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## Movements, diving behaviour and diet of type-C killer whales (*Orcinus orca*) in the Ross Sea, Antarctica

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### ABSTRACT

1. The fish-eating, type-C ecotype, killer whale is a top predator in the Ross Sea, Antarctica. Increasing knowledge on this animal's foraging habitats, diet and movement patterns is listed amongst the research priorities adopted under the framework of the Commission for the Conservation of Antarctic Marine Living Resources (CCAMLR).
2. To contribute to this goal, satellite transmitters were deployed on ten type-C killer whales and skin biopsies were obtained from seven individuals in Terra Nova Bay (TNB; Ross Sea) during austral summer (Jan-Feb) 2015. Hierarchical switching state-space models (hSSSM) were applied to Argos satellite tracking data to describe the movements of tagged whales, which were then paired with available diving data. Stable isotopes analyses were performed on the biopsy samples to describe the diet.

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3. A total of 8,803 Argos locations were available to fit the hSSSM. All whales engaged in potential foraging activity in localized areas along the Ross Sea coastline, followed by uninterrupted travel (i.e. migration) outside Antarctic waters, with no evidence of foraging activity. The pattern of deeper dives matched the occurrence of encamped behaviour indicated by the hSSSM results. The stable isotopes analysis indicated that Antarctic toothfish comprised the majority (35%) of the prey biomass, raising concerns since this species is targeted by commercial fishery in the Ross Sea Region.
4. These results provide new insights into the ecology of type-C killer whales in the Ross Sea Region, underlining a potential threat from commercial fishing in the area. Considering the recent establishment of the Ross Sea Region Marine Protected Area (RSR MPA), these findings will contribute to the required Research and Monitoring Programme of the MPA and provide new empirical evidence to inform conservation measures in the existing TNB Antarctic Special Protected Area (ASPA).

**KEY –WORDS:** Antarctica, diet, type C killer whale, *Orcinus orca*, Ross Sea, satellite tagging, stable isotopes, Terra Nova Bay

## INTRODUCTION

Two killer whale (*Orcinus orca*) ecotypes regularly occur in the Ross Sea, Antarctica: type - B, a mammal-eating form that feeds mainly on seals, and type-C, a fish-eating, dwarf form (Pitman & Ensor, 2003; Pitman et al., 2007; Ainley, Ballard & Olmastroni, 2009). Type-C, also known as the Ross Sea killer whale (hereafter RSKW), is mainly distributed along coastal areas, especially along the fast ice edge and around dense pack ice. It is readily identified from other killer whale ecotypes by its narrow, slanted eye patch, and by being the smallest known killer whale (adult males reach 6.1 m; Pitman et al., 2007).

Information on RSKW occurrence, distribution, and movements is mainly limited to the austral summer and to McMurdo Sound in the western Ross Sea (Andrews, Pitman &

Ballance, 2008; Ainley et al., 2009; Pitman, Fearnbach & Durban, 2018; Pitman et al. 2019). Pitman & Ensor (2003) suggested that RSKW might sometimes get trapped in the advancing winter ice and be forced to overwinter in Antarctica, or alternatively, they may also occur in Antarctica year-round (see also Gill & Thiele, 1997; Pitman et al., 2019). Previously, it was speculated that RSKW range north of the Antarctic Polar Front or routinely undergo long-distance migrations (Pitman & Ensor, 2003; Visser, 2007; Dwyer & Visser, 2011). This was subsequently confirmed for RSKW from the western Ross Sea equipped with satellite transmitters, leading to the hypothesis that the primary driver of this migration is to travel to warmer waters for skin moult (Durban & Pitman, 2012; Pitman et al., 2019).

Knowledge about the diet of RSKW is also scarce and debated. Antarctic toothfish (*Dissostichus mawsoni*) - up to 2 m and approximately 100 kg - is by far the largest fish available for fish-eating killer whales in Antarctic waters. Although other, smaller fish species are also consumed, toothfish has generally been considered the primary prey species of RSKW (Pitman & Ensor, 2003; Ainley et al., 2009; Pitman et al., 2019). Assessing the importance of toothfish in RSKW diet is important because it is also the target of a commercial fishery in the Ross Sea, and that fishery may already have reduced toothfish availability (Ainley et al., 2009; Aimley et al., 2013; but see Pitman, Fearnbach & Durban, 2018). RSKW may be forced to compete with other predators, such as minke whales (*Balaenoptera bonaerensis* Burmeister), Weddell seals (*Leptonychotes weddellii* Lesson) and Adelie and emperor penguins (*Pygoscelis adeliae* Hombron & Jacquinot; *Aptenodytes forsteri* Gray) for smaller fish species (Ichii et al., 1998; Burns & Kooyman, 2001; Lyver et al., 2011; Torres et al., 2013), including, possibly, silverfish (*Pleurogramma antarcticum*) (Ainley et al., 2007; La Mesa, Eastman & Vacchi, 2004; La Mesa & Eastman, 2012). Such a prey switch in RSKW diet might alter the delicate balance of the Ross Sea food web (Ainley et al., 2007).

Shedding light on the ecology of Antarctic predators is challenging, not only due to the high cost and difficult logistics associated with collecting data in a remote and extreme

environment, but also because of the wide-ranging habits of these highly mobile and migratory species. Satellite telemetry can be used in these scenarios to provide valuable insights on the ecology of marine mega-fauna (Block et al., 2011), to identify critical habitats and Important Marine Mammal Areas (IMMAs), to inform management and conservation measures (de Castro et al., 2014), and to design and monitor Marine Protected Areas (Hays et al., 2019).

The Ross Sea is amongst the least anthropogenically affected regions of the world's ocean (Halpern et al., 2008). The continental shelf there is one of the most productive areas of the Southern Ocean, an area where the top- and middle trophic levels have not been substantially impacted (Smith, Ainley & Cattaneo-Vietti, 2007), and where the community of top predators - prior to the toothfish fishery - was considered still intact (Ainley, 2010). These unique characteristics and the outstanding ecological values of the region have consistently attracted international scientific interest and led to the establishment of the Ross Sea Region Marine Protected Area (RSR MPA) by the Convention for the Conservation of Antarctic Marine Living Resources (CCAMLR) in October 2016. The area has also been declared an Important Marine Mammal Area (IMMA, [www.marinemammalhabitat.org](http://www.marinemammalhabitat.org)) due to its recognized importance for both seals and cetaceans, and includes areas in which seals are protected under the Antarctic Seals Treaty, as well as several Antarctic Specially Protected Areas (ASPA) that include marine portions.

The aim of this paper is to present the results of a satellite telemetry study and stable isotope analysis conducted on RSKW in the austral summer of 2015 and provide insights on movements, diving activity, foraging areas, and diet of this little-known ecotype in the western Ross Sea. These results may represent useful tools for the management measures needed in the framework of the RSR MPA, as well as important steps towards the assessment of RSKW vulnerability to commercial toothfish fishing in the Ross Sea.

## MATERIALS AND METHODS

### *Study area and data collection*

Satellite tagging was conducted from mid-January to mid-February 2015 off the Italian Research station Mario Zucchelli (MZS; 74° 41' 42" S - 164° 07' 23" E), located in Terra Nova Bay (TNB), in the Ross Sea, Antarctica (Figure 1). Visual searches for killer whales were conducted during helicopter flights along the ice edge. Once a killer whale pod was spotted, the helicopter moved forward in their travel direction to locate a suitable landing site close to the ice edge, where the pod was expected to pass and where tagging operations would be attempted. Smart Position Only (SPOT) and depth-recording satellite transmitters (SPLASH) in the Low Impact Minimally Percutaneous External-electronic Transmitters (LIMPET; Wildlife Computers, Inc. Redmond, WA) configuration were deployed. Both types of transmitters provide animal position through the Argos system (<http://www.Argos-system.org>), available from CLS Service. The transmitters were equipped with two darts (68 mm L), containing two sets of six outwardly-folded petals, and were deployed with a 150 lb draw weight recurve crossbow (Vixen Excalibur II) and a 20 inch carbon fibre arrow (Andrews, Pitman & Ballance, 2008). Shooting distance ranged between 3 - 9 m and transmitters were deployed on the dorsal fin of both adult males and females. To allow *a posteriori* evaluation of the position of the transmitter and the animal's immediate reaction to deployment, each tag deployment was recorded on a high-resolution digital camera mounted on the crossbow. The instruments were programmed to send 600 transmissions per day over three daily temporal windows (02-04, 06-17, 19-21), for a total of 17 hours per day. Time intervals were selected in relation to the availability of ARGOS satellites in the study area, between January and February 2015.

SPLASH transmitters (PTT: 143823, 143824, 143825, 143826) also recorded diving data, summarized and compressed on the tags to improve transmission over bandwidth- and time-limited Argos satellite connections. Pressure measurements (a proxy for depth) were summarized in two separate logs. The data log returned the maximum depth reached in each recorded dive (accuracy:  $\pm 1\%$  of depth reading), as well as the dive durations and surface intervals between dives. A separate log returned a coarse-resolution time series of depth

recordings collected at 1.25-minute intervals. To extend battery life, the time-series log was duty cycled to collect data on one in every six days. These dive and depth measurements were assigned to one of three regions (Closs Bay, Ross Sea Coast, Offshore migration), based on the Argos location fix that was nearest in time to the mid-point time of each record. The proportion of time spent at different depth ranges was compared among the three regions by compiling histograms of depth measurements from the time series log.

Finally, to investigate killer whale diet, individuals were biopsied using biopsy tips (8x60 mm) mounted on a 55 cm-long carbon fibre dart (both manufactured by CETA-DART V/FINN LARSEN), also launched by a 150 lb draw weight recurve crossbow. Skin biopsies were frozen and stored at -20° for stable isotope analysis.

To comply with the "Protocol on Environmental Protection to the Antarctic Treaty", Annex II, art.3, a permit to deploy satellite transmitters and to collect biopsy samples on protected species was issued by the Italian Antarctic Research Programme (PNRA) on behalf of the Italian Ministry of Foreign Affairs (September 2014).

## Data analysis

### *Satellite telemetry*

Bayesian hierarchical switching state-space models (hSSSM) were fitted to Argos satellite tracking data to characterize the horizontal movement and behaviour of tagged whales (Jonsen, Flemming & Myers, 2005; Jonsen et al., 2013). These models classify the behavioural state at each time step as either transiting or Area Restricted Search (ARS). ARS is believed to emerge when animals forage in a patchy environment (Tinbergen, Impekoen & Franck, 1967; Kareiva & Odell, 1987), but feeding while in transit cannot be excluded. Movements with high persistence (i.e. high autocorrelation in speed and angle) and low turning angle are assumed to correspond to transiting behaviour, while low persistence and high turning angles reflect ARS behaviour (Jonsen, Flemming & Meyers, 2005). The model was fitted to locations with varying quality, as indicated by the Argos

positioning algorithm (Lopez & Malardé, 2011). Poor quality locations of class "Z" were removed prior to modelling to facilitate convergence. Package *bsam* for R (Jonsen et al., 2013; R Development Core Team, 2013) was used to fit hSSSM by means of Markov Chain Monte Carlo (MCMC) algorithms implemented via JAGS. A 6 h time step, which was larger than 98% of observed time steps, was chosen for the analysis. Preliminary modelling suggested that this time step allowed the characterization of the two behavioural modes, while resulting in successful convergence of the model. Two chains were run in parallel for 110,000 iterations; 100,000 iterations were discarded as burn-in, and 1 in every 10 observations was retained for the remaining 10,000 samples, to reduce autocorrelation. Convergence was assessed by inspecting trace, autocorrelation, and posterior density plots. Point estimates and uncertainty for model parameters were derived from 2,000 samples from the joint posterior distribution (1,000 samples per chain). The behavioural state of whale  $k$  in each time step  $t$  ( $b_{k,t}$ ) is a binary variable that can take a value of 1 (transiting) or 2 (ARS). Following Jonsen, Myers & James (2007), the behavioural state at each location was classified as ARS if the posterior mean of  $b_{k,t}$  was greater than 1.75, as transiting if the mean was smaller than 1.25, and as uncertain otherwise.

#### *Diet: stable isotope mixing models*

RSKW skin samples were dried at 40 °C for 24 h, and then powdered with a mortar and pestle. Lipids were removed from the samples by rinsing the powdered tissue several times with a chloroform/methanol (2:1) solution. Stable isotopes analysis was carried out using a Flash EA 1112 Series elemental analyser coupled to a Delta C isotope ratio mass spectrometer via a ConFlo III interface (Thermo Finnigan, Bremen, Germany). Secondary standards were run before and after the skin samples. All results were expressed as parts per thousand (‰) delta values ( $\delta^{15}\text{N}$  or  $\delta^{13}\text{C}$ ) referenced to atmospheric nitrogen for  $\delta^{15}\text{N}$  and Vienna Pee-Dee Belemnite for  $\delta^{13}\text{C}$ . Average analytical precision was  $< 0.1\text{‰}$  for  $\delta^{13}\text{C}$  and  $< 0.3\text{‰}$  for  $\delta^{15}\text{N}$  (for more details see Borrell et al., 2012).

A Bayesian mixing model was applied using package SIAR for R (Stable Isotope Analysis in R; Parnell et al., 2010; Phillips, 2012) to estimate the proportional contribution of each potential prey to killer whales diet. The variables used in the mixing model were:  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values for each sampled killer whale,  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  mean values with standard deviations (SDs) for each potential prey species or group, and appropriate discrimination factors with SDs.

A list of potential prey species of RSKW in the area was compiled. The isotopic ratios from these likely prey were extracted from the recent literature (Table 1). When there was more than one isotopic ratio per species (i.e., reported in multiple papers), the mean was calculated, in order to reduce laboratory bias (Table 1). Species that were taxonomically similar and had similar isotope values were pooled (Phillips, 2012), forming two groups (encircled in Figure 5a).

Discrimination factors between prey and odontocete skin have only been experimentally calculated for captive bottlenose dolphins (Browning et al., 2014; Gimenez et al., 2016). In the current study, discrimination factors relating to middle lipid content diet (6%) were used ( $2.09 \pm 0.07\text{‰}$  for  $\delta^{15}\text{N}$  and  $1.28 \pm 0.16\text{‰}$  for  $\delta^{13}\text{C}$ ; Browning et al., 2014), as the % lipids in selected prey species ranged from 15% in *Dissostichus mawsoni* to 2.6 % in *Trematomus pennellii* (Lenky et al., 2012).

## Results

### *Satellite telemetry*

Out of 13 shooting attempts, ten satellite transmitters, four SPLASH and six SPOT, were successfully deployed on individuals belonging to two RSKW groups (Table 2). Tags transmitted on the whales for 19 - 44 days (mean=28.6 d; SD=8.79).

The first two transmitters (PTT 143833, 143834) were deployed on whales from a group of 25 RSKW (hereafter Group 1) on 14 January (Table 2). Subsequently, during 14-22 January, three SPLASH (PTT 143823, 143824, 143825) and three SPOT (PTT 143828,

143830, 143831) transmitters were deployed on six RSKWs from the same group. Individuals from Group 1 then left the TNB area and were replaced by another group of 20 individuals (hereafter Group 2). Two individuals from Group 2 were tagged on 25-26 January with a SPOT (PTT 143832) and a SPLASH tag (PTT 143826), respectively. Three more deployments were attempted, but PTT 143829 detached soon after shooting due to the unfit attachment on the dorsal fin, and both PTT 143835 and 143836 were lost during shooting.

Out of 11 successful applications, four whales showed no reaction, and seven individuals showed a slight startle response by accelerating after tag attachment. Nevertheless, tagged whales were resighted over a period of 8 (group 1) and 2 days (2) in the same tagging area. These qualitative findings indicate that reactions to tag deployment were only short-term and did not alter the whales' overall behaviour and residency in the area.

A total of 8,803 Argos locations were available to fit the hSSSM (median by individual: 876; range: 598-1219). Visual inspection of the trace and density plots confirmed that the model converged adequately after discarding the burn-in iterations. The two behavioural modes were identified correctly, as suggested by the successful discrimination of the associated state-dependent parameters (Table 3). The corrected tracks and corresponding behavioural states were reconstructed based on the posterior distribution of the parameters (Figure 2a,b).

The two groups of whales differed in their spatial movements and habitat use patterns. All tagged individuals from Group 1 spent 8 d in Closs Bay after tagging, where they showed consistent ARS behaviour (Figure 2b). They then travelled north along the western edge of the Ross Sea and engaged in additional ARS activity around the Mariner Glacier and Coulman Island, for 1 d only (Figure 2b). They continued following the coast northwards to approximately 72.5°S, south of Cape Hallet, where they left the Ross Sea and coastal area on

25 January; no further ARS behaviour was detected in any of the tracks, as the group travelled directly north.

Both individuals from Group 2 engaged in ARS behaviour in the tagging area (Closs Bay) for 2 d after tagging. They then started to travel north on 27 January and reached Lady Newnes Bay on 28 January. ARGOS data indicate they returned to Closs Bay on the same day, where subsequently they engaged in ARS behaviour for another 6 d. Later, the two individuals spent more time engaged in apparent foraging activities outside Closs Bay, near several ice tongues in Lady Newnes Bay (Figure 2b).

Similar to Group 1, the two animals from Group 2 did not engage in ARS behaviour north of 73°S, where they started the northward travel on 11 February.

The diving activity of three animals from Group 1 and one from Group 2 is shown in Table 4, in Figure 3 (a,b,c,d) and Figure 4. Individuals in Group 1 typically performed dives in excess of 150 m within Closs Bay, with a maximum depth of 292 m. The individual in Group 2 engaged in deep dives (>150 m) both within Closs Bay (max 246 m) and in the proximity of ice tongues in Lady Newnes Bay (max 452 m). Deep dives were congruent with ARS behaviour as indicated by the hSSSM results. Less dive activities were recorded once the individuals of either group left the Ross Sea coast (Table 4, Figure 3 a,b,c,d and Figure 4).

The proportion of time tagged individuals from Group 1 spent in different dive depth bins showed a similar pattern (Figure 4a), with limited use of deeper strata outside Closs Bay. Unlike individuals from Group 1, the Group 2 individual engaged in comparable deep-diving activity in Closs Bay and along the Ross Sea Coast; the similarity of the diving profiles in these two areas is evident in the histograms for time allocated at different depths (Figure 4b).

In summary, surface relocations and dive data indicate that Group 1 foraged mainly in Closs Bay and after leaving the area, the group rapidly moved towards Cape Hallet performing only shallow dives. Individuals from Group 2 moved differently and spent more time

foraging (i.e. deep diving and engaging in ARS behaviour) in Closs Bay, around Cape Washington and near ice tongues in Lady Newnes Bay.

The offshore migration of the 10 tagged whales ended in the New Zealand sub-tropical waters after travelling 4,900 km; detailed information on this northwards travel are described in Pitman et al. (2019).

#### *Diet analysis*

Seven skin biopsies were collected, including three from tagged individuals. All skin samples were processed and analysed.  $\delta^{13}\text{C}$  values ranged from  $-24.27\text{ ‰}$  to  $-23.14\text{ ‰}$  (mean  $\pm$  SD:  $-23.9 \pm 0.4\text{ ‰}$ ) and  $\delta^{15}\text{N}$  values ranged from  $14.12\text{ ‰}$  to  $15.45\text{ ‰}$  (mean  $\pm$  SD:  $14.5 \pm 0.5\text{ ‰}$ ) (Figure 5). The stable isotope ratios of three potential prey species and two clusters of potential prey species were extracted from the literature (Table 1): 1) Antarctic toothfish (*D. mawsoni*):  $-24.54\text{ ‰}$  -  $-13.7\text{ ‰}$  for  $\delta^{13}\text{C}$  /  $\delta^{15}\text{N}$  values; 2) striped rockcod (*T. hansonii*):  $-24.40\text{ ‰}$  -  $-12.50\text{ ‰}$ ; 3) Jonah's icefish (*N. ionah*):  $-26.1\text{ ‰}$  -  $-11.1\text{ ‰}$ ; 4) dusky rockcod (*T. newnesi*), bald notothen (*P. borchgrevinki*) and Antarctic silverfish (*P. antarcticum*):  $-24.42\text{ ‰}$  -  $-10.45\text{ ‰}$  and 5) emerald rockcod (*T. bernachii*) and sharp-spined notothenia (*T. penellii*):  $-22.5\text{ ‰}$  -  $-10.85\text{ ‰}$ . The estimate of the diet-tissue isotopic discrimination factor (Browning et al., 2014) was added to these values to plot them in Figure 5b, in order to show the mixing space defined by the sources, as recommended by Philips (2012).

The mean values of the probability density functions are the most likely level of contribution to the diet, but solutions could fall anywhere within the credibility intervals (Parnell et al., 2010).

The potential prey species and their relative abundance indicated by the SIAR mixing model demonstrate that Antarctic toothfish made up the largest contribution to the diet of RSKW (mean: 34.5%), followed by Jonah's icefish (33.2%) and striped rockcod (19.1%). All other prey have been combined, since they could not be distinguished individually, given their

very similar isotope ratios; this group, which includes Antarctic silverfish and *P. borchgrevinki*, contributed 13.2% to the diet (Figure 6).

## DISCUSSION

### *Satellite telemetry*

Satellite telemetry provided a description of the movements, habitat use and diving activity of fish-eating, type-C killer whales in the western Ross Sea, Antarctica. The Bayesian state-space model revealed discrete, largely non-overlapping ARS behaviour of killer whale individuals along the coastline, as well as transiting behaviour in the open ocean. ARS behaviour is assumed to correspond to foraging, socializing, or resting activities (Bailey et al., 2009; Jonsen et al., 2007), but feeding while in transit cannot be ruled out (Pitman et al., 2019). In this study, long bouts of ARS behaviour occurred in association with dives deeper than 150 m, suggesting that these animals may have been foraging at that depth. This behaviour occurred in Closs Bay and in Lady Newnes Bay, mainly within or close to the Terra Nova Bay ASPA #173 (Cape Washington & Silverfish Bay), #165 (Edmonson Point 5), #106 (Cape Hallett), and surrounding waters. Between these areas, tagged individuals engaged in transiting behaviour. Mean swimming speed along the Ross Sea coastline was between 3.5 and 7.3 km/hr (Pitman et al., 2019).

In contrast with the ARS behaviour exhibited in coastal areas, the state-space model showed that when tagged whales left the Ross Sea coast they engaged in a linear transit towards waters offshore of New Zealand (Figure 2a) with less dive activities (Table 4) - an apparent long-range migration where the travelling speed increased to between 8.1 and 10.6 km/hr (Pitman et al., 2019).

Previous known information on the occurrence and movements of RSKW in the SW Ross Sea during the austral summer come from the Ross Island/Mcmurdo Sound area. There, RSKW occurrence undergoes a westward expansion from Ross Island in mid-November to McMurdo Sound by mid-December (Ainley et al., 2017). Particularly important to the whales in McMurdo Sound is the channel that icebreakers open annually in the fast ice to

connect McMurdo Station to the open ocean; the channel exposes new habitats where killer whales can forage under the fast ice (Andrews, Pitman & Balance, 2008; Ainley et al., 2017; Kim et al., 2018). Pitman, Fearnbach & Durban (2018) used photo-identification techniques and identified a seasonally resident population of 73 killer whales (95% C.I. 57-88) in McMurdo Sound, along with a population of at least 397, more transient individuals. The authors speculated that the transients were either residents of other areas of the Ross Sea that temporarily stopped in McMurdo Sound for socializing or feeding, or were members of a more nomadic RSKW population. Killer whales have been recorded in mid-January in the area of TNB (present study; Lauriano, Fortuna & Vacchi, 2010), which is about 250 km north of McMurdo Sound. There currently is no indication of mixing between pods from McMurdo and TNB, and comparisons of photo-identified individuals from both areas have not yielded any matches (Fearnbach pers. comm.). Additional photo-id work at TNB will be necessary to clarify how connected these populations might be and whether, like McMurdo, TNB also has resident and transient populations occurring in the area.

#### *Prey species*

According to the current stable isotopes results, Antarctic toothfish was the main prey of RSKW, accounting for 35% of ingested biomass; previously, all the information about Toothfish in RSKW diet were based on field observations (Pitman & Ensor, 2003; Ainley, Ballard & Dugger 2006; Ainley et al., 2009; Pitman et al., 2018), where individuals with their heads above water and Antarctic toothfish in their mouths (Thomas et al., 1981) were observed. Since toothfish is such a large species it has always been considered to be the most important, or only, species taken. These data demonstrate, for the first time, the high presence of the Antarctic toothfish in RSKW diet.

However, the results differ from those of Krahn et al. (2008), who inferred lower importance of toothfish for RSKW, based on their similar trophic levels. However, the latter study was based on the analysis of a single toothfish individual, assumed a discrimination factor between killer whale skin and fish of 3.5‰ (which is now believed to be closer to 2‰; Browning et al., 2014), and used data collected during years when concentrated, multi-year fast ice likely reduced killer whale access to toothfish in McMurdo Sound (Pitman et al.,

2018). Our results suggest that RSKW feeds at a higher trophic level than toothfish, and therefore, this species acts as a likely predator of the fish. This observation is supported by the biology of the species: individuals are benthic before reaching 100 cm in length, and then accumulate fat to increase buoyancy (Near et al., 2003). It is only when inhabiting the water column that toothfish feed on silverfish (Eastman, 1985), one of the few other neutrally buoyant species occurring in the Ross Sea, and are targeted by RSKW.

In support of the importance of this species in RSKW diet, a purported decrease of RSKW occurrence along the eastern coast of Ross Island (Cape Crozier; Ainley & Ballard, 2012) was linked to a reduction of toothfish presence in the water column (Ainley et al., 2013). Nevertheless, Pitman and colleagues (Pitman et al., 2018) offered evidence that the McMurdo RSKW population has been stable for at least a century, and that RSKW ‘decline’ reported by Ainley and colleagues (Ainley et al., 2013) could be related to the presence of the iceberg b-15 at Ross Island, which could have disrupted the usual RSKW movement patterns.

In addition to Antarctic toothfish, Antarctic silverfish has been suggested to represent an important prey for RSKW diet (Ainley, Ballard & Dugger, 2006) and to influence their winter presence in the Antarctic (Ballard et al., 2011). Silverfish constitutes 90% of both the abundance and biomass of the mid-water fish fauna in the Ross Sea (La Mesa, Eastman & Vacchi, 2004; O’Driscoll et al., 2011) and outer Terra Nova Bay; a breeding area for the species (Vacchi et al., 2004). However, results from this study suggest that the combined prey group that included silverfish accounted for less than 9% of RSKW diet (Figure 6).

There are considerable differences in the size, weight and behaviour of these two potential prey species. Toothfish can reach a length of 160 cm (Hanchet et al., 2015), while silverfish are generally around 15 cm long (Froese & Pauly, 2019); toothfish can weigh about 3,000 times more and has double the lipid content per gram than silverfish (Lenky et al., 2012). These differences make toothfish a much more suitable and energetically advantageous, albeit much less common, food source for RSKW compared to silverfish.

All the other identified prey species are known to inhabit the continental shelf of the western Ross Sea (La Mesa, Eastman & Vacchi, 2004). They have either a benthopelagic lifestyle (*N. ionah*, Eastman & Hubold, 1999; La Mesa, Eastman & Vacchi, 2004; Kock, 2005), are active in the mid-water column (*T. hansonii*, La Mesa, Eastman & Vacchi, 2004), or are part of benthic (*T. bernacchii* and *T. pennellii*) or cryopelagic (*P. borchgrevinki*, and *T. newnesi*) fish communities.

Although the analysis of satellite telemetry data indicated the occurrence of putative foraging behaviour in the area at the time of the deployments, this cannot be directly related with the diet inferred via stable isotope analysis; isotope ratios in skin samples reflect the diet between 2 and 6 months prior to sampling (Browning et al., 2014; Giménez et al., 2016). Therefore, sampled RSKW could have been feeding anywhere in the Ross Sea prior to biopsy sampling.

Interestingly, when heading offshore towards New Zealand, tagged whales entered Victoria Land in the CCAMLR fishing area 88.1, where *D. mawsoni* represents more than 99% of the reported catch of *Dissostichus* spp. (Ponganis & Stockard, 2007). Nevertheless, the absence of ARS behaviour in the CCAMLR fishing area is evidence that the whales were not feeding during migration. Concerning that transit, it has been hypothesized that Antarctic killer whales, including type-C killer whales, travel north, to warmer waters, for routine skin maintenance and not for feeding or breeding; this behaviour has been recently described for the Antarctic killer whale (Durban & Pitman, 2012; Pitman et al., 2019).

#### *Management considerations*

Three permanent scientific stations are located in ASPA 173 (Cape Washington and Silverfish Bay): Mario Zucchelli (Italy), Gondwana (Germany), and Jang Bogo (Republic of Korea), while another one is under construction (China). The growing development of research stations and their infrastructures is leading to an increase in scientific and logistical activities in the region; for example, a gravel airstrip is under construction approximately 6 km south of Mario Zucchelli Station and 40 km from what has been inferred as a potential and important foraging area for killer whales. Moreover, the Cape Washington area is also

of tourist interest, thanks to the emperor penguins colony. Interactions with this range of anthropogenic activities may cause unprecedented disturbance for RSKW in the area, requiring dedicated strategies for their management and appropriate mitigation measures (Hughes, Pertierra & Walton, 2013).

Tracking data can be a valuable tool to identify priority areas for conservation, and help revise existing management measures (Hays et al., 2019). A larger data set and a longer study period will be needed for a comprehensive description of type-C killer whale movements and behaviours in the Ross Sea and the relative importance of the coastal area for this population clusters. Moreover, in our study, the 10 individuals representing two killer whale pods may not be considered as fully independent units, given the highly cohesive social behaviour of the species. Besides, tagging in a single area, even although this was the only option due to the logistical and safety constraints in flights far from the research station, may have biased the Closs Bay importance.

This study cannot therefore provide a conclusive picture of the extent of the population's area of habitat use and, in absence of more data, the conclusions of the present study should remain conservative and the precautionary approach should be considered. Nevertheless, despite these caveats, the data offer new insights on RSKW ecology and identify the priorities for future data collection.

RSKW putative foraging activity in the Ross Sea overlap with the season of highest human scientific and recreational presence, especially in the area around Mario Zucchelli Station. Moreover, both Italian and Korean stations are supported by several research vessels, cargo ships and flight operations. Boat presence and noise can disrupt cetacean behaviour and lead to changes in the animals' activity patterns (e.g. Lusseau, 2003; Williams et al., 2006; Pirotta et al., 2015). Therefore, even if killer whales in McMurdo Sound benefit from icebreakers, assuming that ship traffic has contributed to a larger population of type-C killer whales there since this exposes new forage grounds for the animals, the exposure and

responses to increasing boat traffic in other areas need to be carefully assessed and any potential negative impacts evaluated.

As far as the aerial activities are concerned, the Cape Washington and Silverfish Bay ASPA (ASPA 173) management plan adopted in 2013, establishes a flight limitation during the emperor penguin breeding season (January to April), as well as the prohibition of aircraft landing in Closs Bay. The main findings of this study have been taken into account in the first revision of the plan in July 2019 (<https://www.ats.aq/devAS/Meetings/Past/87>); the aim of the revision, which is mandatory every 5 years, was to update the biological value of the area to be protected from anthropogenic disturbance. Nevertheless, no specific rules for RSKW have been introduced in the updated management plan, while these preliminary findings clearly show that the ARS behaviour is mostly under the ASPA legal protection and fall in the ‘ship entry by permit’ area and partially in the no-entry zone (ASPA area in Figure 1). A larger sample size is clearly needed to confirm the importance of the area for this killer whale ecotype; nonetheless, a temporal and geographical extension of both naval and aircraft restrictions should be considered as a foreseeable measure in order to cover killer whale presence in this potential feeding area or stopover site along their northward migration.

An update of the distribution and the intensity of human-related stressors, in light of the growing activities in the area, is clearly needed to suggest an improvement of the existing measures in the area, as well as to delineate new ones. According to the article II of the CAMLR convention, the Ross Sea Region Marine Protected Area is designated to contribute to specific conservation objectives. Thereby, the establishment of the Ross Sea Region MPA in 2016 offers, by means of the identification of the priority elements for scientific research and monitoring, a valuable opportunity to further understand the presence and distribution of this fish-eating killer whale ecotype and delineate the value of the habitats along the coast. It also will be crucial to estimate RSKW abundance in the Ross Sea through dedicated photo-identification studies, to investigate the relationship between the McMurdo/Ross Island and Terra Nova Bay areas.

Moreover, the Antarctic toothfish relevance in RSKW diet underlines the potential threat posed by commercial harvesting of this species. In this study, it was not possible to distinguish individually the other smaller species that may characterize RSKW diet (see Pitman et al. 2018), and there is no new evidence that RSKW ever take Antarctic silverfish or *P. borchgrevinki* and the other potential prey, as has been previously assumed (Lauriano et al., 2007). In addition, the presence of the pelagic Channichthyidae Jonah's icefish, a previously unknown prey for the type-C killer whale, deserves further investigation. This icefish is considered the main prey of the Adelie penguin and the main predator of the *P. antarcticum* (La Mesa, Eastman & Vacchi, 2004), which may lead to potential prey competition between these species.

In conclusion, this paper reports preliminary insights on RSKW foraging range and their potential for trophic competition with other predators in the Ross Sea. The ecosystem impact of commercial toothfish fishing in the Ross Sea region, as well as, concrete mitigation measures for the growing anthropogenic pressures represent important gaps still need further investigations.

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**Table 1.** Mean and standard deviation (SD) of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of each prey species used in the Bayesian mixing models

Species	Common Name	n	$\delta^{13}\text{C}$	SD $^{13}\text{C}$	$\delta^{15}\text{N}$	SD $^{15}\text{N}$	Reference
<i>Dissostichus mawsoni</i>	Antarctic toothfish	9	-23.6	0.5	13.5	0.5	Goetz et al., 2017
		1	-26.3		14.0		Krahn et al., 2008
		100	-24.6	0.6	13.6	1.1	Bury et al., 2008
		56	-23.7	0.5	13.7	1.0	Jo et al., 2013
			<b>-24.5</b>		<b>13.7</b>		<b>mean</b>
<i>Trematomus hansonii</i>	Striped rockcod	6	-24.8	0.2	12.3	0.3	Goetz et al., 2017
		2	-24.0	0.3	12.7	0.1	Jo et al., 2013
			<b>-24.4</b>		<b>12.5</b>		<b>mean</b>
<i>Pagothenia borchgrevinki</i>	Bald notothen	8	-23.3	0.5	10.4	0.3	Goetz et al., 2017
		2	-25.2	1.2	10.7	0.8	Krahn et al., 2008
			<b>-24.2</b>		<b>10.5</b>		<b>mean</b>
<i>Trematomus newnesi</i>	Dusky rockcod	11	-24.5	0.5	10.0	0.3	Goetz et al., 2017
		2	-25.0	0.4	10.9	0.7	Krahn et al., 2008
			<b>-24.7</b>		<b>10.5</b>		<b>mean</b>
<i>Pleurogramma antarcticum</i>	Antarctic silverfish	3	-24.3	0.9	9.4	0.2	Goetz et al., 2017
		2	-24.3	0.1	11.4	0.5	Jo et al., 2013
		5	-24.3	0.3	10.3	0.4	Krahn et al., 2008
			<b>-24.3</b>		<b>10.4</b>		<b>mean</b>
		26	-22.3	0.4	11	0.9	Goetz et al., 2017
<i>Trematomus bernacchii</i>	Emerald rockcod						
<i>Trematomus pennellii</i>	Sharp-spined notothenia	3	-22.7	0.5	10.7	0.3	Goetz et al., 2020
<i>Neopagetopsis ionah</i>	Jonah's icefish	2	-26.1	0.1	11.1	0.7	Jo et al., 2013

Table 2 – Details of satellite transmitter deployments

Tag type	PTT ID	Deployment date and duration (days)	Deployment location (Closs Bay)	Age/sex	Group
SPOT5	143833	14-01-2015 (33)	74.678 S;164.963W	A/M	
SPOT5	143834	14 -01-2015 (19)	74.664 S;164.642W	A/M	
MK10	143823	15 -01-2015 (34)	74672 S;165.308W	A/M	
SPOT5	143831	15 -01-2015 (34)	74.670S;164.843W	A/M	
SPOT5	143828	15 -01-2015 (34)	74.665S;164.753W	A/F	1
MK10	143824	18 -01-2015 (24)	74.671S;164.816W	A/M	
MK10	143825	18 -01-2015 (16)	74.677S;165.068W	A/F	
SPOT5	143830	22 -01-2015 (20)	74.653S;164.614W	A/M	
SPOT5	143832	25 – 01-2015 (44)	74.642S; 164.652W	A/M	
MK10	143826	26 – 01-2015 (28)	74.629S; 164.817W	A/M	2

Table 3. Posterior median and 95% Highest Posterior Density Interval (HPDI) for the average turning angle ( $\theta$ ) and move persistence ( $\gamma$ ) for the two behavioural states (transiting and Area Restricted Search, or ARS).

Parameter	Median	95% HPDI
$\theta_{transiting}$	4.2	2.5 - 5.6
$\theta_{ARS}$	198.4	191.9 - 205.5
$\gamma_{transiting}$	0.90	0.88 -0.92
$\gamma_{ARS}$	0.32	0.23 - 0.42

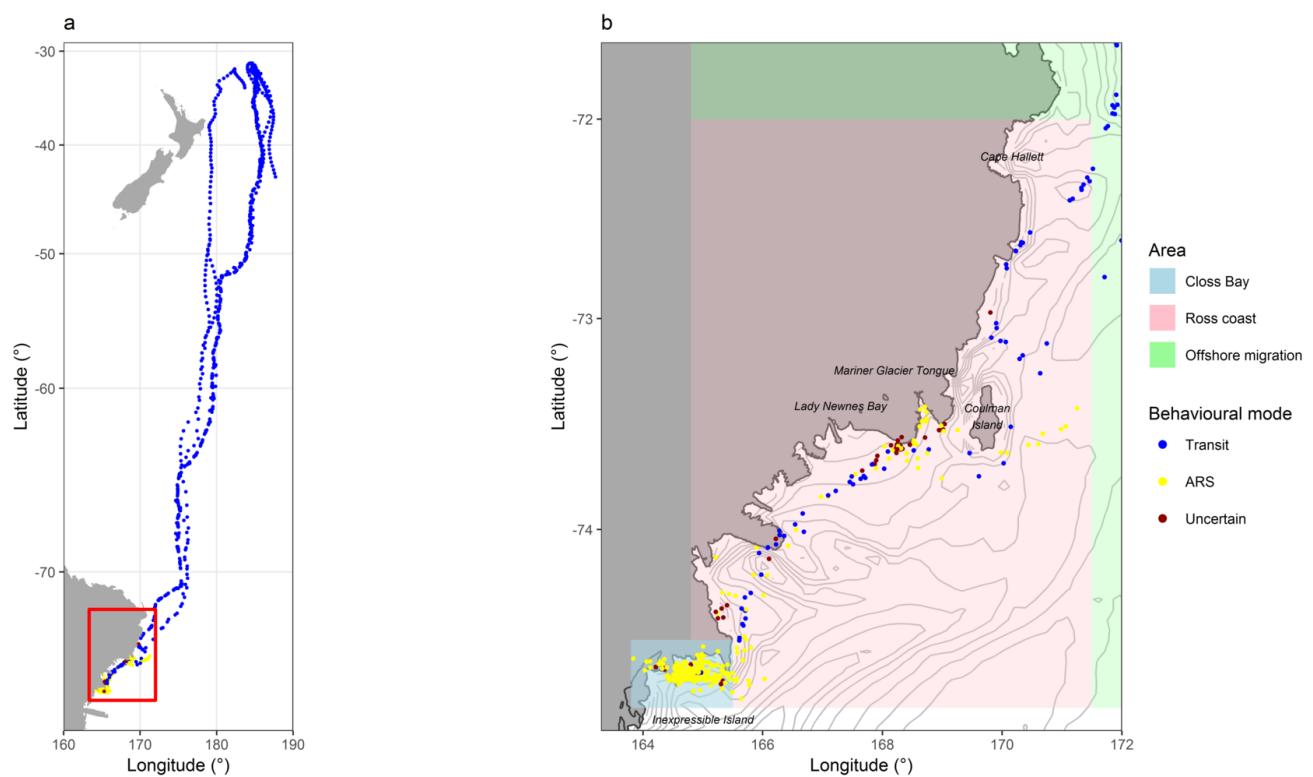
Table 4. Dive metrics of the four tagged whales in the three areas

	# Dives	#Dives per day	Mean duration (minutes)	Max duration	Mean depth (meters)	Max depth
<b>Group 1</b>						
Closs Bay	16,142	1,031.2	2.75	23.18	10.5	292
Ross Sea	5,758	825.88	4.52	42.82	10	158
Offshore migration	3,408	93.69	5.08	35.32	14.5	238
<b>Group 2</b>						
Closs Bay	14,321	2,533.63	2.68	19.48	12.5	246

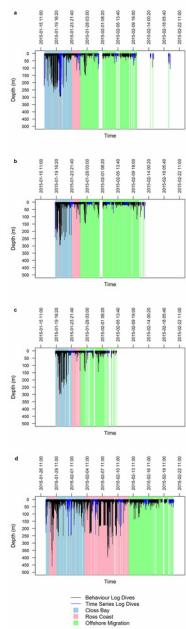
Ross Sea	6,638	689.13	3.48	60.15	12.5	452
Offshore migration	1,301	159.36	5.32	26.55	17	134



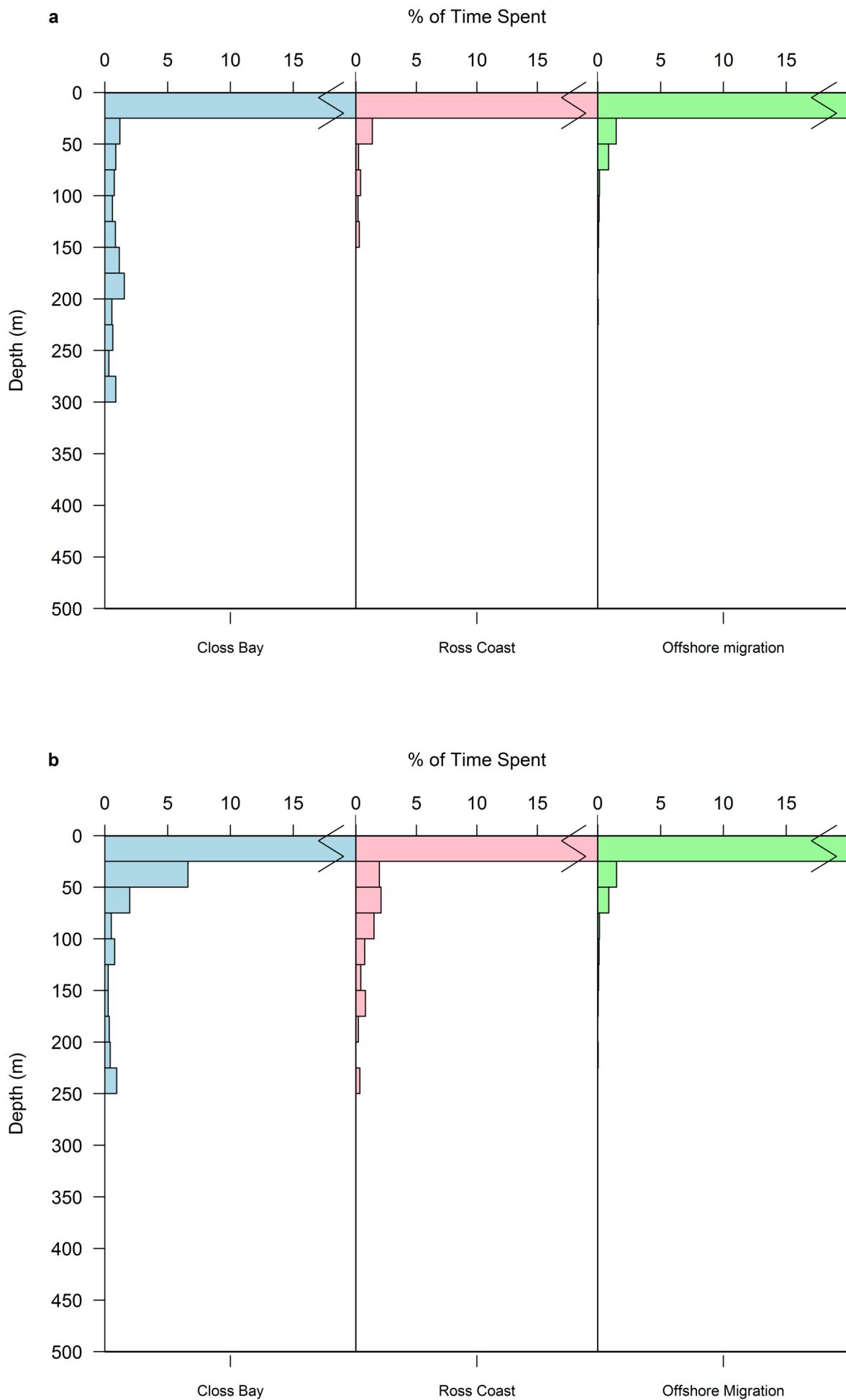
AQC\_3371\_F1.png

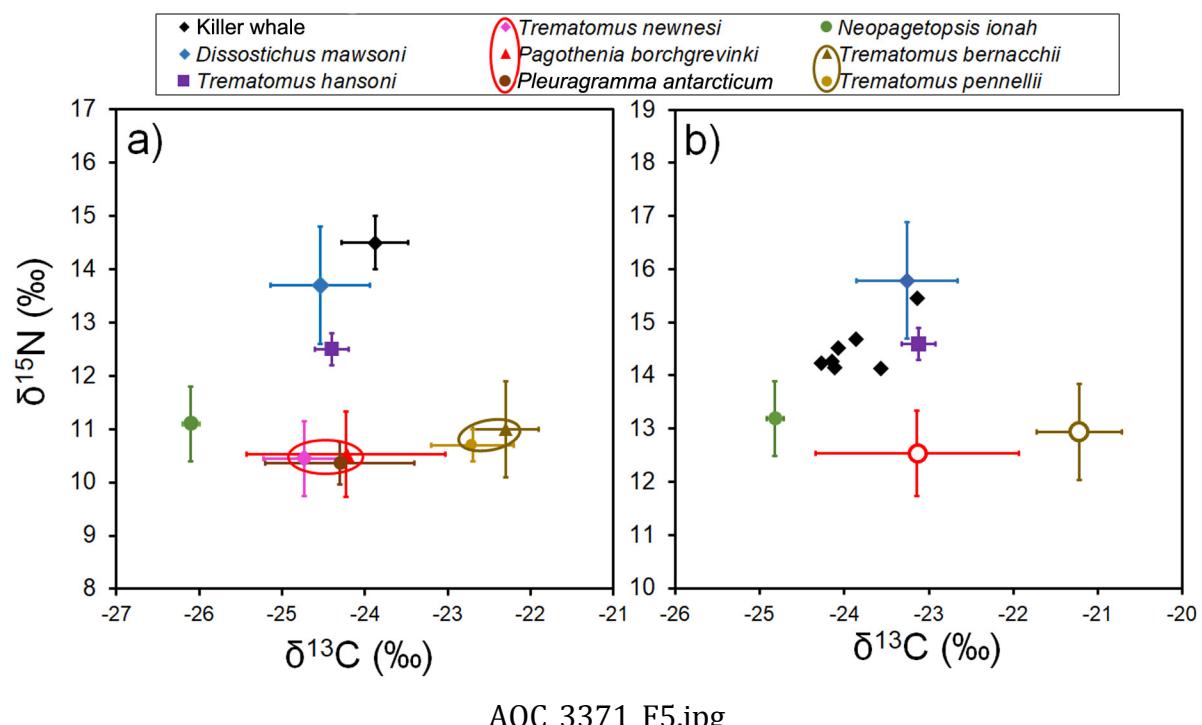


AQC\_3371\_F2.png

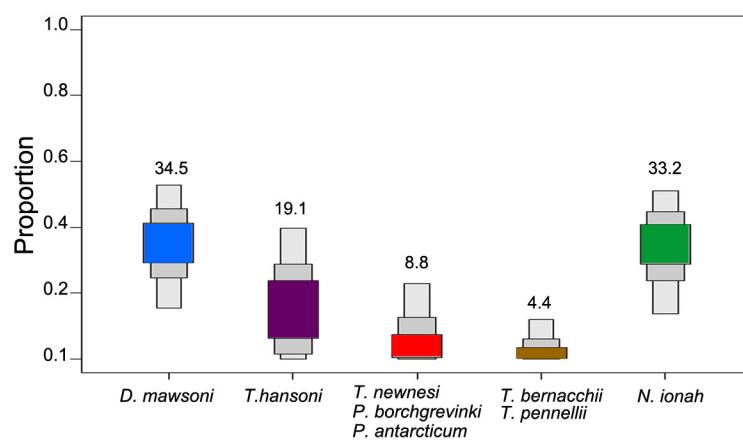


AQC\_3371\_F3.jpg





AQC\_3371\_F5.jpg



AQC\_3371\_F6.jpg