

Comparative seabird diving physiology: first measures of haematological parameters and oxygen stores in three New Zealand Procellariiformes

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ABSTRACT: Within breath-hold diving endotherms, procellariiform seabirds present an intriguing anomaly as they regularly dive to depths not predicted by allometric models. How this is achieved is not known as even basic measures of physiological diving capacity have not been undertaken in this group. To remedy this we combined time depth recorder (TDR) measurements of dive behaviour with haematology and oxygen store estimates for 3 procellariiform species (common diving petrels *Pelecanoides urinatrix urinatrix*; grey-faced petrels *Pterodroma macroptera gouldi*; and sooty shearwaters *Puffinus griseus*) during their incubation phase. Among species, we found distinct differences in dive depth (average and maximal), dive duration and dives h⁻¹, with sooty shearwaters diving deeper and for longer than grey-faced petrels and common diving petrels. Conversely, common diving petrels dove much more frequently, albeit to shallow depths, whereas grey-faced petrels rarely dived whatsoever. Such differences in dive behaviour were reflected in haematological parameters, with sooty shearwaters having higher red blood cell counts and haematocrit (Hct) values compared to common diving and grey-faced petrels; whereas common diving petrels had significantly lower Hct but possessed higher haemoglobin concentrations per cell and greater respiratory oxygen stores than both sooty shearwaters and grey-faced petrels. Such results provide the first insights into the physiological traits underpinning procellariiform dive behaviour, and confirm the trend for deep-diving seabirds to have proportionally lower blood and respiratory oxygen stores than shallow divers.

KEY WORDS: Petrel · Shearwater · Physiology · Time depth recorder · TDR · Allometry

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INTRODUCTION

Breath-hold diving in endotherms is a complex interplay between competing ecological, behavioural and physiological exigencies (Halsey et al. 2006a). Understanding the contribution of each of these demands relies upon accurate characterisation of dive behaviour, most notably dive depth (average and maximal) and duration (Halsey et al. 2007). High-resolution insights into underwater behaviour

of diving endotherms are being realised via the use of time depth recorders (TDRs) which log multiple recordings over extended periods (Wilson & Vandenberg 2012). From this, precise estimates of dive duration and average/maximal dive depth can be ascertained, and, by matching this information to physiological data, a mechanistic insight into the physiological dive capacities of a species is afforded.

Physiologically, there is a general trend for larger endothermic species to attain greater depths (Schreer

& Kovacs 1997). This is because oxygen usage and oxygen stores in the blood, muscle myoglobin and respiratory system scale with mass; thus larger animals use less oxygen and have more oxygen reserves to draw upon when underwater (Halsey et al. 2006b). However, one group of animals that deviate from this relationship are procellariiform seabirds, which can regularly attain depths greater than their mass would predict (Halsey & Butler 2006). The exact physiological mechanisms underpinning this result are unknown as no information exists on procellariiform dive physiology. Moreover, some species of Procellariiformes undertake annual trans-equatorial migrations (e.g. sooty shearwater *Puffinus griseus*) thus placing them in an adaptive valley between being a migratory flight or dive specialist (Shaffer et al. 2006, Elliott et al. 2013). Strategies used to support this dual mobility cannot be understood in the absence of basic physiological metrics of dive capacity.

We undertook a comparative study of dive behaviour and physiology in 3 procellariiform species that breed in New Zealand waters. The species studied were common diving petrels *Pelecanoides urinatrix urinatrix*, grey-faced petrels *Pterodroma macroptera gouldi* and sooty shearwaters *Puffinus griseus*, which exhibit large differences in mass (147, 503 and 848 g, respectively). Moreover, whilst these species are known to have differences in maximal dive depth (Taylor 2008), temporal measures of average dive depth, dive duration and dive frequency are yet to be determined. We attempted to match dive behaviour measurements to measures of oxygen stores (blood and respiratory) and haematology to allow a mechanistic understanding of dive capacity in these species. Finally, for many bird species oxygen carrying capacity changes intraspecifically as a result of ontogeny or migration (Minias et al. 2013), thus we concentrated on birds during their incubation phase in order to standardise comparisons among species. Our specific questions were: Do differences in dive behaviour (average depth and duration) exist among 3 species of Procellariiformes? If so, is this information reflected in key physiological parameters of dive capacity (oxygen stores and haematology)?

MATERIALS AND METHODS

Study location and specimens

The procellariiform species used in this study were from breeding colonies within the Auckland region of New Zealand (Fig. 1). Characterisation of diving behaviour and haematological samples of common diving petrel were taken from a colony located on Burgess Island (35° 54' 10" S, 175° 07' 05" E) Moko-hinau Island group; whereas sooty shearwaters were from a colony on Kauwahaia Island, Bethells beach (36° 53' 13" S, 174° 26' 12" E). For grey-faced petrels, dive behaviour was characterised for adults located on Ihumoana Island (located 400 m south of Kauwahaia Island, Bethells Beach) and haematological samples were taken from adults on Burgess Island. All birds used were adults in the incubation phase of the breeding cycle. Species weights, TDR deployment duration and the number of replicates for quantifying dive behaviour and physiological parameters in this study are summarised in Table 1. Morphological differences exist among these species, with the common diving petrel noted for its shorter wing (reminiscent of the Alcidae) compared to the longer wings of grey-

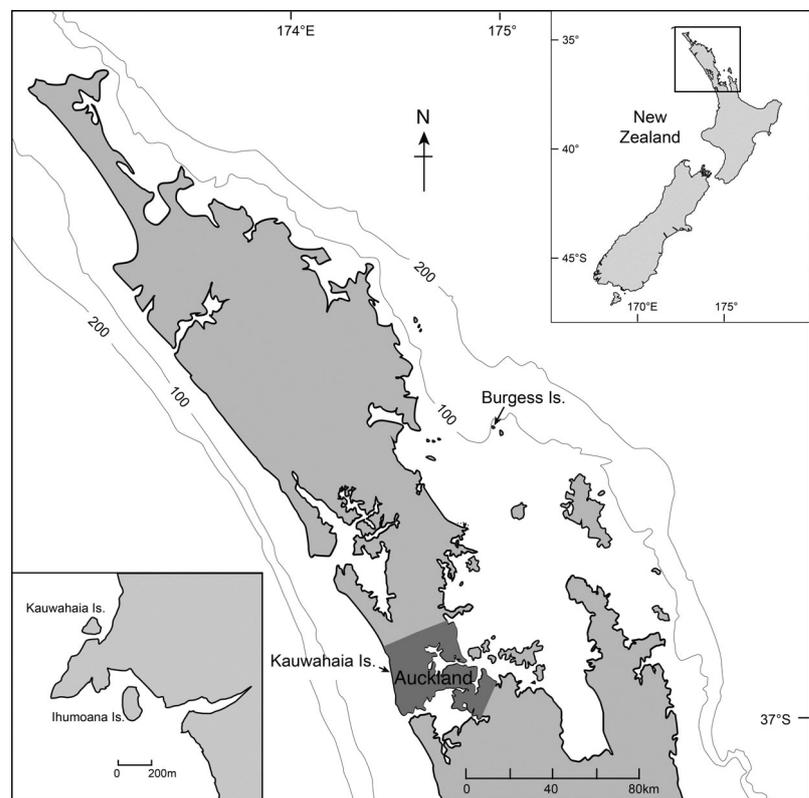


Fig. 1. Location of Burgess Island, Ihumoana Island and Kauwahaia Island study sites in northern New Zealand

Table 1. Weight (mean \pm SE), time depth recorder (TDR) deployment data and number of replicates taken for characterising dive behaviour and haematology/oxygen stores in incubating adult common diving petrels *Pelecanoides urinatrix urinatrix*, grey-faced petrels *Pterodroma macroptera gouldii* and sooty shearwaters *Puffinus griseus*

Species	Weight (g)	TDR deployments (n)	TDR deployment duration (d)	Haematology (n)
Common diving petrel	146.9 \pm 4.9	15	1–2	21
Grey faced petrel	502.9 \pm 16.9	10	30–90	22
Sooty shearwater	847.50 \pm 37.6	10	30–60	10

faced petrels and sooty shearwaters (Warham 1977). Additionally, dietary differences exist between common diving petrels (crustaceans), grey-faced petrels (fish, cephalopods and crustaceans) and sooty shearwaters (euphasiids, cephalopods and fish) (Warham 1990).

Due to natural offsets in breeding phases among species, haematological analyses were undertaken in September 2013 for common diving and grey-faced petrels, whereas sooty shearwaters were sampled in December of that year.

Dividing behaviour

Measures of dive behaviour were obtained using LAT1400 (11 \times 35 mm, 4.8 g) and LAT1500 (8 \times 32 mm, 3.2 g) TDRs (Lotek Wireless). Tags deployed on common diving petrels were applied with waterproof tape to small clusters of central back feathers. The tape was further sealed with superglue to stop tape edges peeling back. These tags were deployed for only 1 to 2 d as the species changes over at the nest daily every 2 d. Tags weighed 2 to 4% of total body weight (~150 g). Tags on the larger species (grey-faced petrel and sooty shearwater) were attached in one of 3 ways: glued and cable tied to 2 colour leg bands; mounted in a custom-made plastic housing attached to the leg by a rubber strap; or taped and glued to the central bag feathers (as per the common diving petrel). These 3 g and 6 g tags weighed <1% of total body weight (on 550 and 800 g birds) and were deployed for up to 3 mo. All birds were returned to the nest after processing and recaptured either on the surface or in their burrow.

Diving data were analysed using Multitrace (Jensen Software Systems) to produce summary statistics for each dive: mean duration (s), mean and maximum depth (m) and mean dives h^{-1} . Sensor (zero-off-set) and drift in the depth values for each tag was corrected manually within Multitrace. The error threshold of the TDRs was 1 m, thus only dives >1 m were used for analysis.

Haematology and oxygen stores

We employed conventional clinical haematology techniques as laid out in Lewis et al. (2006). Blood samples were obtained from the tarsal vein of incubating birds using a 27 gauge needle and 1 ml syringe. Blood samples (approx. 100 to 250 μ l) were collected within 4 min of handling the bird and were placed in lithium heparin-lined tubes to prevent coagulation of the blood. Following this, blood samples were immediately analysed for haematocrit (Hct) and red blood cell count (RBCC) with a portion frozen at $-20^{\circ}C$ for later analysis of haemoglobin concentration ([Hb]) as in accordance with the methods of Elliott et al. (2010). Hct was analysed in triplicate using microhaematocrit capillary tubes which were spun for 3 min. Cell counts were made using an improved Neubauer haemocytometer for 6 subsamples of red blood cells diluted in isotonic saline. Haemoglobin content of thawed samples was measured spectrophotometrically at 540 nm using Drabkins reagent (Sigma-Aldrich), with no turbidity observed in samples. However, upon thawing, 11 common diving petrel samples and one sooty shearwater sample had evidence of coagulation and thus were not analysed for haemoglobin. Mean cell haemoglobin concentration (MCHC) was calculated from [Hb]:Hct, mean cell volume (MCV) from Hct:RBCC, and mean cell haemoglobin (MCH) from [Hb]:RBCC. Oxygen-carrying capacity of whole blood was estimated assuming 1 g bird Hb binds 1.2 ml O_2 as determined by Viscor et al. (1984) for the black-headed gull *Larus ridibundus*.

Due to prohibitive animal ethics surrounding invasive sampling of these nationally important species we are unable to report muscle oxygen stores. However, Elliott et al. (2010) demonstrated that blood and respiratory oxygen stores form 90 to 95% of oxygen stores in seabirds that dive and fly; thus we are able to report the bulk of oxygen stores for these species.

Respiratory oxygen stores were calculated for each bird using:

$$\text{Respiratory oxygen stores} = 0.9 \times 0.176 \times 0.1608M^{0.91} \quad (1)$$

where M is mass (kg) and which utilises the allometric relationship determined by Lasiewski & Calder (1971) and assumes that 17.6% of lung volume was O_2 and that 90% of lung O_2 stores were usable (Elliott et al. 2010).

Blood oxygen stores were estimated as:

$$\text{Blood oxygen stores} = 0.96 \times 1.2 \times (0.95 \times 0.3 + 0.7 \times 0.7) Hb \times 0.123M \quad (2)$$

This incorporates measures of haemoglobin (Hb , g dl^{-1}) and mass. We assumed that blood volume was 12.3% of body mass in g (Croll et al. 1992), that each g of haemoglobin pigment binds 1.2 ml O_2 , 30% of the blood was arterial at 95% O_2 saturation and 70% of blood was venous at 70% O_2 saturation and 96% of O_2 was usable during a dive (Croll et al. 1992).

Statistical analysis

Comparisons among species of maximum dive depth, haematological parameters and oxygen stores were made via 1-way ANOVA. Data were checked for normality and homogeneity of variance via Shapiro-Wilk and Levene's tests, respectively. Values of maximum depth, MCH, MCHC and useable blood oxygen stores were log transformed in order to meet the assumptions of normality for ANOVA. Differences among species were identified using Tukey's post hoc tests.

Kruskal-Wallis nonparametric methods were performed for mean depth, dives h^{-1} , and dive duration as transforming data did not result in assumptions of ANOVA being met. Dunn's post hoc tests were used when nonparametric tests were significant. All statistical analyses were undertaken using Sigmaplot soft-

ware (SYSTAT Software) and $\alpha = 0.05$. Unless otherwise stated all means are given as \pm SE.

RESULTS

Diving behaviour

Contrasting dive behaviours were evident among species, with sooty shearwaters being the more accomplished deep divers (Fig. 2) compared to common diving petrels and grey-faced petrels. Moreover, sooty shearwaters attained an average depth of 6.93 ± 0.94 m compared with 2.93 ± 0.27 m and 1.64 ± 0.18 m for common diving petrels and grey-faced petrels, respectively (Kruskal-Wallis $H = 26.587$, $df = 2$, $p < 0.001$, Fig. 3A). Such patterns were also evident in mean maximum dive depth, with sooty shearwater adults reaching an average maximal depth of 39.16 ± 2.88 m compared to 10.75 ± 0.66 m and 2.41 ± 0.30 m for common diving petrels and grey-faced petrels (1-way ANOVA $F_{2,35} = 253.02$, $p < 0.001$, Fig. 3B). Accordingly, sooty shearwater dives were significantly longer, taking an average of 39.73 ± 2.60 s compared to 19.31 ± 1.38 s and 4.15 ± 0.60 s for common diving petrels and grey-faced petrels, respectively (Kruskal-Wallis $H = 32.20$, $df = 2$, $p < 0.001$, Fig. 3C). However, in terms of diving frequency, common diving petrels spend a proportionally longer amount of time underwater (at a depth greater than 1 m) with average dives h^{-1} being 76.29 ± 5.02 compared to 0.005 ± 0.001 and 0.22 ± 0.025 for grey-faced petrels and sooty shearwaters (Kruskal-Wallis $H = 32.93$, $df = 2$, $p < 0.001$, Fig. 3D). Of the dives recorded, 80% of dives greater than 1 m occurred during daylight hours.

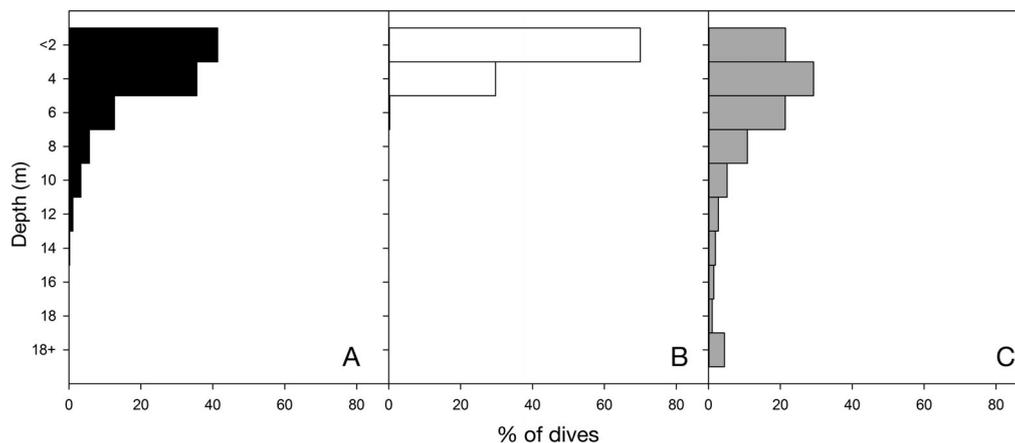


Fig. 2. Percentage of dives by depth for foraging trips of incubating adult (A) common diving petrels *Pelecanoides urinatrix*, (B) grey-faced petrels *Pterodroma macroptera gouldi* and (C) sooty shearwaters *Puffinus griseus*

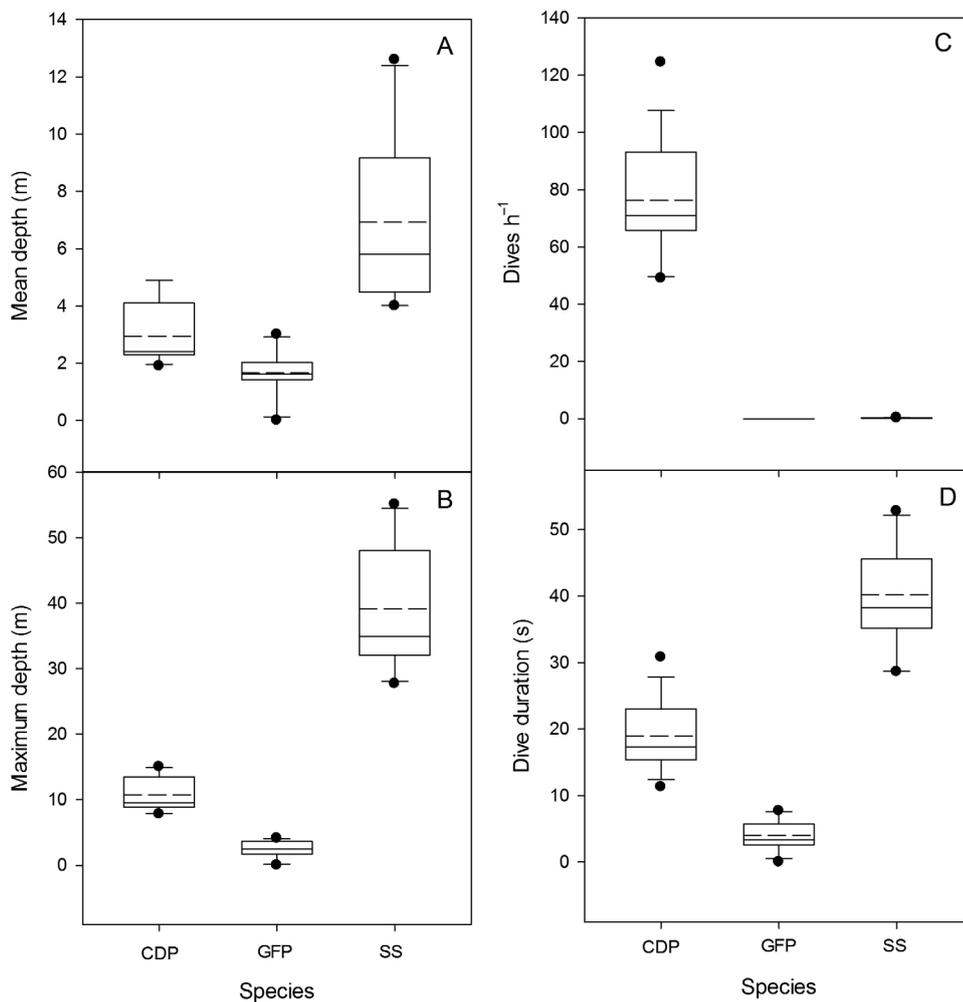


Fig. 3. Dive behaviour of incubating adult common diving petrels *Pelecanoides urinatrix urinatrix* (CDP), grey-faced petrels *Pterodroma macroptera gouldi* (GFP) and sooty shearwaters *Puffinus griseus* (SS). Note: mean values shown by dashed line, median values by solid line, 25 and 75% percentiles by edges of box, and 10th and 90th percentiles by the error bars. Dots denote outliers

Haematology and oxygen stores

Fig. 4 provides a comparison of haematological variables among species with incubating sooty shearwaters possessing higher red blood cell counts than either common diving petrels or grey-faced petrels (1-way ANOVA $F_{2,50} = 3.767$, $p = 0.0306$, Fig. 4A). Additionally, Hct values significantly differed among all species, with sooty shearwaters having higher Hct values than grey-faced petrels, which in turn were higher than common diving petrels (1-way ANOVA $F_{2,50} = 10.856$, $p \leq 0.001$, Fig. 4B). Although haemoglobin values differed among species, these were not statistically significant (1-way ANOVA $F_{2,39} = 1.295$, $p = 0.286$, Fig. 4C).

Of the haematological parameters derived from the above variables, MCHC was highest in common diving petrels followed by grey-faced petrels and then sooty shearwaters (1-way ANOVA $F_{2,39} = 11.925$,

$p \leq 0.001$, Fig. 4D). Despite differences in MCH values among species these were highly variable, thus no statistically significant differences were found (Kruskal-Wallis $H_2 = 5.24$, $p \geq 0.05$, Fig. 4E) and a similar lack of significance was observed in values of red blood cell volume (1-way ANOVA $F_{2,50} = 0.06286$, $p = 0.93915$, Fig. 4F).

Mean oxygen carrying capacity of blood did not differ among species (1-way ANOVA $F_{2,39} = 1.435$, $p = 0.250$, Fig. 5A). However, whilst estimated blood volume was significantly higher in sooty shearwaters (1-way ANOVA $F_{2,39} = 270.24$, $p = 0.0001$, Fig. 5B) differences in estimates of usable blood oxygen stores were not statistically significant among species (1-way ANOVA $F_{2,39} = 3.237$, $p > 0.05$, Fig. 5C). Nonetheless, common diving petrels had higher estimated respiratory stores than grey-faced petrels, which in turn were higher than sooty shearwaters (1-way ANOVA $F_{2,39} = 975.1$, $p = 0.0001$, Fig. 5D).

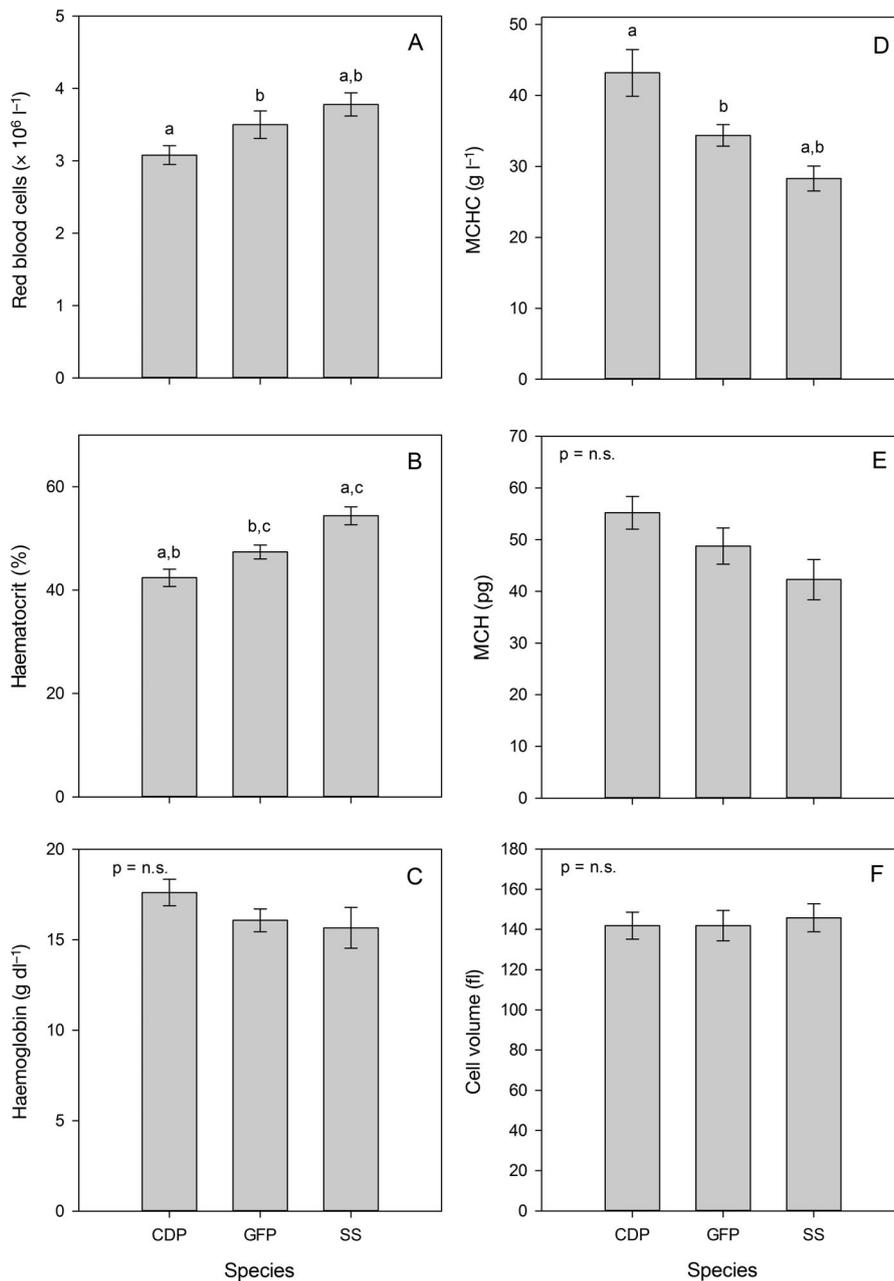


Fig. 4. Haematological parameters (mean \pm SE) of incubating adult common diving petrels *Pelecanoides urinatrix urinatrix* (CDP), grey-faced petrels *Pterodroma macroptera gouldi* (GFP) and sooty shearwaters *Puffinus griseus* (SS). Similar superscripts denote statistically significant differences between species ($p < 0.05$); n.s.: not significant. MCHC: mean cell haemoglobin concentration; MCH: mean cell haemoglobin

DISCUSSION

We sought to test whether differences in dive behaviour existed among 3 species of New Zealand Procellariiformes and whether such differences were reflected in key physiological parameters known to support dive capacity. From our data it is apparent that grey-faced petrels infrequently dive to depths greater than 1 m, with some birds making as little as 7 dives over an 80 d period. An earlier study by Taylor (2008) recorded grey-faced petrels reaching an average maximal depth of 4.7 m (com-

pared to 2.4 in our study) with a range of 0.7 to 23.6 m (cf. 0.0 to 4.1 in this study). Reasons for the discrepancies between our dataset and that of Taylor (2008) could be a result of increased resolution from methodological advances. Taylor (2008) deployed capillary tube maximum depth gauges which do not offer the fine-scale temporal resolution offered by the electronic depth recorders used in this study (Elliott & Gaston 2009, Wilson & Vandenberg 2012). Alternatively, inter-annual differences in prey availability between these 2 studies could be a factor as dive depths of birds in Taylor's (2008)

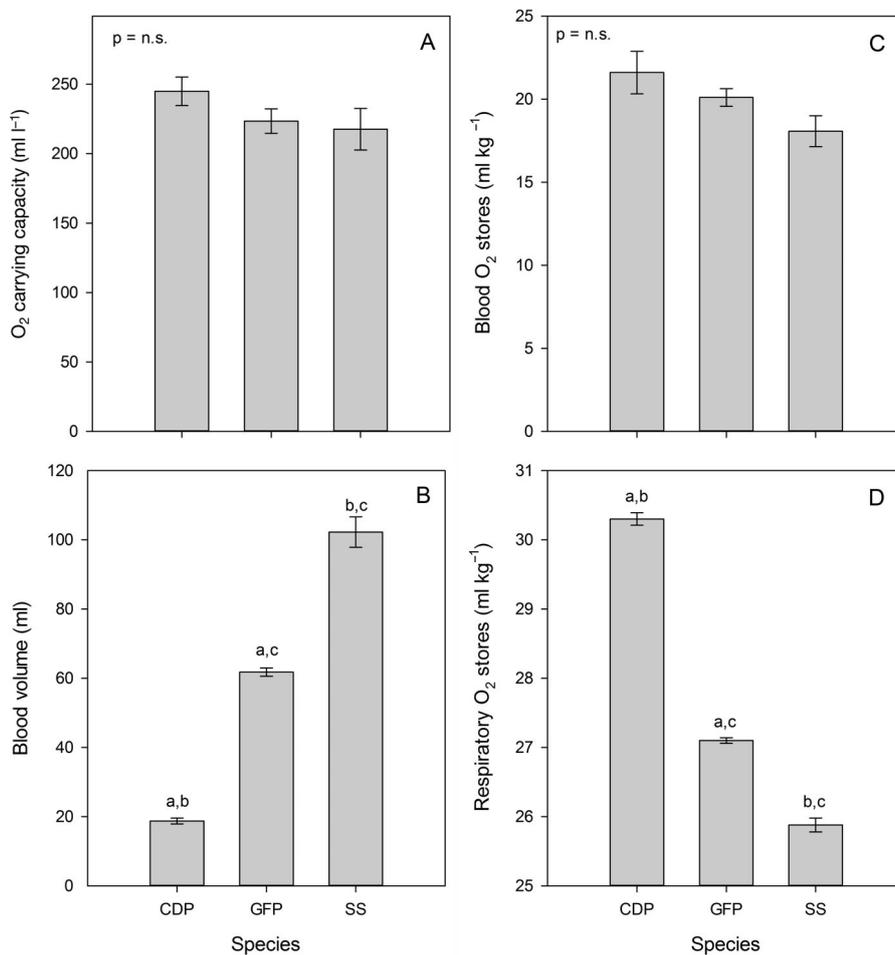


Fig. 5. Oxygen storage (respiratory and blood) parameters (mean \pm SE) of incubating adult common diving petrels *Pelecanoides urinatrix urinatrix*, grey-faced petrels *Pterodroma macroptera gouldi* and sooty shearwaters *Puffinus griseus*. Note: differing y-axis scale. Similar superscripts denote statistically significant differences between species ($p < 0.05$); n.s.: not significant

study were recorded in the late 1990s. Nonetheless, from our data, diving below 1 m does not appear to be a significant foraging behaviour for grey-faced petrels, with incubating adults undertaking comparatively shallow dives very infrequently.

Sooty shearwaters foraged at greater depth in terms of both average and maximal depths. Our data slightly differs compared to previous TDR accounts of dive performance for this species, with Shaffer et al. (2006) recording average depths of 10.7 m (northern hemisphere) and 14.2 m (southern hemisphere) compared to our 6.93 m. However, in order to sample over a complete annual cycle, the tags used by Shaffer et al. (2006) recorded pressure once every 432 s (unlike once every second in our study). This meant that birds could have been in the early, mid or deepest stage of the dive when the sample was taken. Moreover, the deepest we recorded a sooty shearwater diving was 55.1 m, somewhat less than the 68.2 m of Shaffer et al. (2006) and 67 m of Weimerskirch & Sagar (1996) recorded previously using maximal depth gauges. Thus far, the deepest dive re-

corded by this species sits at 93 m (Taylor 2008), revealing that this species has impressive dive capabilities.

Dive behaviour of common diving petrels mirrors recent work by Navarro et al. (2014) on conspecifics at South Georgia. Like us, Navarro et al. (2014) attached TDRs to common diving petrels and estimated mean and maximal dive depth to be 2.1 and 10.4 m, respectively (cf. 2.9 and 10.75 m in our study). Interestingly, dive duration was longer in our temperate populations compared to the higher-latitude South Atlantic populations (19.31 vs. 10.1 s, respectively) and may reflect the differing levels of productivity between temperate and sub-polar regions that requires adult birds to extend underwater time in less productive, lower-latitude waters. Using MDGs, Taylor (2008) recorded a mean maximal dive depth of 10.9 m, a value very similar to our study. However, maximum dive depth of this species varies among studies, with 22 m recorded by Taylor (2008) and 64 m by Bocher et al. (2000). Furthermore, Bocher et al. (2000) noted that maximal dive depths of common

diving petrels at the Kerguelen Archipelago varied through the year in relation to prey availability.

Diving abilities of air-breathing vertebrates are generally related to body mass, with larger animals having greater physiological capacity for deeper and longer-duration dives (Halsey et al. 2006b). To account for this, allometric relationships have been developed to calculate the maximal dive depth and duration for alcids and penguins (Schreer & Kovacs 1997, Watanuki & Burger 1999) and for birds and mammals as a whole (Halsey et al. 2006b). For our study species, maximum dive depths were more accurately predicted using Schreer & Kovacs (1997) allometric equation for penguins, giving values of 11 and 38 m for common diving petrels and sooty shearwater species, respectively (cf. 10.7 and 39.1 m in our data). Grey-faced petrels were predicted to dive to 26 m, depths which were not recorded in our study but closely resemble the 23 m recorded by Taylor (2008). Despite being phylogenetically more related to procellariiforms than penguins, allometric equations developed for alcids overestimated the maximal dive depth we observed for procellariiforms (Table 2). Reasons for this are difficult to discern, but may relate to differences in prey as animals of a similar mass are known to exhibit different maximum dive depths brought about by prey location in the water column, i.e. sperm vs. grey whales (Halsey et al. 2006a). Thus, while mass provides a useful way of predicting potential depth attained by a species, these must be matched with field data as variation in such estimates can be brought about by the ecology, behaviour and physiology of the species.

Haematology and oxygen stores

Compared to 36 other avian families studied by Hawkey et al. (1991), the procellariiform species in our study had higher than average red blood cell counts (3.08 to 3.78 vs. $2.79 \times 10^6 \text{ l}^{-1}$) and lower than average cell volumes (141.89 to 145.78 vs. 169 fl). Such a result suggests the metabolic rates of the species we studied are comparatively higher than the majority of avian species as an inverse correlation between metabolic rate and cell size has been identified for birds (Breuer et al. 1995). Reasons for this are thought to relate to the enhanced oxygen delivery that smaller volume red blood cells offer due to greater cell surface area and reduced oxygen diffusion distances (Jones 1979).

The 3 species we studied were equivalent in terms of [Hb], RBCC and oxygen carrying capacity. How-

ever, the common diving petrel possessed lower cell numbers and Hct values, yet appears to compensate for this with higher MCHC values. Low Hct values are potentially disadvantageous in that the level of oxygen delivery can be reduced to exercising tissues (Jenni et al. 2006). However, reduced Hct also reduces blood viscosity, allowing an increase in blood flow rate which can offset any reductions in oxygen delivery (Breuer et al. 1995). Furthermore, low viscosity blood offers energetic efficiencies in that the blood is easier for the heart to circulate (Jenni et al. 2006). In contrast, the deeper-diving sooty shearwater possessed higher Hct and RBCC values. Deep-diving northern elephant seals *Mirounga angustirostris* demonstrate a similar strategy, maximising blood oxygen storage capacity via high Hct values at the expense of oxygen transport through the body (Hedrick et al. 1986). Whether sooty shearwaters have adapted the same strategy to support their comparatively deeper dive capability is unknown and deserves further investigation. Given that haemoglobin content is thought to be higher in birds that dive to depth (Croll et al. 1992), we might reasonably predict higher haemoglobin values in the deeper-diving sooty shearwater. However, we observed the opposite with lower (albeit statistically non-significant) [Hb] for this species. Thus it may be that sooty shearwaters offset such lower haemoglobin levels by increasing circulating red blood cells.

Body oxygen stores within diving endotherms comprise the circulatory and respiratory systems in addition to myoglobin within the musculature (Butler 2001). In the present study we could not quantify muscle myoglobin, thus cannot go on to calculate theoretical aerobic dive limits. However, compared to dive specialists such as penguins, muscle oxygen stores are low in seabirds that dive and fly (Elliott et al. 2010). This may derive from space constraints within muscle cells as a balance is sought between myoglobin oxygen stores and the mitochondrial densities necessary for migration (Croll et al. 1992). As a result the blood and respiratory system comprise 90 to 95 % of total oxygen stores in seabirds that both fly and dive (Elliott et al. 2010, Yamamoto et al. 2011), thus we were still able to characterise the majority of body oxygen stores of our study species. Comparing our estimates with alcids reveals that our study species had equivalent respiratory oxygen stores but slightly lower blood oxygen stores (Table 3). The deeper-diving sooty shearwater had lower estimated respiratory oxygen stores compared to common diving petrels and grey-faced petrels. Elliott et al. (2010) found similar results within diving alcids, with the

Table 2. Dive depth (maximal and average) and duration (average) for common diving petrels *Pelecanoides urinatrix urinatrix*, grey-faced petrels *Pterodroma macroptera gouldii* and sooty shearwaters *Puffinus griseus* calculated using published allometric equations and compared to our observed values. M = mass (kg). See Table 4 for full species names

Parameter	Equation derived from	Allometric equation	Common diving petrel	Grey-faced petrel	Sooty shearwater	Source
Mean dive depth (m)	–	Observed	2.93	1.64	6.94	This study
	Birds	$10.5M^{0.398}$	4.89	7.99	9.83	Halsey et al. (2006b)
Max. dive depth (m)	–	Observed	10.75	2.41	39.16	This study
	Mammals and birds	$9.4M^{0.327}$	5.02	7.51	8.90	Halsey et al. (2006b)
	Penguins	$42.66M^{0.71}$	10.93	26.19	37.93	Schreer & Kovacs (1997)
	Alcids	$158.49M^{1.04}$	21.56	77.54	133.43	Schreer & Kovacs (1997)
Mean dive duration (s)	–	Observed	19	4	39	This study
	Mammals and birds	$21.2M^{0.368}$	10.47	16.46	19.95	Halsey et al. (2006b)

deep-diving thick billed murre *Uria lomvia* having lower estimated respiratory oxygen stores than the shallow-diving Cassin's auklet *Ptychoramphus oaleuticus* and ancient murrelet *Synthliboramphus antiquus*. This is thought to be a response to the increased buoyancy-related costs that larger respiratory oxygen stores would incur for species diving to depth.

Finally, although little evidence of diving capacity could be found, the grey-faced petrel appears intermediary on many of the blood parameters measured here (i.e. RBCC, [Hb], MCV and oxygen carrying capacity). Such a result presumably reflects the fact that this species still has to physiologically match flight-related costs, and also provides an elegant counterpoint to common diving petrels and sooty shearwaters, both of whom rely on dive foraging but with marked differences in frequency, depth and duration.

A synthesis of published procellariiform and alcid haematology data (mostly used to identify haematological baselines in pollutant or parasitic studies) has been matched to available dive depth data for breeding birds (Table 4). Based on these studies it is apparent that alcids dive deeper in terms of both maximal depth (although not statistically significant due to large variation) and average depth. Moreover, such differences are matched with a higher [Hb] in the alcids (Table 5). Given the lack of haematological comparisons in seabirds in general it is hard to interpret this result. Increased haemoglobin in Passeriformes is associated with increased meta-

bolism (Palomeque et al. 1980); thus, whether alcids possess a higher metabolism than the procellariiform species used in the present study is unknown but deserves further study.

Thus our results show that distinct differences in dive behaviour are reflected in haematological variables and estimated oxygen stores of our study species. The common diving petrel undertakes frequent shallow dives and, although having lower numbers of red blood cells, has a higher amount of haemoglobin per cell, thus offsetting any disadvantage from low cell numbers. Such a strategy could be to keep a low-viscosity blood circulating that is high in haemoglobin, thus allowing efficient oxygen uptake upon its frequent surfacing. Moreover, of the species we studied, respiratory oxygen stores are highest in common diving petrels, as buoyancy related costs are potentially of minor cost for this species. Conversely, the deeper-diving sooty shearwater has higher values of Hct and RBCC in addition to lower respiratory oxygen stores. Such a result suggests that the reduced haemoglobin levels species are offset via maximising oxygen-carrying units and a reduced

Table 3. Published values of body oxygen stores of diving and flying procellariiform and alcid species, with an emperor penguin *Aptenodytes forsteri* for comparison

Species	Useable oxygen stores (ml kg^{-1})			Source
	Respiratory	Blood	Muscle	
Common diving petrel	30.30	21.6	–	This study
Grey-faced petrel	27.10	20.1	–	This study
Sooty shearwater	25.88	18.07	–	This study
Cassin's auklet	30.1	26.9	3.6	Elliott et al. (2010)
Ancient murrelet	29.3	25.9	3.4	Elliott et al. (2010)
Thick-billed murre	25.1	23.5	2.8	Elliott et al. (2010)
Rhinoceros auklet	26.8	21.7	5.9	Yamamoto et al. (2011)
Emperor penguin	10.1	18.0	24.9	Kooyman & Ponganis (1998)

Table 4. Haematological parameters (all values are mean \pm SE [unless otherwise indicated]) matched with maximal dive values recorded for alcid and procellariiform species with varying dive behaviour. Note species within each order/family organised by increasing dive depth. RBCC: red blood cell count; Hct: haematocrit; [Hb]: haemoglobin concentration; MCHC: mean cell haemoglobin concentration; MCH: mean cell haemoglobin; TDR: time depth recorder; MDG: maximal depth gauge; (-): no data available

Species	Dive depth (m) Max	Mean	Observation method	Mass (g)	RBCC (10^6 l^{-1})	Hct (%)	[Hb] (g dl^{-1})	MCHC (g l^{-1})	MCH (pg)	Cell volume (fl)	Source
Procellariiformes											
Southern giant petrel <i>Macronectes giganteus</i> ^d				3200	-	43.5 \pm 3.5	14.64 \pm 2.6	33.8 \pm 3.8	-	-	Milsom et al. (1973) ^b
Wilson storm petrel <i>Oceanites oceanicus</i> ^e				40	4.0 \pm 0.03 ^c	49.3 \pm 0.8 ^c	17.0 \pm 0.4 ^c	34.7 \pm 1.1 ^c	43.1 \pm 2.0 ^c	124.6 \pm 4.6 ^c	Myrcha & Kostelecka-Myrcha (1980) ^b
Grey-faced petrel <i>Pterodroma macrotrema gouldi</i>	2.48 \pm 0.4	1.6 \pm 0.1	TDR	502.9 \pm 16.9	3.5 \pm 0.2	47.4 \pm 1.3	16.1 \pm 0.6	34.4 \pm 1.5	48.8 \pm 3.5	141.9 \pm 7.6	This study ^{a,b}
Cory's shearwaters <i>Calonectris diomedea</i>	9.8 \pm 2.4;	4.3 \pm 3.2	TDR	716	-	41.81 \pm 2.87 ^c	17.23 \pm 9.67 ^c	-	-	-	Paiva et al. (2009) ^a , Navarro et al. (2008) ^b
Common diving petrel <i>Pelecanoides urinatrix</i>	10.7 \pm 0.7	2.9 \pm 0.3	TDR	146.9 \pm 4.9	3.1 \pm 0.1	42.4 \pm 1.7	17.6 \pm 0.7	43.2 \pm 3.3	55.2 \pm 3.2	141.9 \pm 6.7	This study ^{a,b}
Sooty shearwater <i>Puffinus griseus</i>	39.2 \pm 2.9	6.9 \pm 0.9	TDR	847.5 \pm 37.6	3.8 \pm 0.2	54.4 \pm 1.7	15.7 \pm 1.1	28.3 \pm 1.8	42.38 \pm 3.9	145.8 \pm 7.0	This study ^{a,b}
Short-tailed shearwater <i>Puffinus tenuirostris</i>	58 \pm 11	not recorded	MDG	588.4 \pm 72.6 ^c	1.7	57 \pm 6.1 ^c	17.2 \pm 3.2 ^c	-	52.3 \pm 9.3 ^c	178.0 \pm 44.2	Weimerskirch & Cherel (1998) ^a , Arnold et al. (1999) ^b
Alcidae											
Cassin's auklet <i>Ptychoramphus aleuticus</i>	22.4 \pm 4.0 ^c	8.9 \pm 3.1 ^c	TDR	171 \pm 2	-	53.1 \pm 1	20.7 \pm 0.6	-	-	-	Elliott et al. (2010) ^{a,b}
Ancient murrelet <i>Synthliboramphus antiquus</i>	24.4 \pm 5.6 ^c	9.4 \pm 2.8 ^c	TDR	197 \pm 7	-	51 \pm 1	19.6 \pm 0.9	-	-	-	Elliott et al. (2010) ^{a,b}
Rhinoceros auklet <i>Cerorhinca monocerata</i>	60	not recorded	TDR	560	-	43.9 \pm 2.8	17.2 \pm 4.6	-	-	-	Kuroki et al. (2003) ^a , Yamamoto et al. (2011) ^b
Thick-billed murre <i>Uria lomvia</i>	171 \pm 11 ^c	18 \pm 19 ^c	TDR	980	-	52.8 \pm 2.3 ^c	18.0 \pm 1.8 ^c	-	-	-	Croll et al. (1992) ^{a,b} , Fort et al. (2013) ^a

^aDive data; ^bHaematology; ^cSD; ^dShallow surface diver (depth unknown); ^eSurface feeder

Table 5. Synthesis of published values as a comparison of alcid and procellariiform diving (time depth recorders) and haematological parameters. Values are mean \pm SE. Hct: haematocrit; [Hb]: haemoglobin concentration; M-W: Mann-Whitney

Parameter	Alcids (n = 4)	Procellariiformes (n = 7)	Test
Max. depth (m)	69.1 \pm 34.7	17.2 \pm 8.5	M-W, p = 0.07
Avg. depth (m)	14.3 \pm 3.0	2.6 \pm 0.9	M-W, p = 0.01
Hct	50.2 \pm 2.2	48 \pm 2.3	t-test, p = 0.53
[Hb]	18.6 \pm 0.7	16.5 \pm 0.4	t-test (t = -3.025, df = 10, p = 0.014)

respiratory store allows deeper depths to be attained for potentially less cost.

Future work needs to characterise muscle oxygen stores in order to allow total body oxygen stores to be estimated, thereby allowing theoretical aerobic dive limits to be calculated for these species. The use of species caught as fisheries by-catch could be a source of muscle material, particularly for sooty shearwaters, which are known to interact with commercial fishing fleets.

Acknowledgements. We thank the School of Biological Sciences PBRF fund for support given to B.J.D., M.J.R., R.L.S., L.R. and T.J.L. Access and accommodation to Ihumoana and Kauwahaia Islands was kindly granted by John and Barbara Lusk and the Woodward family. We also wish to acknowledge Ngāti Rehua for access to the Mokohinau Islands, Chris Gaskin for assistance with all manner of logistics and research discussions. M.J.R. acknowledges the support from Wendy Rayner during the completion of this study. This work was undertaken in accordance with the Animal Welfare Act (1999) via permit no. AEC 218 issued by the Department of Conservation Animal Ethics Committee.

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*Editorial responsibility: Scott Shaffer,
San Jose, California, USA*

*Submitted: September 25, 2014; Accepted: January 8, 2015
Proofs received from author(s): March 3, 2015*