds in low latitude tropical waters, where they spend the winter and spring mating and calving (CLAPHAM & MEAD 1999, REEVES et al. 2002). Their relatively coastal habits have made them vulnerable to whaling activities, especially after 1900, when modern techniques were implemented (FINDLAY 2001). Currently the humpback whale is classified as "least concern" by the International Union for Conservation of Nature, since

ZOOLOGIA 33(2): e20150133 | DOI: 10.1590/S1984-4689zool-20150133 | May 6, 2016



populations have shown clear signs of recovery following the cessation of whaling activities (BARLOW & CLAPHAM 1997, STEVICK et al. 2003, ANGLISS & OUTLAW 2005, CALAMBOKIDIS et al. 2008, ZERBINI et al. 2010, 2011, WARD et al. 2011). Nevertheless, continuous monitoring is needed to provide updated information for the development of management plans, and to evaluate the potential effects of other anthropogenic activities known to influence the recovery of this species, e.g., ship strikes, incidental catches in fishing gear, and oil and gas extraction (Rocha-CAMPOS & CÀMARA 2011, MARTINS et al. 2013).

The social organization of the humpback whale is characterized by small, unstable, but well defined groups, either on feeding or breeding grounds (CLAPHAM 2009). These groups are the target in line transect surveys, and for this reason, robust estimates of abundance depend on accurate counts, or estimation, of group sizes (BARLOW et al. 1998, GERRODETTE et al. 2002).

Efforts to assess errors in the estimation of cetacean group sizes from ships have been made only for small cetaceans (CLARK 1984, Scott et al. 1985, Gerrodette & Perrin 1991, Gilpatrick Jr 1993, Barlow et al. 2001, GERRODETTE et al. 2002). The primary difficulty in such assessments is to obtain reliable data on the true size of groups (GERRODETTE & PERRIN 1991). Aiming to develop correction factors, BARLOW et al. (1998) ascertained the accuracy and precision of estimates of oceanic dolphin group sizes from a ship, by comparing observers' estimates with aerial photographs of the same groups. They found an overall negative bias of 7% in shipboard estimates of mean group size. They also noted that a less expensive method than using helicopters would be preferable for correcting mean group size. Similarly, Scott et al. (1985) calculated the relative error of observers estimating dolphin group sizes from boats, estimating not just the error, but the variance among estimates of different observers. Their results demonstrated that the interpretation of observer's estimates is problematic, and can be highly variable, with most observers tending to underestimate the size of large groups by 10-30%. Since groups of large whales usually have fewer, but larger individuals that swim relatively slower than dolphins, estimation of large whale group sizes may be affected by slightly different issues, but field observation conditions are likely to be similar.

Therefore, it is expected that estimates of group size will vary according to the observation platform and conditions. To obtain the most robust estimates, it is important to develop an understanding of such variation, and how it can be accounted for in correction factors. However, little is known about observer errors in group size estimation for large cetaceans. In this study, an experiment was carried out to assess the magnitude of observer errors in estimating humpback whale group sizes in shipboard line transect surveys.

Data were obtained in August/September 2012 during a PMBS (Whales Satellite Monitoring Project) ship survey designed to deploy satellite transmitters and to estimate humpback whale abundance in their wintering grounds, off the Brazilian coast. This period corresponds to the usual peak of abundance for the species in the area (MARTINS et al. 2001). The survey covered the continental shelf, from an approximate depth of 10 m near the shore to the 500 m isobath, between the coasts of Salvador (~13°S), state of Bahia, and Cabo Frio (~23°S), state of Rio de Janeiro, and included the Abrolhos Bank (~19°S), an enlargement of the shelf where about 80% of the population is found every year (ANDRIOLO et al. 2010). The observation platform was the oceanographic research vessel N/Pq Atlântico Sul (Universidade Federal do Rio Grande) searching at a constant speed of about nine knots.

Humpback whales were continuously searched from 5h30min to 17h00min. The research team consisted of nine trained observers in total. Three primary observers (POs), one tracker (T), one data recorder, and resting positions (2 hours minimum) were swapped every 30 minutes. The PO positions were port, center and starboard, all located on the flying deck at a 9.5 m high platform. The primary observer in the central position was responsible for searching animals over the trackline and between 10° of each side of it, while lateral observers searched between 10° of the opposite side and 90° of its own side. This overlap of detection fields near and over the trackline was adopted to minimize chances of violating the g(0) = 1 assumption, which is essential to conventional distance sampling (BUCKLAND et al. 2001). On the other hand, the T was placed at a 12.6 m high observation platform, located at the crow's nest. Each observer was equipped with a reticuled binocular 7x50 Fujinon, an angle board for bearing reading and a radio communicator.

Data relevant for estimating abundance were collected by the POs and recorded on Wincruz software (written by R. Holland, SWFSC, NOAA, USA) by the data recorder. Detections were made using binoculars and the naked eye. The reticules between the sighting and the horizon, and radial angles between the sighting and the trackline were collected right after each detection. Radial distance r was obtained as described by (LERCZAK & HOBBS 1998) and the perpendicular distance was calculated as $x = r \cdot sin(\theta)$, where θ is the radial angle of the group relative to the ship's direction. Group size estimates were first collected by POs at the moment of detection and verification was usually made when the groups became closer to the ship, which travelled along the trackline. Therefore, for some of the sighted groups, there is a first PO estimate (PO1) and a last PO estimate (PO2). The PO2 is considered as the best estimate made by the PO, since it was made when the groups were closer to the ship and for a longer period of time. It was used to either confirm or to correct the first estimate. In high density areas, where there could be multiple detections, off-effort observers were placed in the primary observer's platform to assist in tracking detected groups and to avoid double counting.

Immediately after a detection was reported by a PO, the data recorder relayed the information to the T, except for group size. This observer was then responsible for tracking the group until it passed abeam, to obtain and record an independent estimate of group size. New relative positions, sea conditions, as measured by the Beaufort scale, and a measure of confidence



(high or low) that the T was tracking the same group as the PO, were also recorded. The T was only alerted to a new group when the current tracking had finished. To ensure independence between the estimates made by the POs and T, a different radio channel was used for communication between the latter and the data recorder. Because of its higher observation platform, a greater area of sea in view (1.6 km greater "horizon range" over POs), and the dedicated effort towards counting individuals in a group, it was assumed that the T provided more accurate estimates of group size.

In order to compare group size estimates made by the T and the POs, groups were considered in the analysis only if they met the following criteria: (1) groups with at least one re-sighting (observed at least twice) made by the T, and (2) with high confidence of being the correct group identification. Groups meeting these criteria were assumed to provide accurate information and three different ratios were calculated:

- $R1_i = T_i/PO2_i$ = ratio between the tracker's estimate and the last primary observer's estimate for each group i;
- $R2_i = T_i/PO1_i$ = ratio between the tracker's estimate and the first primary observer's estimate for each group i;
- $R3_i = PO2_i/PO1_i$ = ratio between the last and the first primary observer's estimate for each group i (PO's self-correction).

Due to the non-normal distribution on the data, the three ratio data sets were compared with the Wilcoxon pairwise test ($\alpha = 0.05$). The same test was used to compare group size estimates by T, PO1 and PO2. In order to investigate the potential of correction that a T approach as adopted in this study may have on abundance estimation, a correction factor CF was calculated taking the ratio between the mean of T group sizes and the mean of PO2 group sizes. A bootstrap coefficient of variation for the CF was calculated trough 10,000 iterations, with groups as resampling units. All analysis were performed using software R (R CORE TEAM 2013).

The independent observer recorded 136 groups, only 39 of which met the criteria for high confidence on the correct identification and at least one re-sighting made by the T. The most frequent value for R1 (ratio of the T estimate over the last/ best estimate of the PO) was 1 (43.6%), and group size estimates made by the T were lower in 30.8% and higher in 25.6% of cases, than the PO. Results of Wilcoxon pairwise test comparing group size estimates (T, PO1 and PO2) and ratios (R1, R2 and R3) are presented in Table 1. The CF found was 0.99 (bootstrap CV = 10.0%, bootstrap 95% CI = 0.92-1.21).

In the present work group sizes collected by the T did not differ from those collected by the POs in their last estimates (PO2), which means that both provided similar data. R2 and R3 also did not differ because the T corrected the PO1 in the same way that the PO self-corrects in the final estimate. This suggests that, despite the greater platform height and increased effort in collecting group sizes data, T's corrections did not improve the final estimate. This is also supported by the CF of 1, which would not improve an abundance estimate, if applied.

Table 1. Wilcoxon pairwise test results comparing group size estimates (T, PO2 and PO1) and ratios (R1, R2 and R3). See text for descriptions. * Significant values ($\alpha = 0.05$).

	Mean	CV%	P value	
			PO2	PO1
Т	1.897	62.6	0.874	*0.004
PO2	1.923	71.9	-	*0.001
PO1	1.308	50.1	-	-
			R2	R3
R1	1.175	71.6	*0.001	0.127
R2	1.626	84.1	-	0.829
R3	1.603	70.3	-	-

The extension of observation efforts when off-effort observers were allocated to the primary platform in high density areas may have improved group size estimation, as they contributed to the quality of PO's self-corrections, helping to track and to confirm/correct the size of already detected groups. We recommend that the self-correction approach described here should be adopted as a standard procedure for line transect sampling studies when large whales are the target species.

Previous studies (CLARK 1984, GERRODETTE & PERRIN 1991, BARLOW et al. 1998, GERRODETTE et al. 2002) have demonstrated that estimates of cetacean group sizes may have high variance among different observers, even when made from the same ship. The main source of error in those cases were the particular characteristics of observer's estimations, based on previous experience. Limited sample size precluded evaluation of such individual features here. It is clear, however, that the variability of the resultant average group size estimates in line transect ship surveys for humpback whales are affected by more than only the natural variability in group sizes.

Although our findings support that a tracker position, as adopted in the present study, does not improve abundance estimation for humpback whales, they highlight the importance of primary observers' efforts in collecting the best group size data as possible. This may be extended to other large whale species at some level, according to the degree of similarity on diving and surface behavior they share with the present species (CROLL et al. 2001, DOUGLAS et al. 2008). Nevertheless, when ship surveys are carried out to estimate the abundance of large whales, allocating off-effort observers to help tracking already detected groups may be essential to collect reliable data on group sizes.

ACKNOWLEDGMENTS

The authors wish to thank the Universidade do Rio Grande (FURG) and the N/Pq Atlântico Sul crew for support during field work. Several researchers assisted on data collection, including Igor Morais, Franciele Castro, Jonatas Prado, Federico Sucunza, Marco Aurélio Crespo, Natália Mamede, Luiz Cláudio Alves, Daniela Godoy, Suzana Stutz and Ygor Geyer. This manuscript



was greatly improved thanks to the comments of Professor Philip Hammond and from an anonymous reviewer. Júlio Baumgarten and Luciano Dalla Rosa provided valuable comments on its earlier version. PMBS was sponsored by Shell Brasil. Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) supported GA Bortolotto with a scholarship during his master's degree at the Universidade Estadual de Santa Cruz, under the supervision of DD. Cetacean Society International granted GA Bortolotto during the development of this work. The authors of this paper are members of Instituto Aqualie. ANZ is a Research Biologist at Cascadia Research Collective. This study was conducted under permits issued by CNPq (grant #CMC 026/02-028/03) and the Brazilian Environmental Agency (IBAMA, permit #009/02/CMA/ IBAMA, process #02001.000085/02-27, ICMBio #11523-1).

LITERATURE CITED

- ANDRIOLO A, MARTINS CCA, ENGEL MH, PIZZORNO JL, MÁS-ROSA S, FREITAS AC, MORETE ME, KINAS PG (2006) The first aerial survey to estimate abundance of humpback whales (*Megaptera novaeangliae*) in the breeding ground off Brazil (Breeding Stock A). Journal of Cetacean Research and Management 8(3): 307-311.
- ANDRIOLO A, KINAS PG, ENGEL MH, MARTINS CCA, RUFINO AM (2010) Humpback whales within the Brazilian breeding ground: Distribution and population size estimate. **Endangered Species Research 11**: 233-243.
- ANGLISS RP, OUTLAW RB (2005) Alaska marine mammal stock assessments, 2005. U.S. Department of Commerce, NOAA Technical Memorandum NMFS-AFSC-161. Available online at: http://www.nmfs.noaa.gov/pr/pdfs/sars/ak2005.pdf [Accessed: 31/08/2015]
- BARLOW J, CLAPHAM PJ (1997) A new birth-interval approach to estimating demographic parameters of humpback whales. Ecology 78(2): 535-546.
- BARLOW J, GERRODETTE T, PERRYMAN W (1998) Calibrating group sizes estimates for cetaceans seen on ship surveys. National Marine Fisheries Service Center Administrative Report LJ-98-11. Available online at: https://swfsc.noaa.gov/uploadedfiles/ divisions/prd/programs/etp_cetacean_assessment/lj_02_08. pdf [Accessed: 31/08/2015]
- BARLOW J, GERRODETTE T, FORCADA J (2001) Factors affecting perpendicular sighting distances on shipboard line-transect surveys for cetaceans. Journal of Cetacean Research and Management 3(2): 201-212.
- BUCKLAND ST, ANDERSON DR, BURNHAM KP, LAAKE JF, BORCHERS DL, THOMAS L (2001) Introduction to distance sampling: Estimating abundance of biological populations. New York, Oxford University Press, 432p.
- CALAMBORIDIS J, BARLOW J (2004) Abundance of blue and humpback whales in the Eastern North Pacific estimated by capture-recapture and line-transect methods. **Marine Mammal Science 20**(1): 63-85.

- CALAMBOKIDIS J, FALCONE EA, QUINN TJ, BURDIN AM, CLAPHAM PJ, FORD JKB, GABRIELE CM, LEDUC R, MATTILA D, ROJAS-BRACHO L, STRALEY JM, TAYLOR BL, URBÁN J, WELLER D, WITTEVEEN BH, YAMAGUCHI M, BENDLIN A, CAMACHO D, FLYNN K, HAVRON A, HUGGINS J, MALONEY N (2008) SPLASH: Structure of populations, levels of abundance and status of humpback whales in the North Pacific. Olimpia, Cascadia Research, final report contract AB133F-03-RP-00078 prepared for U.S. Department of Commerce. Available online at: https://swfsc.noaa.gov/uploadedFiles/Divisions/ PRD/Projects/Research_Cruises/Hawaii_and_Alaska/SPLASH/ SPLASH-contract-Report-May08.pdf [Accessed 31/08/2015]
- CLAPHAM PJ (2009) Humpback whale, p. 173-196. In: PERRIN WF, WÜRSIG B, THEWISSEN JGM (Eds.) Encyclopedia of Marine Mammals. New York, Elsevier Academic Press, 1352p.
- CLAPHAM PJ, MEAD JG (1999) *Megaptera novaeangliae*. Mammalian Species 604: 1-9.
- CLARK WG (1984) Analysis of variance of photographic and visual estimates of dolphin school size. La Jolla, National Marine Fisheries Service Center Administrative, Report LJ-84-11C, 36p.
- CROLL DA, ACEVEDO-GUTIÉRREZ A, TERSHY BR, URBÁN-RAMÍREZ J (2001) The diving behavior of large whales: is dive duration shorter than predicted? **Comparative Biochemistry and Physiology Part A 129**: 797-809.
- Douglas AB, Calambokidis J, Raverty S, Jeffries SJ, Lambourn DM, Norman SA (2008) Incidence of ship strikes of large whales in Washington State. Journal of the Marine Biological Association of the United Kingdom 88(6): 1121-1132.
- FINDLAY KP (2001) A review of humpback whale catches by modern whaling operations in the Southern Hemisphere. Memoirs of the Queensland Museum 47(2): 411-420.
- GERRODETTE T, PERRIN C (1991) Calibration of shipboard estimates of dolphin school size from aerial photographs. La Jolla, National Marine Fisheries Service Center Administrative, Report LJ-91-36, 26p. Available online at: http://lajolla. noaa.gov/om/library/publications/AR/LaJolla/1991/LJ-91-36. pdf [Accessed: 29/06/2015]
- GERRODETTE T, PERRYMAN W, BARLOW J (2002) Calibrating group size estimates of dolphins in the Eastern tropical Pacific Ocean. La Jolla, Southwest Fisheries Science Center National Marine Fisheries Service, NOAA, Report LJ-02-08, 26p. Available online at: https://swfsc.noaa.gov/uploadedFiles/Divisions/PRD/Programs/ETP_Cetacean_Assessment/LJ_02_08. pdf [Accessed: 31/08/2015]
- GILPATRICK JR JW (1993) Method and precision in estimation of dolphin school size with vertical aerial photography. Fishery Bulletin 91(4): 641-648.
- HAMMOND PS (2010) Estimating the abundance of marine mammals, p. 42-67. In: BOYD IL, BOWEN WD, IVERSON SJ (Eds.)
 Marine Mammal Ecology and Conservation: A handbook of techniques. New York, Oxford University Press, 450p.
- JOHNSTON DW, FRIEDLANDER AS, READ AJ, NOWACEK DP (2012) Initial density estimates of humpback whales *Megaptera novaeangliae* in the inshore waters of the western Antarctic Peninsula



during the late autumn. Endangered Species Research 18(1): 63-71.

LERCZAK JA, HOBBS RC (1998) Calculating sighting distances from angular readings during shipboard, aerial, and shore-based marine mammal surveys. **Marine Mammal Science 14**(3): 590-598.

MARTINS CCA, ANDRIOLO A, ENGEL MH, KINAS PG, SAITO CH (2013) Identifying priority areas for humpback whale conservation at Eastern Brazilian Coast. Ocean & Coastal Management 75: 63-71.

- MARTINS CCA, MORETE ME, ENGEL MH, FREITAS AC, SECCHI ER, KINAS PG (2001) Aspects of habitat use patterns of humpback whales in the Abrolhos Bank, Brazil, breeding ground. **Memoirs of the Queensland Museum 47**(2): 563-570.
- R CORE TEAM (2013) R: A language and environment for statistical computing. Vienna, R Foundation for Statistical Computing. Available online at: http://wwwR-projectorg [Accessed: 9/12/2013]
- REEVES RR, STEWART BS, CLAPHAM PJ, POWELL JA (2002) Guide to marine mammals of the world. New York, Chanticleer Press, 527p.
- Rocha-Campos CC, Câmara IG (2011) Plano de ação nacional para conservação dos mamíferos aquáticos: grandes cetáceos e pinípedes. Versão III. Brasília, ICMBio, Diretoria de Conservação da Biodiversidade, Coordenação Geral de Espécies Ameaçadas, Série Espécies Ameaçadas 14, ISBN: 978-85-61842-16-1, 156p.
- Scott MD, Perryman W, Clark WG (1985) The use of aerial photographs for estimating school sizes of cetaceans. Inter-American Tropical Tuna Commission Bulletin 18(5) 381-419.
- SECCHI ER, DALLA ROSA L, KINAS PG, SANTOS MCO, ZERBINI AN, BASSOI M, MORENO IB (2011) Encounter rates and abundance of humpback whales in Gerlache and Bransfield Straits, Antarctic Peninsula. Journal of Cetacean Research and Management 3(Special Issue): 107-111.
- STEVICK PT, ALLEN J, CLAPHAM PJ, FRIDAY N, KATONA SK, LARSEN F, LIEN J, MATTILA DK, PALSBØLL PJ, SIGURJÓNSSON J, SMITH TD, ØIEN N, HAMMOND PS (2003) North Atlantic humpback whale abun-

dance and rate of increase four decades after protection from whaling. **Marine Ecology Progress Series 258**: 263-273.

- THOMAS L, BUCKLAND ST, BURNHAM KP, ANDERSON DR, LAAKE JL, BORCH-ERS DL, STRINDBERG S (2002) Distance sampling. Encyclopedia of Environmetrics 1: 544-552.
- WARD E, ZERBINI AN, KINAS PG, ENGEL MH, ANDRIOLO A (2011) Estimates of population growth rates of humpback whales (*Megaptera novaeangliae*) in the wintering grounds off the coast of Brazil (Breeding Stock A). Journal of Cetacean Research and Management 3(Special Issue): 145-149.
- ZERBINI AN, ANDRIOLO A, ROCHA JM, SIMÕES-LOPES PC, SICILIANO S, PIZZORNO JL, WAITE JM, DEMASTER DP, VANBLARICOM GR (2004) Winter distribution and abundance of humpback whales (*Megaptera novaeangliae*) off Northeastern Brazil. Journal of Cetacean Research and Management 6(1): 101-107.
- ZERBINI AN, CLAPHAM PJ, WADE PR (2010) Assessing plausible rates of population growth in humpback whales from life-history data. Marine Biology 157(6): 1225-1236.
- ZERBINI AN, WARD E, KINAS PG, ENGEL MH, ANDRIOLO A (2011) A Bayesian assessment of the conservation status of humpback whales (*Megaptera novaeangliae*) in the Western South Atlantic Ocean (Breeding stock A). Journal of Cetacean Research and Management 3(Special Issue): 131-144.

Submitted: 3 September 2015 Received in revised form: 8 December 2015 Accepted: 28 December 2015 Editorial responsibility: Paulo da Cunha Lana

Author Contributions: AA, ANZ, DD and GAB designed the experiment; AA, DD and GAB conducted the experiment; DD and GAB analyzed the data; AA, ANZ, DD and GAB wrote the paper.

Competing Interests: The authors have declared that no competing interests exist.