Fish shoal dynamics in North-Eastern New Zealand POP2019-02

Milestone 5: Final report summarising analysis of zooplankton samples collected 2019 - 2020

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Cover image: Skipjack tuna with Buller's shearwater in foreground in pursuit of small fish. *Photo: Chris Gaskin.*

Figure 1 (above): Towing the zooplankton net through a dense school of feeding trevally from the RV *Hawere*. *Photo: Chris Gaskin.*

CONTENTS

1 STUDY AIMS

The aim of this study was to characterise the biological composition of workups by determining the associations among the presence of zooplankton, shoaling fish, and feeding seabirds. This was achieved by looking at the associations between zooplankton prey, such as krill, and their fish and seabird predators. Some key environmental parameters that potentially affect the spatial and temporal distribution of zooplankton and their predators were recorded. The abundance and composition of zooplankton in fish shoals was determined utilising a combination of zooplankton nets and underwater video to identify key species involved with triggering fish shoaling. These data were examined in relation to interannual, seasonal and spatial parameters. This report presents a summary of the analysis of zooplankton samples collected in the 2019 – 2020 sampling season and their relationships with different types of seabird feeding events. It forms a continuation of the fish shoal and zooplankton research conducted in the two previous sampling seasons (2017 – 2018 & 2018 – 2019, Gaskin[1], 2019, Gaskin & Adams, 2019).

1.1 This report

This final report for POP2019-02 Fish shoal dynamics in North-Eastern New Zealand project updates the interim report (Kozmian-Ledward et al, 2020) with lab work delayed by COVID and analyses not completed until after that report was released.

2 INTRODUCTION

2.1 Background

A notable feature of north-eastern North Island waters are the large numbers of seabirds feeding in “workups” – multi-species feeding aggregations containing zooplankton and fish. There is a need to understand the processes that drive workup formation and dynamics as many seabird species, predominantly red-billed gull (*Larus novaehollandiae scopulinus*), white-fronted tern (*Sterna striata*), Australasian gannet (*Morus serrator*), fairy prion (*Pachyptila turtur*), Buller’s shearwater (*Puffinus bulleri*), and fluttering shearwaters (*P. gavia*), are potentially dependent on shoaling fish to drive prey to the sea surface, making them accessible as a food source. There is poor knowledge of both the relationship between the diet of surface-foraging seabirds, and what prey items are being made available to seabirds from workups. This is limiting our understanding of the mechanisms through which any changes in the distribution and/or abundance of workups may be driving seabird population changes (population status and annual breeding success). For several seabird species that interact with workups, their recent population abundance data are also incomplete or unknown which limits our assessment of population trends over time.

North-eastern North Island waters also support extensive purse-seine fisheries, due to the presence of the large shoals of fish. Fish species include kahawai (*Arripis trutta*), trevally (*Pseudocaranx georgianus*), skipjack tuna (*Katsuwonus pelamis*), jack mackerel (*Trachurus declivis*), blue mackerel (*Scomber australasicus*), saury (*Scomberesox saurus*), pilchard (*Sardinops sagax*) and anchovy (*Engraulis australis*). By targeting fish species which are also part of workups utilised by various seabird species; purse-seine fisheries potentially negatively impact these seabird populations. However, the degree to which this may occur is unknown, therefore it is important that we better understand the relationship between seabird population trends and changes in abundance and distribution of fish shoals. Note that in this report, fish ‘shoal’ and ‘school’ are used somewhat interchangeably. Technically, the term ‘shoal’ refers to a loose aggregation of fish, sometimes comprising different species, whereas a ‘school’ is a group of fish of the same species swimming together in synchrony.

2.2 Seabird feeding associations

Zooplankton occupy a key position in the pelagic food web (Fig. 2), transferring the organic energy produced by phytoplankton to higher trophic levels such as fish, seabirds, and baleen whales (Harris et al., 2000; Frederiksen et. al., 2006). Zooplankton abundance and diversity are determined predominantly by oceanographic (e.g. temperature, upwelling zones) and biological factors (e.g. predation) which result in a large amount of spatial and temporal variability (Zeldis & Willis, 2015).

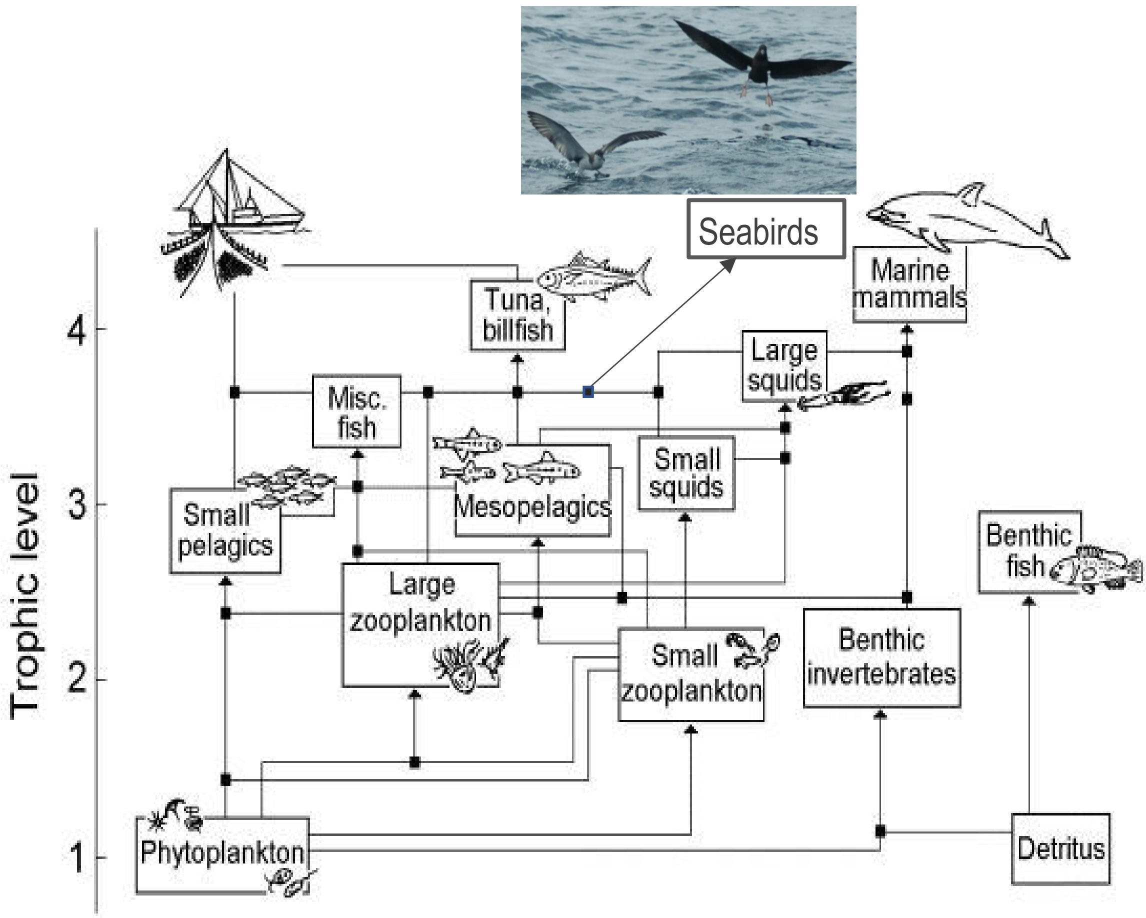


Figure 2: Generalised food web showing trophic levels and interactions between zooplankton, pelagic fish, seabirds, fishing, and other functional groups. *Modified from http://www.personal.kent.edu/~mkeatts/marinefoodwebs.htm with photo by Lily Kozmian-Ledward.*

Pelagic crustaceans such as krill, amphipods and copepods are often targeted as prey by seabirds particularly at those times when they occur at high densities near the sea surface. For example, on Canada’s West coast, the seasonal surface aggregations of *Neocalanus* sp. (large-bodied copepod), form an important food source for breeding Cassin’s Auklets (*Ptychoramphus* *aleuticus*) (Bertram et al., 2017). In Australian waters, the coastal krill *Nyctiphanes australis* and the pelagic amphipod *Paraprone clausi* have been noted as important prey for short-tailed shearwaters (*Ardenna tenuirostris*) when these zooplankters swarm at the surface during the summer (Montague et al., 1986). Seabirds may prey on zooplankton directly, as in the above examples, or indirectly by feeding on small pelagic planktivorous fish.

In north-east North Island, NZ, the previous years of research and observations related to this project have determined prey types of various seabird species feeding in association with surface shoaling fish schools (Table 1). Of the zooplankton, *N. australis* (krill) appears to be an important prey for many seabirds including Buller's and fluttering shearwater and white-fronted terns. Australasian gannets feed on a variety of planktivorous fish species that include krill in their diet. Krill are also targeted by larger shoaling fishes such as kahawai, trevally and skipjack tuna. Analysis of stomach contents of kahawai and trevally in last season's work (2018-2019) found that the predominant prey was krill (Gaskin & Adams, 2019).

Table 1. Summary of seabird prey items described in previous studies by NNZST and associates. Field observations include direct identification of prey captured/carried at sea and at colonies, and later analysis of photographs taken. Regurgitations and faecal samples were obtained from seabirds in their colonies.

Seabird	Prey types	Samples	References
Buller's shearwater	Krill, squid. Scraps from marine mammal feeding (false-killer whales, pilot whales, pelagic bottlenose dolphins, fur seal). Potential small fish species.	Regurgitations, field observations.	Gaskin (2019[2]), Gaskin & Adams (2019), Kozmian-Ledward et al. (2019[1]).
Fluttering shearwater	Pelagic crustaceans, predominantly krill. Juvenile/larval fish. Scraps from marine mammal feeding (false-killer whales, pilot whales, pelagic bottlenose dolphins)	Regurgitations, field observations.	Gaskin & Adams (2019), Kozmian-Ledward et al. (2019[1]).
Fairy prion	Pelagic crustaceans, predominantly krill. Juvenile/larval fish. Scraps from fur seal feeding.	Regurgitations, field observations.	Doyle & Adams (2019[2]), Gaskin & Adams (2019), Kozmian-Ledward et al. (2019[1]).
Australasian gannet	Arrow squid, anchovy, pilchard, saury, redbait, jack mackerel, blue mackerel, flying fish, kahawai.	Regurgitations, field observations.	Adams (2019), Gaskin (2019[2])
Red-billed gull	Potential krill (also opportunistic foragers on intertidal and land-based food sources).	Regurgitations (pellets), field observations.	Gaskin (2019[2]), Kozmian-Ledward et al. (2019[1])
White-fronted tern	Small fish (anchovy, potential pilchard, sardine), potential krill, juvenile squid	Dropped prey, faecal samples – DNA analysis, field observations.	Doyle & Adams (2019[1]), Gaskin (2019[2]), Kozmian-Ledward et al. (2019[1]).

Flesh-footed shearwater	Potential saury	Field observations.	Gaskin (2019[2])
Black petrel	Scraps from marine mammal feeding (false-killer whales, pilot whales, pelagic bottlenose dolphins)	Field observations.	Gaskin & Adams (2019)
Cook's petrel	Scraps from marine mammal feeding (false-killer whales).	Field observations.	Gaskin & Adams (2019)
White-faced storm petrel	Scraps from marine mammal feeding (false-killer whales, pelagic bottlenose dolphins, fur seal).	Field observations.	Gaskin & Adams (2019)

Observations made during previous years of zooplankton sampling trips and on other seabird research trips have identified various types of seabird feeding events associated with fish shoal activity (Table 2). Other types of events can also be characterised where fish shoals are not involved but there is prey available to seabirds (Table 3). At these feeding events, seabirds utilise a variety of feeding techniques depending on the prey being targeted (Fig. 3). Numbers of seabirds attending these events will vary considerably from tens of thousands to a few hundred, even just tens on occasions. Despite these observations, there is still poor knowledge of the diet of surface-foraging seabirds and what prey items are being made available to seabirds from fish workups.

Table 2. Seabird feeding events involving fish shoals (modified from Gaskin 2017). Seabird species acronyms and full names given below.

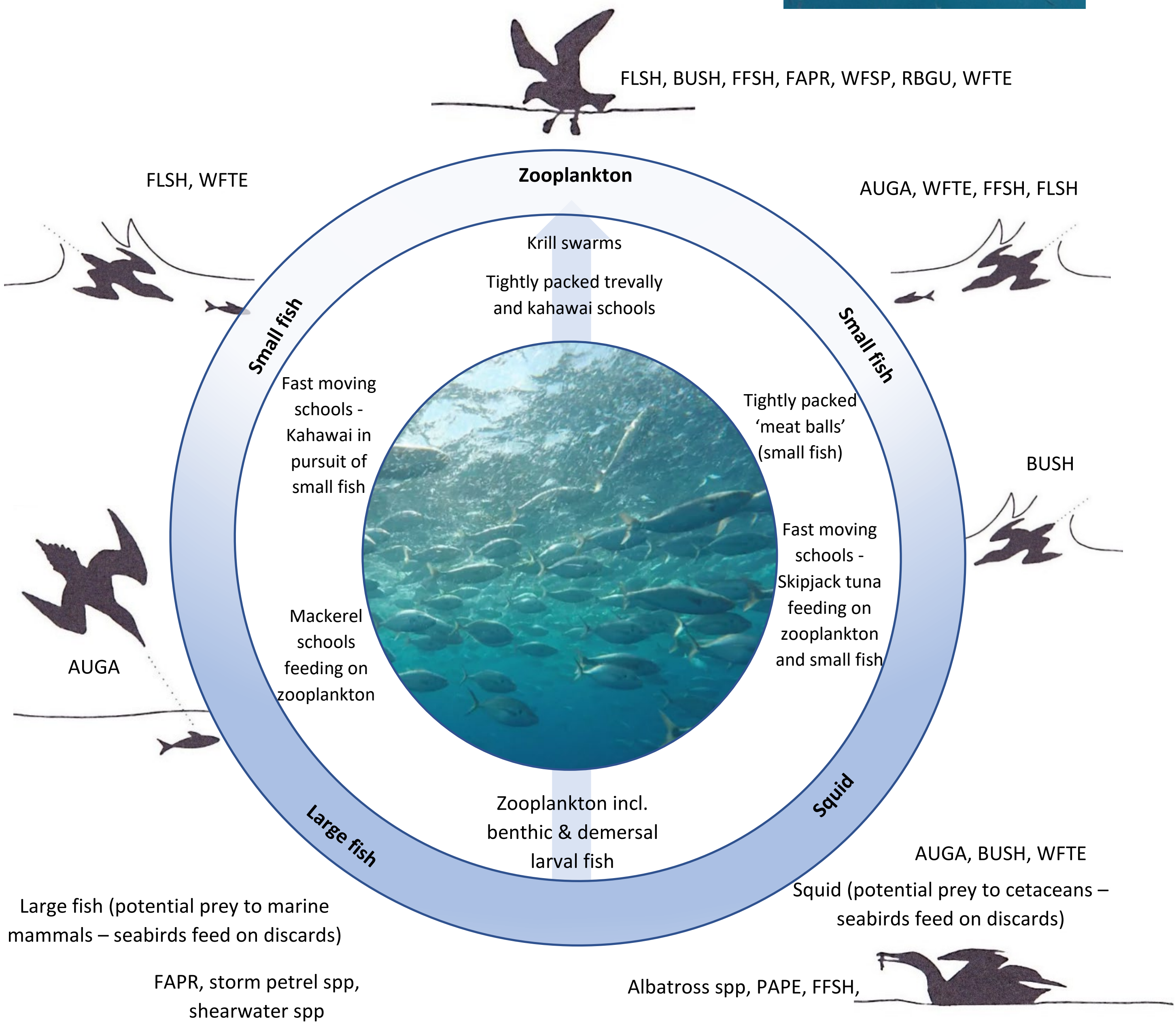
Event type	Fish species	Seabird species	Activity
Mixed fish shoal	Trevally (often the dominant fish species), kahawai, blue maomao, kingfish. Can be just trevally schools.	BUSH, FLSH, FAPR, RBGU, WFTE (plus sometimes SOSH, FFSH, STSH, WFSP, COPE, GRNO)	Tightly packed, very active dense schools, sometimes with several schools merging to form very large schools. Birds either forage in the wake of the schools, or sometimes feed ahead of and around the schools. Fish will erupt explosively if disturbed either from below (e.g. predatory fish) or from above (e.g. birds flying low over school). Shearwaters and prions have been filmed diving in the wake of school activity.
Kahawai school	Kahawai	FLSH, WFTE RBGU, FAPR	Fast-moving schools, birds moving in 'leap-frogging' formations, shearwaters plunging and diving. Also, tightly packed schools separate from trevally schools in the same vicinity.

Saury school	Saury	AUGA, FFSH (BLPE, SOSH)	Shearwaters and gannets diving on saury. Can occur in association with common dolphins.
Jack mackerel school	Jack mackerel	AUGA	Schools most commonly identified by gannets coming to the surface with prey. Fish occasionally seen breaking the surface.
Blue mackerel school	Blue mackerel	AUGA, FLSH, BUSH, FAPR	Very eruptive mobile schools, one minute here, the disappearing to appear somewhere else.
Baitfish shoal	Pilchard, anchovy, koheru	AUGA, FLSH, BUSH (FFSH, WFSP, COPE)	Often tightly packed schools, sometimes forming spinning 'bait balls' close to the surface. Birds plunging/diving and pursuing prey underwater. Can occur in association with common dolphins.
Tuna school	Skipjack tuna	BUSH, FLSH, AUGA, RBGU, occasional WFTE	Fast-moving fish sometimes jumping clear of water. Shearwaters following at speed, leap-frogging from one emergent feeding area to the next.

Table 3. Other types of events where seabirds are observed feeding in the absence of fish shoal activity. (modified from Gaskin 2017).

Event type	Seabird species	Activity
Krill patches	BUSH, FLSH, FAPR, CODP, WFSP, SOSH	Mainly krill and salps with birds actively feeding from the surface, often well-spread, occasionally across several sq. kms.
Current lines	FAPR, FLSH, WFSP	Current lines containing planktonic crustaceans, salps and juvenile fish. Birds actively feeding without prey being visible at the surface.
Common dolphins	FLSH, AUGA, FLSH, BUSH	In contrast to baitfish shoal activity – more sedate feeding activity by the dolphins (with occasional surges). Attendant birds on the surface peering below, sometimes diving in pursuit of prey, or flying to where new action takes place.

AUGA: Australasian gannet, **BLPE**: black petrel, **BUSH**: Buller's shearwater, **CODP**: common diving petrel, **COPE**: Cook's petrel, **FAPR**: fairy prion, **FFSH**: flesh-footed shearwater, **FLSH**: fluttering shearwater, **GRNO**: grey noddy, **RBGU**: red-billed gull, **SOSH**: sooty shearwater, **STSH**: short-tailed shearwater , **WFSP**: white-faced storm petrel, **WFTE**: white-fronted tern.



FLSH, BUSH, FFSH, FAPR, WFSP, RBGU, WFTE
FLSH, WFTE
Zooplankton
AUGA, WFTE, FFSH, FLSH
Krill swarms
Tightly packed trevally and kahawai schools
Small fish
Small fish
Fast moving schools - Kahawai in pursuit of small fish
Tightly packed 'meat balls' (small fish)
BUSH
Fast moving schools - Skipjack tuna feeding on zooplankton and small fish
Mackerel schools feeding on zooplankton
AUGA
Zooplankton incl. benthic & demersal larval fish
Squid
Large fish
AUGA, BUSH, WFTE
Squid (potential prey to cetaceans – seabirds feed on discards)
Large fish (potential prey to marine mammals – seabirds feed on discards)
FAPR, storm petrel spp, shearwater spp
Albatross spp, PAPE, FFSH,

Figure 3 (preceding page): Feeding associations observed over this three-year study (2017 – 2020). Photos (clockwise from top left): Buller's and flesh-footed shearwaters feeding on krill patches; small fishes feeding on krill; NZ fur seal feeding on a John Dory with attendant fairy prion and Cook's petrel; pilot whales with flesh-footed shearwaters.

2.3 Study area

The study area is located off the north-eastern North Island, including the northern Hauraki Gulf (Fig. 4). This includes most of the areas where research work was conducted in previous years projects (INT2016-04 and POP2017-06) and extending out to include the waters around Kawau, Te Hauturu-o-Toi/Little Barrier Island and Aotea/Great Barrier Island. Research on seabird feeding associations and diet has been conducted in this area for several years due to the islands here being important breeding areas for 27 species who then forage in the surrounding waters (Gaskin & Rayner 2013, Forest & Bird 2014).

The wider Hauraki Gulf area is a highly productive marine ecosystem whose productivity is influenced by both wind and current driven circulation. Offshore winds during spring cause upwelling of cool, nutrient rich waters, which, together with increasing daylight, promote high levels of phytoplankton production (Booth & Sondergaard, 1989; Sharples & Greig, 1998). During the summer, the Gulf and the coast are influenced by the warm, nutrient-poor surface waters of the East Auckland Current (EAUC), which are pushed inshore by easterly winds (Chang et al., 2003; Sharples, 1997). The EAUC, combined with downwelling caused by the onshore winds, reduces primary productivity during late summer and autumn (Chang et al., 2003). Physical barriers such as headlands and islands enhance local upwelling, together with tidal currents in the Jellicoe, Cradock and Colville Channels that can attain up to 3 knots (Black et al., 2000; Royal NZ Navy Hydrographic Office Chart NZ53). Sea Surface Temperature (SST) typically ranges from 12.5 to 22° C across the Hauraki Gulf (Paul 1968). A full summary of oceanography of the region is provided in the earlier report for this contract (Taylor & Gaskin, 2020).

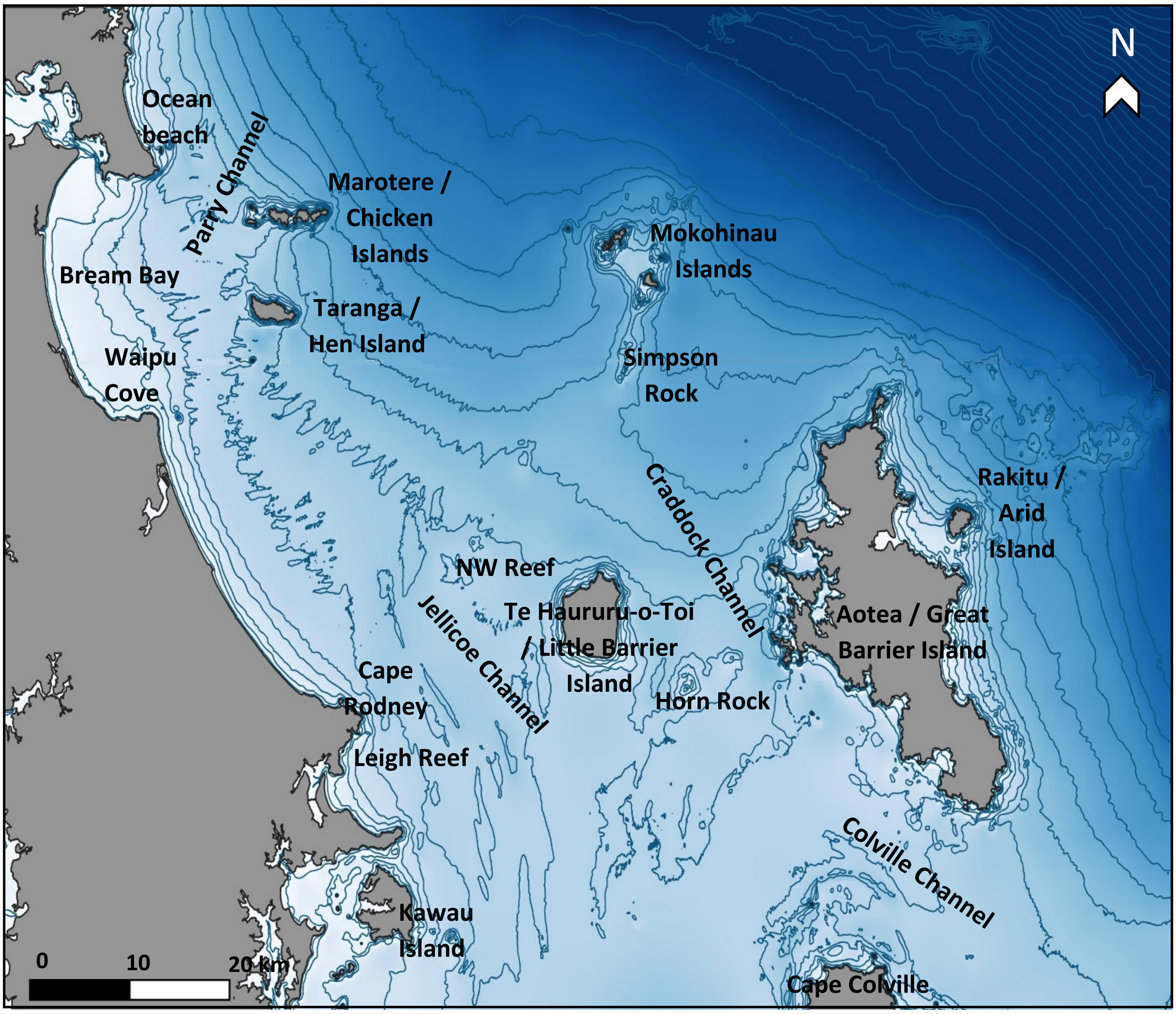


Figure 4. Study area.

3. METHODS

The proposed methodology for Objective 1 of the fish shoal dynamics in north-eastern North Island project was detailed in the Milestone 1 report (Kozmian-Ledward et al., 2019[3]). The methodology was generally conducted as proposed, but with a few modifications, some of which were due to the Covid-19 pandemic. Covid-19 resulted in the cancellation of the second half of the fieldwork season (March – May).

The final design of the "high-speed" zooplankton net was different from that described in the proposed methodology (Kozmian-Ledward et al 2019). Instead of a nested net, the new net was made to the same design as the old "low-speed" net, but with a coarser mesh (1.32 mm versus 0.25 mm) to enable faster towing speeds. It was determined that a nested net would not have worked in this application. The high-speed net was not available until January due to difficulties in obtaining the high strength precision mesh and delays with the net makers.

Instead of broadly categorising zooplankton sampling locations into "workup" and "no workup" as was done in the previous years of work and proposed in Milestone 1, events were categorised into several more detailed types based on seabird and fish activity described by Gaskin (2017) (Tables 2 & 3). These included event types where seabirds were feeding but surface shoaling fish were not present. Figure 5 shows the various inter-linked factors from which data was collected and analysed for this project. The methods for each type of data collection and analysis are given below.

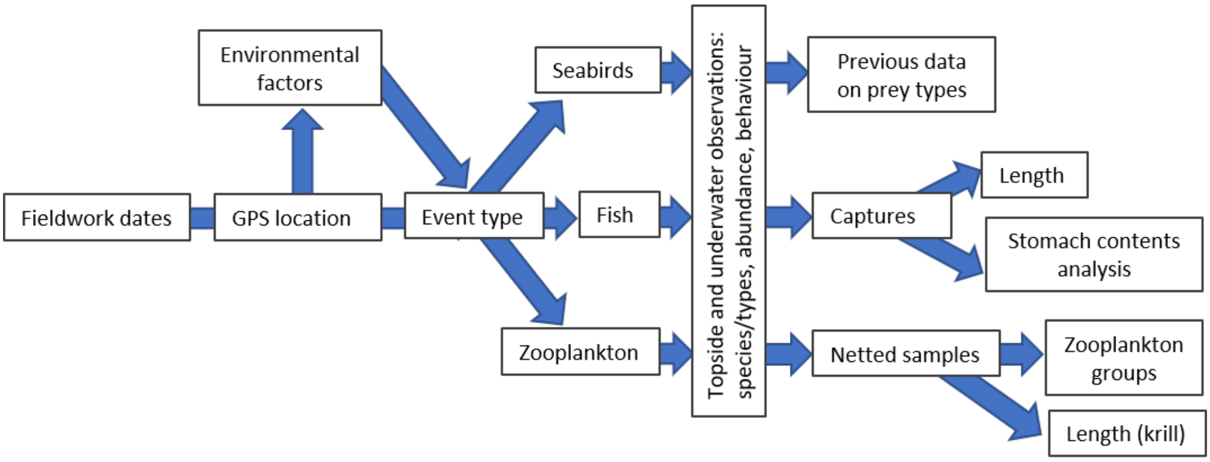


Figure 5: Flow diagram outlining the various inter-linked factors from which data was collected and analysed in this project.

3.1 Field methods

Nine fieldwork days were conducted between 22 November 2019 and 28 February 2020. Figure 6 shows the fieldwork dates and vessel tracks for each day. Day trips were conducted from the charter vessel *El Pescador* (1 day) and the volunteer vessel *Waimania* (3 days) out of Marsden Cove and Omaha respectively. Two multi-day trips (of 2- and 3-day duration) were conducted from the research vessel *Hawere* from Ti Point. The RV *Hawere* is a 15 m research vessel run by the University of Auckland's Leigh Marine Laboratory. Using this bigger vessel allowed us to do overnight trips and this combined with fast vessel speed meant that a large area could be covered to search for fish workup and seabird feeding activity. Four to five team members including the skipper were on these trips, including a dedicated fisher as well as sufficient personnel to undertake the various research tasks. The large back deck/cockpit provided a good working space for sample collection and a small RIB could be stowed and easily deployed without inhibiting plankton net deployment.

Note: The COVID 19 crisis meant trips scheduled for late March, April and early May were not undertaken. These dates coincide with chick-rearing stages for Buller's shearwater, one of the key study species for the indirect effects projects (INT2016-04, POP2017-06 and POP2019-02).

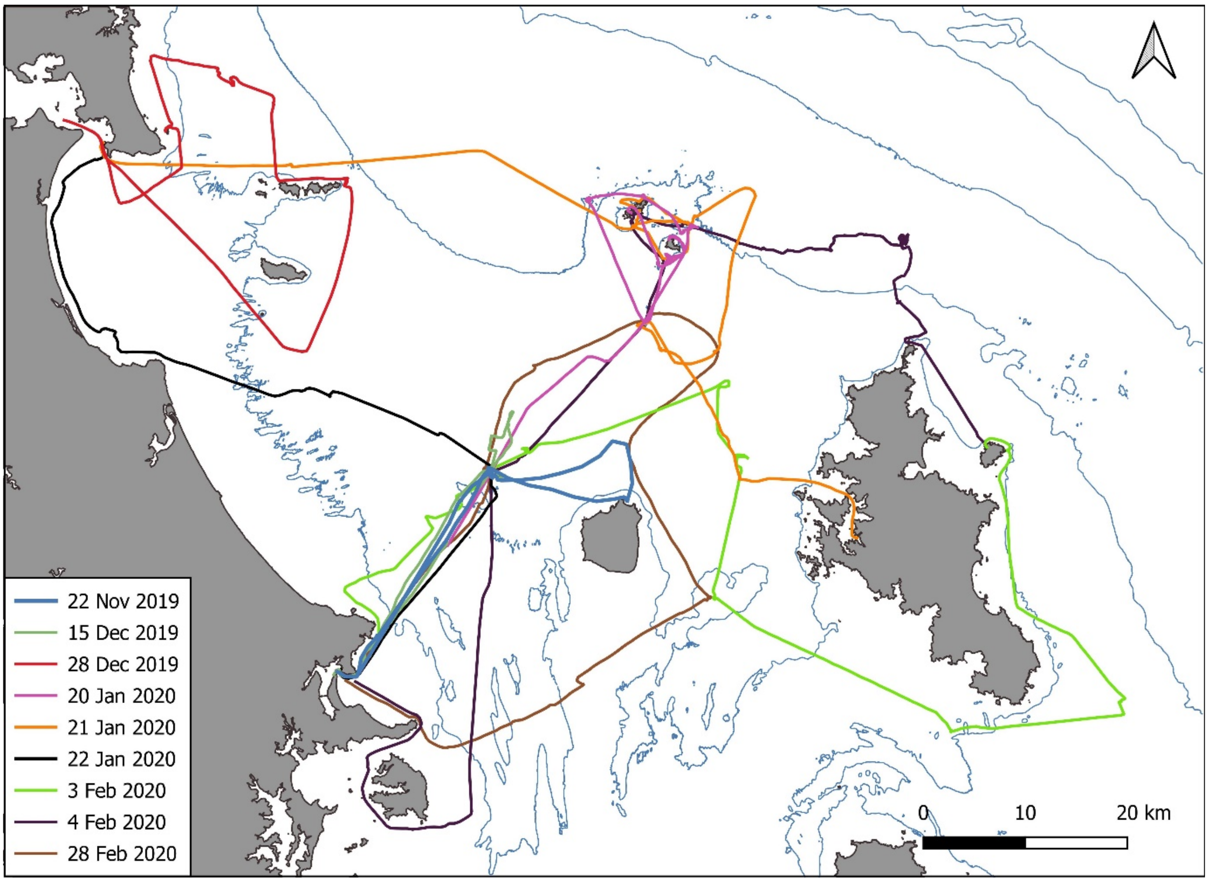


Figure 6: Vessel track-lines for each fieldwork day conducted. Note, an evening passage was undertaken between the Mokohinau Islands and Port Fitzroy, Great Barrier Island, on the 20th January but is not shown on the map due to it occurring mostly in the dark.

Research trips this season were conducted primarily for this project and therefore the sampling work was not opportunistic as it had been previously – i.e. working in with other at sea surveys, island transfers and seabird birdwatching trips. The field methodology was generally conducted in a similar way to the previous two seasons (2017-2018 Gaskin, 2019[1] and 2018-2019 Gaskin & Adams, 2019) but extended to include additional variables described in Milestone 1 and detailed below.

The vessel route was determined by searching for seabird feeding/foraging activity, and where fish activity was observed occurring at or near the surface of the sea. While underway, observers continually scanned the horizon using binoculars and naked eye to search for workups by looking for the presence of seabirds, marine mammals, or disturbances at the sea surface by shoaling fish. Specific locations were targeted where workup activity has been previously located such as Leigh Reef, North-West Reef, Simpson Rock, Mokohinau Islands, Taranga/Hen and Marotere/Chicken Islands, and Parry Channel/Bream Head area. Finding workups can be challenging and the use of high-speed vessels plus the extended range of the RV *Hawere*, together with utilising calm conditions (Beaufort 3 or less) where possible, increased chances of finding multiple workups in a day. Events where there was no surface fish shoaling activity, but birds were feeding such as surface krill patches and current lines (i.e. flow lines visible at the surface, and sometimes with accumulations of algae and other natural debris such as feathers and vegetation) were also opportunistically sampled while looking for workups. Searches for workup activity and subsequent sampling were only conducted during daylight hours. Where possible, the research trips were conducted during calm conditions (Beaufort 3 or less) but this was exceeded at times. The vessel track was recorded on a handheld GPS, at 1-minute intervals except for the first survey trip (22 Nov 2019) where it was recorded at 5-minute intervals.

The vessel track was recorded on a handheld GPS (Garmin GPS 72H) and on arrival at an event the position and time were recorded together with information on the type of activity occurring. Fish species were recorded where possible with their behaviour, for example if they were forming dense shoals feeding at the surface or the activity was quieter and mostly sub-surface. The species of seabirds were recorded, approximate numbers and their behaviour. The presence of other marine megafauna (e.g. cetaceans, manta rays) were recorded. High resolution photographs were taken where possible of the activity and species present. Zooplankton sampling was conducted, and fish were caught during feeding events - further details on these methods are described below. The floating underwater camera rig was deployed at many events to identify fish species in the shoals and to record activity occurring underwater.

Oceanographic data was recorded at many events; a YSI meter was used to measure the SST and salinity, and water clarity was measured using a Secchi disc to the nearest meter. Water samples were taken for chlorophyll-*a* determination with two replicate samples taken at various events/sites. For each replicate, one litre of seawater was filtered through a 0.45 µm, cellulose nitrate filter (25 mm diameter). Filters were kept frozen at -20°C until they could be analysed in the laboratory.

3.2 Zooplankton sampling

Most of the zooplankton sampling was undertaken by horizontal surface net tows (just below the sea surface) using conical plankton nets towed approximately 30 m behind the vessel (n=48) (Fig. 7). Two additional samples were collected using a fine mesh hand net (150 µm mesh) and one vertical haul was conducted. A zooplankton net capable of being towed at faster speeds was designed and built for this season's work and the old 'low-speed' net was also used at times. The duration of the zooplankton tows was generally 5-6 min with the start and finish time recorded to the nearest minute.

The new high-speed net has a mesh size of 1.32 mm and mouth diameter of 750 mm and was towed at around 5 knots. The rationale for having a net that could be towed at a faster speed was to be able to sample the patchy and mobile zooplankton more effectively. With a greater tow speed and therefore manoeuvrability compared to the old net, it was hoped that it would be easier to position the net to pass through the areas of greatest activity and reduce potential net avoidance by larger zooplankton such as krill. For various reasons, the new net was not available until January 2020, therefore the old net was used throughout trips in November and December.

The old low-speed net has a mesh size of 0.25 mm and mouth diameter of 780 mm and was towed at around 2 knots. Both plankton nets were used with a flowmeter (General Oceanics 2030R) mounted in the centre of the net mouth. The addition of the flowmeter this season meant that the volume of water passing through the net mouth was recorded, therefore allowing the number of individual zooplankton per cubic meter of water to be calculated. The flowmeter was not available on two days of sampling.

As in the last season (2018-2019), a tow camera was integrated into the bridle of the net to film any activity at the net mouth. The tow camera consists of a GoPro Hero+ inside a PVC tube, closed at one end, open at the camera end with buoyancy and lead integrated to provide a steady tow. The low-speed net had a tow camera for all trips, but the high-speed net did not have a dedicated tow camera fitted until the last trip when a second dedicated tow camera was made.

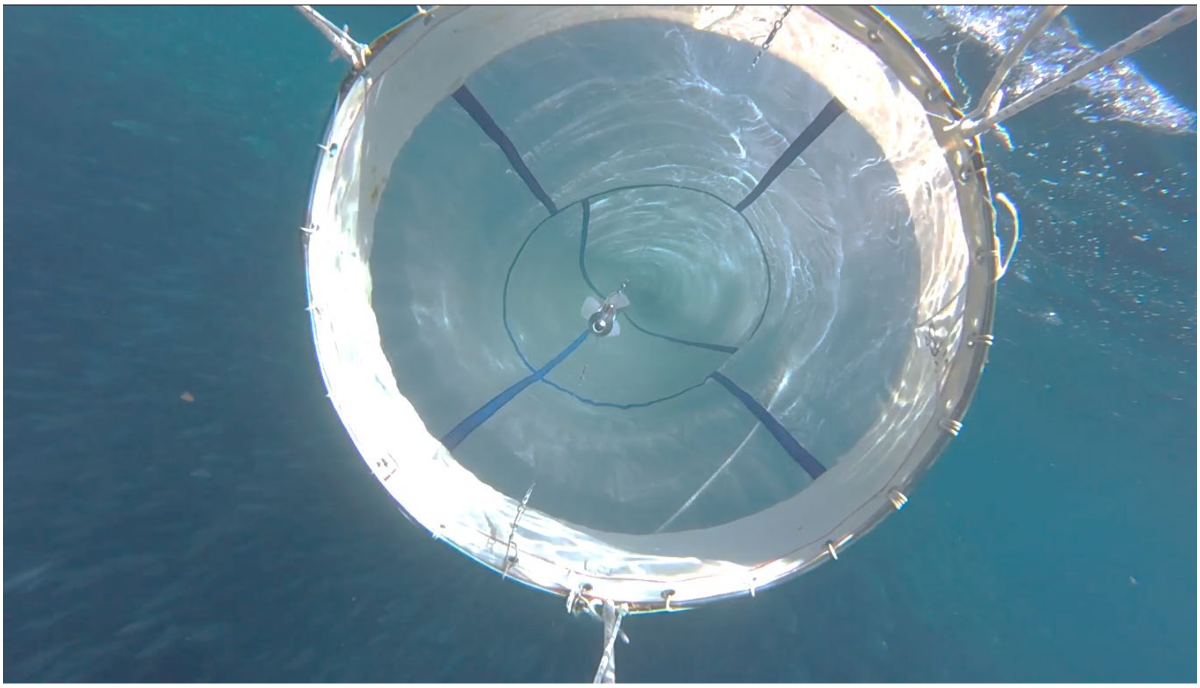


Figure 7: Plankton net with flowmeter, videoed from net camera attached to bridle. Schooling fish are visible in lower left background. *Screenshot from videography: NNZST*

Generally, only one plankton tow was conducted per event encountered. On several occasions however, more than one tow was conducted and with the different nets to compare performance. Control tows were done in one of two ways; either in the vicinity of a previously sampled event where activity was no longer occurring, or as an isolated sample collection where no activity was occurring at locations where activity had been seen on previous days/times.

On the completion of a zooplankton tow, the sample was washed down into the cod end of the net and then transferred to a fine sieve to remove excess water. On several occasions, the sample was so large it had to be transferred to a bucket or fish bin for processing (Fig. 8). The total volume of the sample was recorded, and a subsample taken (typically 300 ml) if the sample was large. Samples for enumeration were preserved in 100% ethanol. Samples were also taken for energy and macronutrient analysis and were kept frozen at -20°C for later analysis.

Figure 8: Krill emptied from the zooplankton net into a 10-litre bucket. *Photo: Lily Kozmian-Ledward*.

3.3 Fish captures

Fish were caught on rod and line (with bait and/or lures) from workups to obtain stomach contents and muscle tissue samples (Fig 9).

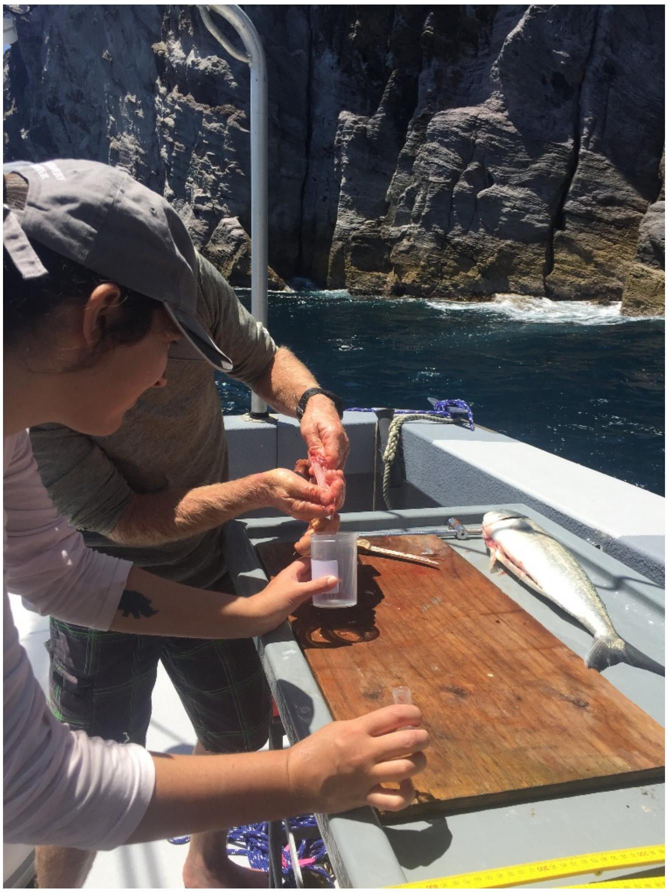


Figure 9. Collecting a stomach sample from a caught kahawai. *Photo: Chris Gaskin*

It had been anticipated that the high-speed net might capture some small 'bait' fish as well as zooplankton, but, aside from larval and small juvenile fish, this did not happen. This may have been due to these fishes not being present at events sampled, or fish avoiding the net. Fishing was undertaken by a dedicated person on the trips undertaken on the RV *Hawere* only as this vessel had sufficient space on the working deck. Fishing was either conducted from the main vessel or from a small outboard powered RIB. When fish were caught, those required for sampling were euthanised immediately by pithing with a spike into the brain cavity. Any other fish caught were returned immediately back to the sea. The length (fork length) and species of all fish landed was recorded. All manipulations were conducted in accordance with the Animal Ethics (AE) permit detailed below and data on fish catches will be reported to the AE Committee. The stomach contents of each fish were immediately removed and stored in 100% ethanol at room temperature for later laboratory analysis. Many of the fish captured had empty or nearly empty stomachs despite being caught where they were presumably feeding. It is possible that they regurgitated their stomach contents between being hooked and landed on the boat. A small sample of fish muscle (approx. 10g) was also removed for later stable isotope or energetic analyses and stored at -20 °C.

Fish captures were covered under the following permits:

- Special Permit 679, Fisheries New Zealand which allows the taking of marine life for the purpose of research.
- Animal Ethics Application 14829, AgResearch with the maximum number of fish captured and killed during the whole research period capped at 440. The total number of fish caught during this season was 18 with 1 released alive and 17 killed.

3.4 Laboratory methods

All samples were stored and processed at the Leigh Marine Laboratory (University of Auckland). The laboratory processing of the zooplankton and fish stomach content samples were done in the same way as the 2018-2019 season (Kozmian-Ledward et al., 2019[2]), with zooplankton samples being subsampled as required and counted into seven taxonomic groups: Copepoda, Malacostraca, Krill Nauplii, Thaliacea, Appendicularia, Fish Eggs and Other (Kozmian-Ledward et al., 2019[2]). A summary of the taxa details of zooplankton included in each of these groups are given in Appendix 1 of Kozmian-Ledward et al. (2019). Larval fish were extracted during the counting process for later identification by Dr. T. Trnski (Auckland Museum). High-resolution photographs of various zooplankton types and larval fishes are presented in Appendices 2 and 3 of Kozmian-Leward et al. (2019[2]). Microplastics were also removed from samples. From each sample containing krill, 10 individuals (if present) were randomly selected, photographed and the length (excluding antennae) measured from the photos using the open-source program Image J (Schindelin et al., 2012). The filters containing the chlorophyll samples were kept at -20 °C until they were analysed using the spectrophotometric laboratory methods and equations from Parsons et al. (1984) to determine the amount of chlorophyll *a* amount in mg m^{-3}.

3.5 Data analysis

The raw counts for each zooplankton group per sample were corrected for the degree of subsampling (in the field and the laboratory) and for the volume filtered by the net, by converting the flowmeter readings using the following equations. Abundances were then expressed as number of zooplankton per cubic metre of seawater sampled.

Equation 1: Distance = Difference in counts x Rotor constant (26,873) / 999999

Equation 2: $\text{Volume } m^{-3} = 3.14 \times (\text{Net diameter})^2 \times \text{Distance} / 4$

To allow comparison with previous years data (and for those samples taken this year without flowmeter data), the proportional abundance (as a percentage of the total count of individuals) was also calculated for each zooplankton group per sample.

Data on zooplankton, fish and seabirds were collected as described in the Milestone 4 report (Kozmian-Ledward et al., 2020). Methods for the additional analysis of data are given here.

3.6 Categorical analysis

Categorical analyses were undertaken to determine statistically significant associations between zooplankton, fish, seabirds and physical variables. Data for these analyses were derived from those sampling events for which the full suite of data was available, i.e., zooplankton tows with a flowmeter in addition to seabird and fish observations. These sampling events comprised several types as described in the interim report: those with active fish shoaling and seabird feeding activity (mixed fish shoal, kahawai school and tuna school events), and 'quieter' events with little to no fish and/or seabird activity (current line, krill patch and control events).

For each sampling event, the abundance of each major group of zooplankton was standardised as the number of organisms per cubic metre of water filtered by the zooplankton net. These zooplankton groups were the same as those described in the interim report: Copepoda, Malacostraca, Krill nauplii, Thaliacea, Fish eggs and Other.

The species of seabirds and fish present at each sampling event were categorised as dominant (most abundant) or secondary (present in good numbers but not the most abundant) based on visual observations of birds from the vessel and fish from the underwater video recordings.

Seabird species present at sampling events and included in the analyses are listed below. Identification code is given in brackets:

- Australasian gannet (AUGA)
- Black petrel (BLPE)
- Buller's shearwater (BUSH)
- Cook's petrel (COPE)
- Diving petrel (DIPE)
- Fairy prion (FAPR)
- Flesh-footed shearwater (FFSH)
- Fluttering shearwater (FLSH)
- Little penguin (LIPE)
- Red-billed gull (RBGU)
- Short-tailed shearwater (STSH)
- Sooty shearwater (SOSH)
- White-faced storm petrel (WFSP)
- White-fronted tern (WFTE)

Fish species present at sampling events and included in analysis:

- Albacore tuna

- Blue knifefish
- Blue maomao
- Juvenile fish species
- Kahawai
- Kingfish
- Koheru
- Mackerel species
- Pink maomao
- Snapper
- Two-spot demoiselle
- Trevally
- Skipjack tuna

The physical variables: depth, distance from shore, seabed slope, and rugosity were obtained from a bathymetry raster (NIWA NZ bathymetric grid at 250 m resolution) and coastline layer (LINZ Topographic dataset). GIS layers for seabed slope and a ruggedness index (Riley et al., 1999) were created from the bathymetric raster using tools in QGIS. GIS layers were sampled using QGIS to obtain data on each physical variable at the GPS position for which each event was first encountered. Tidal information was calculated to the nearest hour +/- high water from each event start time using Auckland tide times (LINZ).

Unfortunately, on account of a truncated field season, the relatively small number of sampling events in relation to the number of different response variables that we were attempting to compare, only associations between zooplankton, fish and seabirds could be included in the analyses described below. Consequently, comparisons of the biological response variables (fish, birds, zooplankton) with the physical variables (depth, distance from shore, seabed slope etc) were excluded from the final analyses to retain sufficient statistical power. Permutational multivariate analyses of variance (PERMANOVA) using distance matrices were performed using Vegan (version 2.5-6; Oksanen et al., 2019) to identify any significant differences between the zooplankton and for each category of birds and fish. Significant differences ($P < 0.05$) were detected for the categories, secondary birds, primary fish, and secondary fish. There were no significant associations identified for any combination of three variables from zooplankton, bird and fish categories.

A generalized linear model (GLM) using Quasi-Poisson was then used to identify the significant interactions within these categories and the resulting data were explored using emmeans (version 1.4.3; Lenth, 2019). All statistical analyses were run in R Studio® (version 3.6.1; R Core Team, 2018).

3.7 Krill length

The body length of krill can be used as a proxy for life-cycle stage. For example, Brinton et al. (2000) gives size ranges for the different life-stages of *N. australis*, one of the most common krill species found in New Zealand waters. For each zooplankton sample containing krill, 10 individuals (if present) were randomly selected by eye and the body length (excluding antennae) measured as described in Kozmian-Ledward et al 2020 (the interim report for POP2019-02).

3.8 Prey selectivity of fishes

Where fish were caught in conjunction with zooplankton tows Ivlev's selectivity index (Ivlev, 1961) was used to compare the relative proportions of zooplankton groups between fish gut contents and the surrounding waters as measured from the associated zooplankton net sample from the same sampling location. Where more than one zooplankton tow was undertaken at a relevant sampling event, the relative proportions of the zooplankton groups present were combined by averaging before comparing them to the fish gut contents from that event.

Ivlev's selectivity index was calculated using the formula below:

$$E_i = (r_i - P_i) / (r_i + P_i)$$

Where, Ei is the Ivlev's selectivity index, ri is the relative abundance of prey i in the gut of the fish caught and Pi is the relative abundance of the prey in the water at the event sampled. Observed values range from -1 to 1, where -1 indicates prey avoidance, 0 indicates that a prey type is being ingested at the same proportion as it is found in the environment, and 1 indicates a preference for a specific prey type.

4 RESULTS

Nine survey trips were conducted between 22 November 2019 and 28 February 2020 covering an area between Kawau Island, Bream Islands, Mokohinau Islands, Great Barrier Island and Little Barrier Island (Fig. 6).

4.1 Seabird feeding events

Fifty-two seabird feeding events were recorded over all the survey trips. Thirty-five of these were surface fish shoal events (mixed shoal, kahawai school or tuna school) (Table 2, Fig. 10), and 17 were of other types (common dolphin, current line, krill patches or "unknown") (Table 3, Fig. 12). Where an event was spread over a wide area more than one observation/data collection was often made and designated A, B, C etc. (e.g. the single tuna school event). Occasions where cetaceans were seen with no seabird association (bottlenose dolphins, n = 3; Bryde's whales, n = 2; common dolphins, n = 1) were recorded but not included in this analysis. Dorsal fin identification photos of Bryde's whales and bottlenose dolphins, together with location and behavioural information from these events and were sent to Assoc. Prof. R. Constantine (University of Auckland) who curates fin ID catalogues for these species.

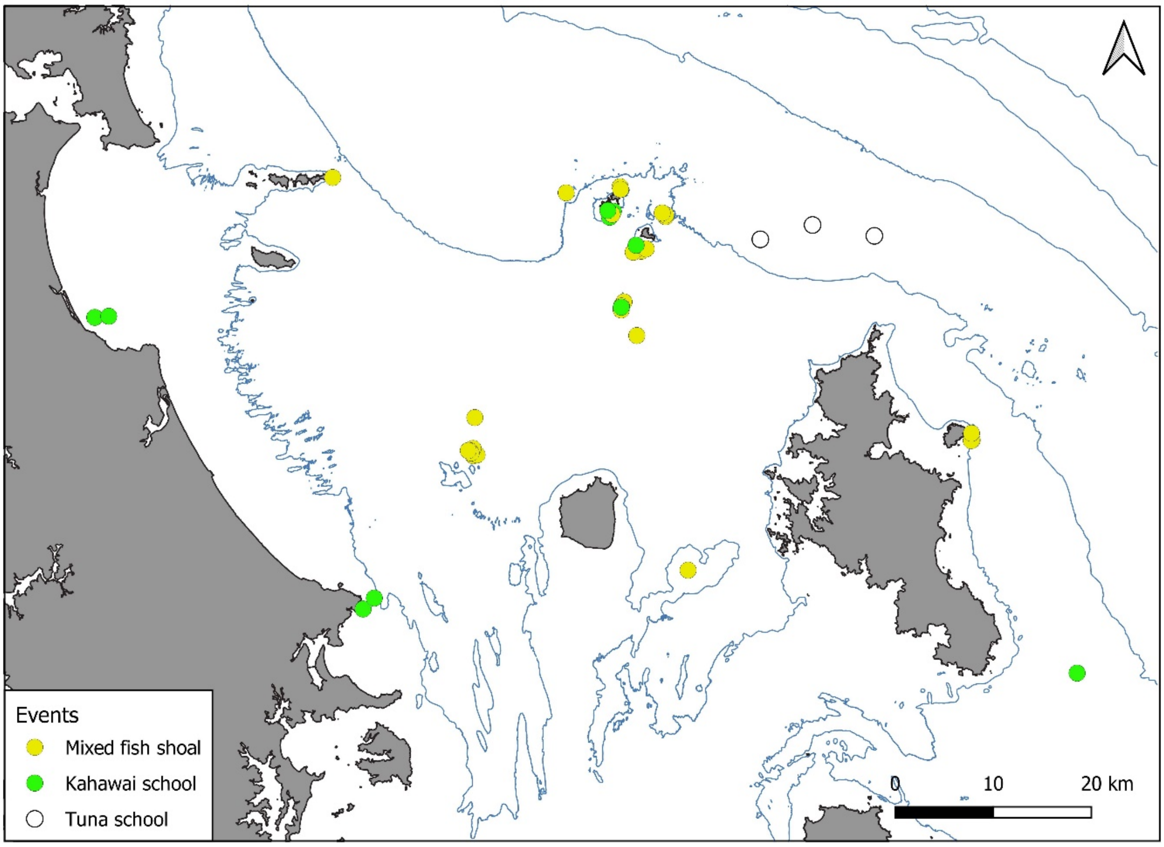


Figure 10. All seabird feeding events encountered during the field research period.

4.1.1 Mixed fish shoal

Twenty-four mixed fish shoals were found throughout the research period, all located at least 12 km away from the mainland (Fig. 11). Key areas were the Mokohinau Islands and Northwest Reef with events also found at Horn Rock, Arid Island and Coppermine Islands. These locations are all in areas of current flow around islands or over underwater reefs and pinnacles. Activity ranged from highly dynamic with multiple fish shoals and large numbers of birds feeding to small quieter shoals that were easily disturbed by the boat. The seabirds present and their activity generally followed that described in Table 2.

Figure 11. Mixed kahawai and trevally school. *Screenshot from videography: NNZST*

4.1.2 Kahawai school

Ten kahawai schools were found throughout the research period. Nine of these schools were in depths of 10 – 50 m, near the mainland coast (off Leigh and Waipu Cove), at Northwest Reef and in the Mokohinau area. An additional school was also found in deeper water (80 m) in the Colville Channel. As with the mixed fish shoal events, fish and seabird dynamism varied between events. Seabirds present and their activity generally followed that described in Table 2.

4.1.3 Tuna school

A single tuna school event was found in February, north of the 100 m depth contour, with widespread and scattered activity, extending at least 15 km along the track line. Three separate observations (data recordings) were made over the course of an hour, while travelling through the scattered school. The tuna here were mixed albacore and skipjack, rather than just skipjack described in Table 2, however, the activity was similar. The majority of seabirds were seen were Buller's shearwaters, chasing prey (likely small fish) near the water's surface (cover image). The birds appeared attracted to the splashes made by the tuna.

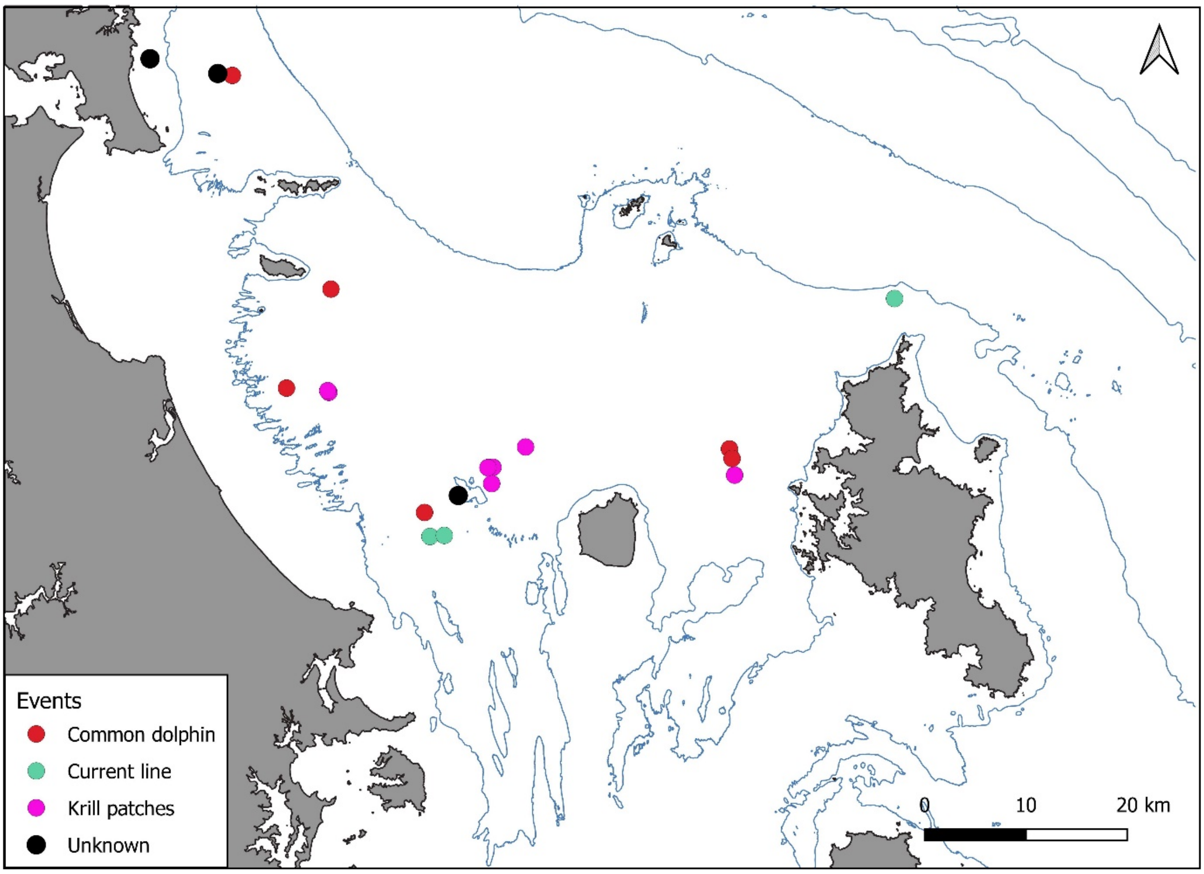


Figure 12. All other events encountered during the field research period.

4.1.4 Common dolphins

Five common dolphin events were encountered from late December onwards, all in areas greater than 50 m depth and in open water. Dolphin activity was generally sedate, with some feeding activity with seabirds following, sometimes spread over a wide area, and tended to comprise groups of less than 50 dolphins. Seabird species associating with the common dolphins included fluttering, flesh-footed, Buller's and short-tailed shearwaters and gannets.

4.1.5 Current lines

Three areas of current lines were encountered throughout the research period, all on calm days in the Jellico Channel and off northern Aotea Great Barrier Island, both are areas of higher current flow. White-faced storm petrels were the most common bird present, feeding on unknown small prey.

4.1.6 Krill patches

Six areas with krill patches were encountered in late January and early February, all in areas of current flow, and during calm conditions. Krill could be seen at the surface over large areas with scattered fluttering shearwaters, Buller's shearwaters and flesh-footed shearwaters feeding while sitting on the water. On one occasion (22 January 2020) there were large numbers of birds, mostly Buller's shearwaters, spread across a wide area in very calm conditions, feeding in scattered small groups (<10), pecking at the krill at the surface (Fig. 13). Small fish (mackerel sp) could be seen at times also feeding on the krill (Fig. 14). On one occasion near Northwest Reef (3 February), a manta ray (*Mobula birostris*) was observed feeding on the krill – doing 'somersaults' at the surface and also detected by the underwater camera rig swimming beneath a krill patch (Fig.15).

Figure 13. Buller's shearwaters feeding on krill. *Photo: Chris Gaskin*

Figure 14. Small mackerel sp. feeding on krill at the surface. *Screenshot from videography: NNZST*

Figure 15. Manta ray swimming beneath a krill patch just below the surface. *Screenshot from videography: NNZST*

4.1.7 Unknown

On three occasions, the seabird feeding activity seen did not fit any of the categories and no fish were seen at the surface. These events were classified as "unknown". On the 28 December 2019, two events were encountered off Ocean Beach where fluttering shearwater were undertaking prolonged dives, potentially pursuing small fish. Fish were seen mid-water on the depth sounder. On 3 February 2020 in the Jellico Channel, Buller's shearwaters were feeding at the surface and Australasian gannets were diving.

4.2 Environmental measurements

Sea surface temperature ranged between 19.3 – 22.7°C and showed a general increase during the research period (Fig. 16C). A much lower SST than others (on that day) was recorded at E-59 (20.6°C, Colville Channel, 03/02/2020) together with low water clarity (11 m) (Fig. 16B) and high chlorophyll concentration (0.76 mg/m_3) (Fig. 16A), indicating the upwelling of cooler, nutrient-rich water here. A slightly higher SST than others (on that day) was recorded at E-31 (20.0°C, Maori Rocks, 20/01/2020) together with a higher water clarity and low chlorophyll concentration (0.21 mg/m_3) which may indicate the influence of warm, nutrient-poor EAUC water. This same pattern was also seen at E-59 (Simpson Rock, 28/02/2020). Unfortunately, the salinity measurements taken were later deemed to be inaccurate due to incorrect calibration and therefore are not shown.

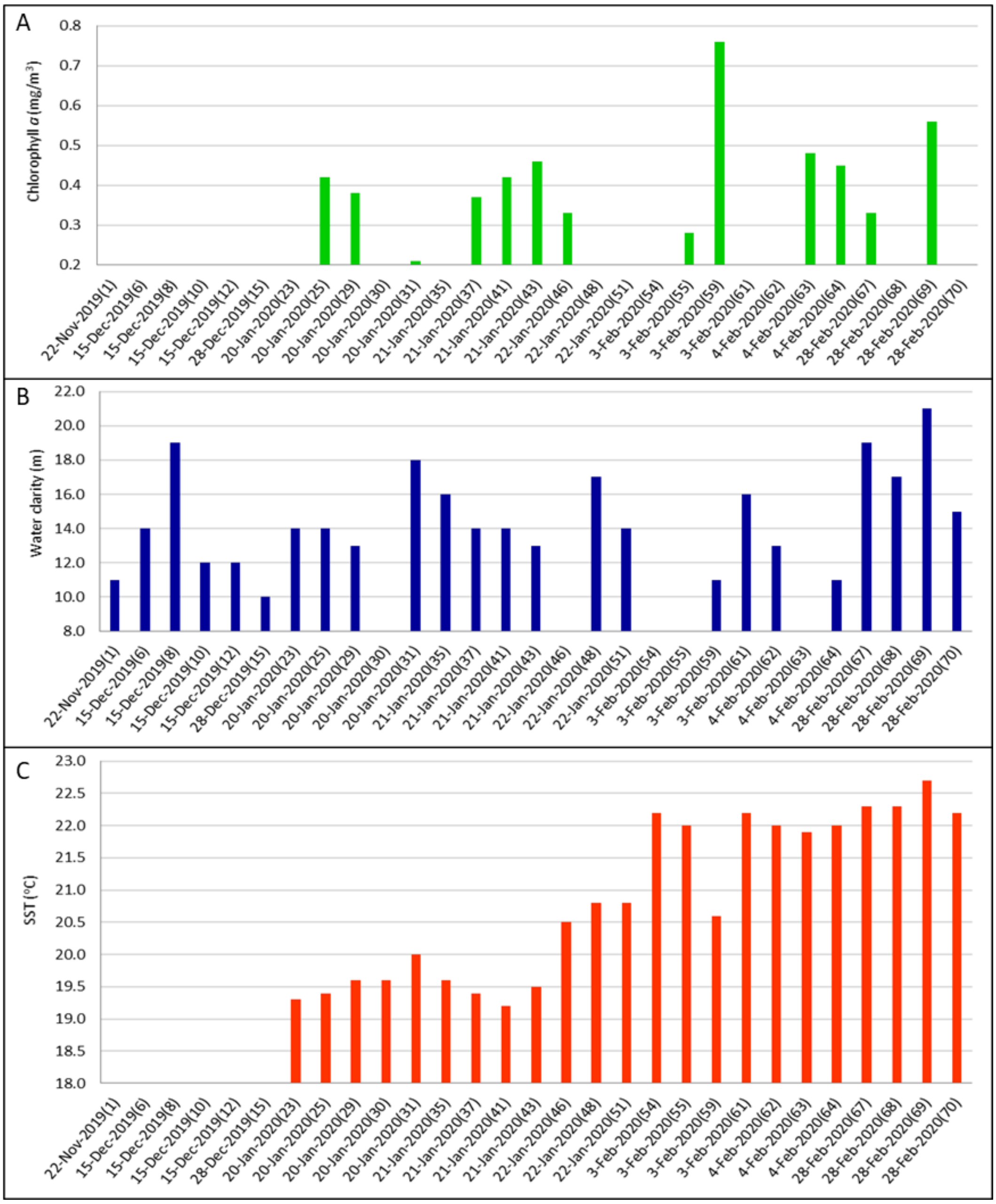


Figure 16. Environmental readings taken at event locations; from top: A. chlorophyll *a*, B. water clarity and C. SST. Note that the y-axes do not start at zero.

4.3 Zooplankton samples

A total of 50 zooplankton samples were collected at 33 seabird feeding events and at eight control sites (Table 4). Samples were taken at three types of fish shoal events (Figs. 18, 19, 20, 21): mixed fish shoal (n = 21), kahawai school (n = 9) and tuna school (n = 1). Zooplankton samples were also taken at three other event types (Figs. 22, 23, 24): krill patches (n = 8), current lines (n = 2), and unknown (n = 1). Of the control tows undertaken, four were direct controls to zooplankton tows conducted in mixed fish shoals, and four were indirect controls i.e. done in areas where mixed fish shoal activity had been seen on previous occasions. Twenty-four samples were taken in total using the low-speed net and 23 with the high-speed net. Additionally, two samples were collected with a fine-mesh hand net and one via a vertical haul from 30 m depth using the low-speed net.

General observations across all zooplankton samples:

- Copepoda present in 68% of samples, generally low proportions/abundances.
- Malacostraca present in 96% of samples, often at high proportions/abundances. Krill at various life stages often the most common, also decapod shrimp larvae, stomatopod larvae, amphipods, crab megalopa and zoeae.
- Nauplii (krill) present in 22% of samples, at both low and high proportions.
- Thaliacea present in 100% of samples, often at high proportions/abundances. The majority were salps of varying sizes.
- Appendicularia present in 8% of samples, generally at low proportions/abundances.
- Fish eggs were present in 56% of samples generally at low proportions/abundances.
- Zooplankton in the Other group were present in 66% of samples, generally at low proportions/abundances. Other zooplankton included siphonophores, arrow worms, cladocera, pteropods, barnacle and echinoderm larvae, and larval fish.

As would be expected, the coarser mesh of the high-speed net resulted in generally lower catches of the smaller zooplankton in the following groups: Copepoda, Nauplii, Appendicularia, Fish eggs and Other.

Table 4: Summary of zooplankton samples: event type and sampling method.

Event type	Number of events sampled	Number of zooplankton samples				
		Low-speed net	**High-speed net**	**Hand net**	**Vertical haul**	**Total**
Trevally / Mixed shoal	16	11	10	0	0	21
Kahawai school	8	4	5	0	0	9
Tuna school	1	0	1	0	0	1
Krill patches	5	2	3	2	1	8
Current line	2	2	0	0	0	2
Unknown	1	1	0	0	0	1
Control	8	4	4	0	0	8
Total	41	24	23	2	1	50

4.3.1 Fish-shoal events

4.3.1.1 Mixed fish shoals

Twenty-nine zooplankton samples were taken using either the high- (n = 10) or low-speed net (n = 11) at 16 of the 24 mixed fish shoals encountered (Table 4, Fig. 17). Relative abundance was calculated for all samples (Fig. 18) and abundance (number of zooplankton per m^3) for 21 samples (as samples from 22/11/19 and 28/12/19 had no flowmeter data) (Fig. 19). At four fish shoal events (1, 3, 15, 25), replicate samples were taken. Direct control samples were taken for four events (1, 15, 25, 31). Four indirect control samples were also taken (events 6, 8, 67, 70).

Samples were generally dominated by either Malacostraca (predominantly krill) or Thaliacea (predominantly salps). Malacostraca were generally more abundant in samples taken between 28 December and 3 February with 79% of these samples containing a relative proportion between 50 and 98% Malacostraca. Abundance calculations for the same timeframe give values up to 90 Malacostraca zooplankton per m^3. Locations with high proportions/abundances of Malacostraca were various sites around the Mokohinau Islands, E side Coppermine Island and NE Arid Islands. The maximum Thaliacea abundance was 21 ind. per m^{-3}.

Two samples, both taken with the low-speed net, were dominated (% abundance) by Nauplii (NW Reef, 22 November). Abundance of Copepoda was generally low (≤ 4.37 ind. per m^{-3}). Appendicularia was abundant in only one sample only, with 25.12 ind. per m^{-3} at Event 33 (20 January at Maori Rocks). This event also had the highest abundance of Fish eggs for all Mixed shoal events (4.50 ind. per m^{-3}). All other samples had ≤ 0.63 ind. per m^{-3} fish eggs. Zooplankton abundance from the Other category were all low, ≤ 0.11 ind. per m^{-3}.

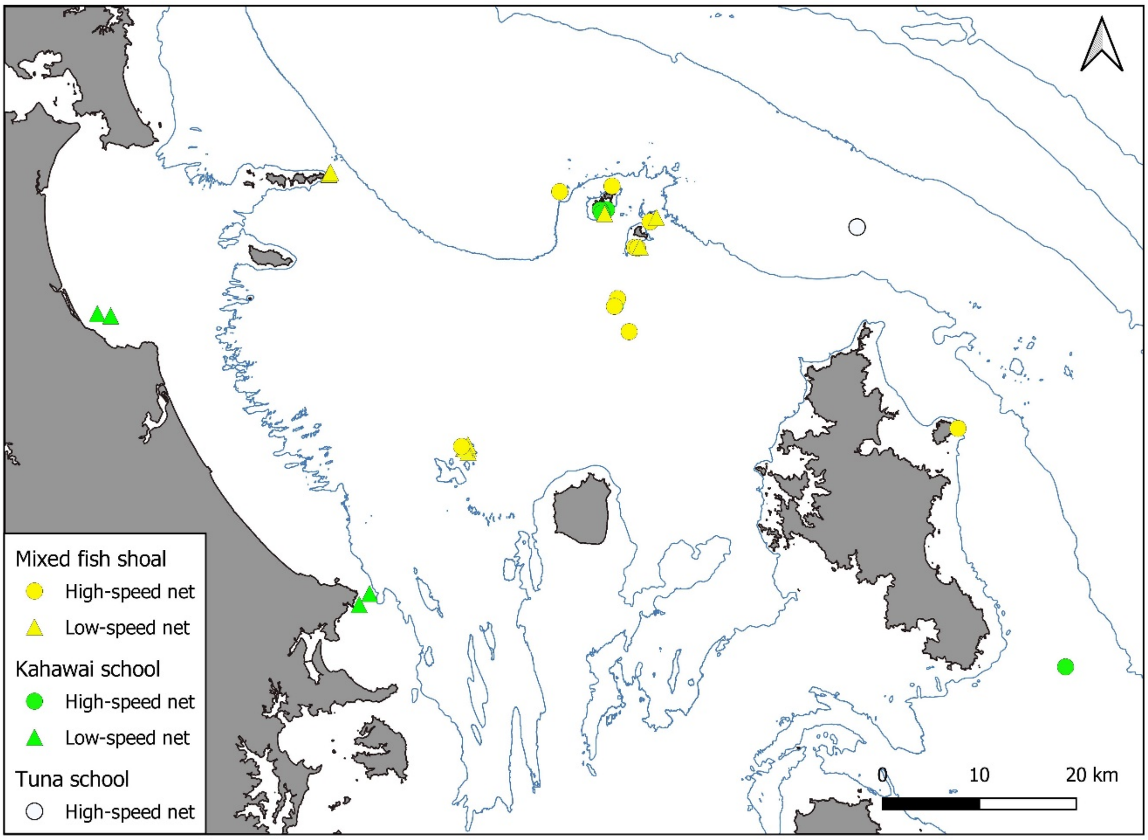


Figure 17: Location of zooplankton samples taken at fish shoal events and control locations with sampling method defined.

All the control samples whether indirect or direct, had less Malacostraca than samples taken at Mixed fish events. Of the direct controls, 2 had flowmeter data (25 & 31) and can be compared by abundance values. At event 25 the mean abundance of the three zooplankton tows conducted at fish shoal activity was 69.4 ind. per m^3 while the control tow only contained 1.1 ind. per m^3. For event 31 there were much less Malacostraca overall but still a far lower abundance in the control tow, 7.7 versus 0.02 ind. per m^3. For the other two direct controls (1 & 15), only relative abundance data can be compared but in both cases the control percentage was much lower than the corresponding samples taken the fish shoal activity. The indirect control samples were taken in the region of NW Reef (6, 8, 67) and Horn Rock (70). All had low total abundances of zooplankton (≤ 4.5 ind. per m^3) and low abundances of Malacostraca (≤ 0.2 ind. per m^3) compared to the majority of the samples taken in Mixed fish events.

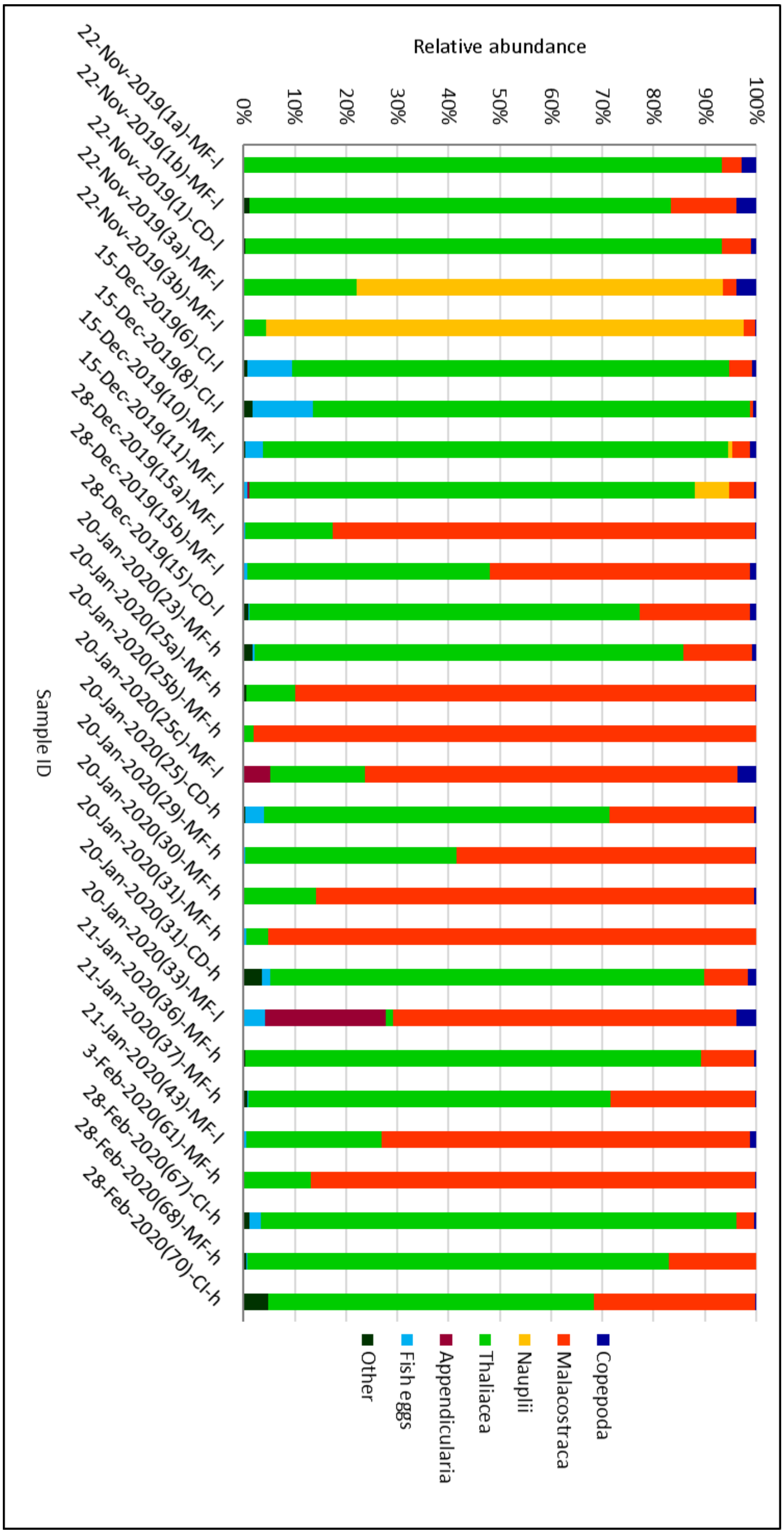


Figure 18: Relative abundance of zooplankton groups in samples taken from mixed fish shoals and controls. The sample ID gives the date, event number, event type (MF – mixed fish, CD – direct control, CI – indirect control) and sampling method: h – high-speed net, l – low-speed net). Where more than one sample was taken at an event this is designated as a, b, etc.

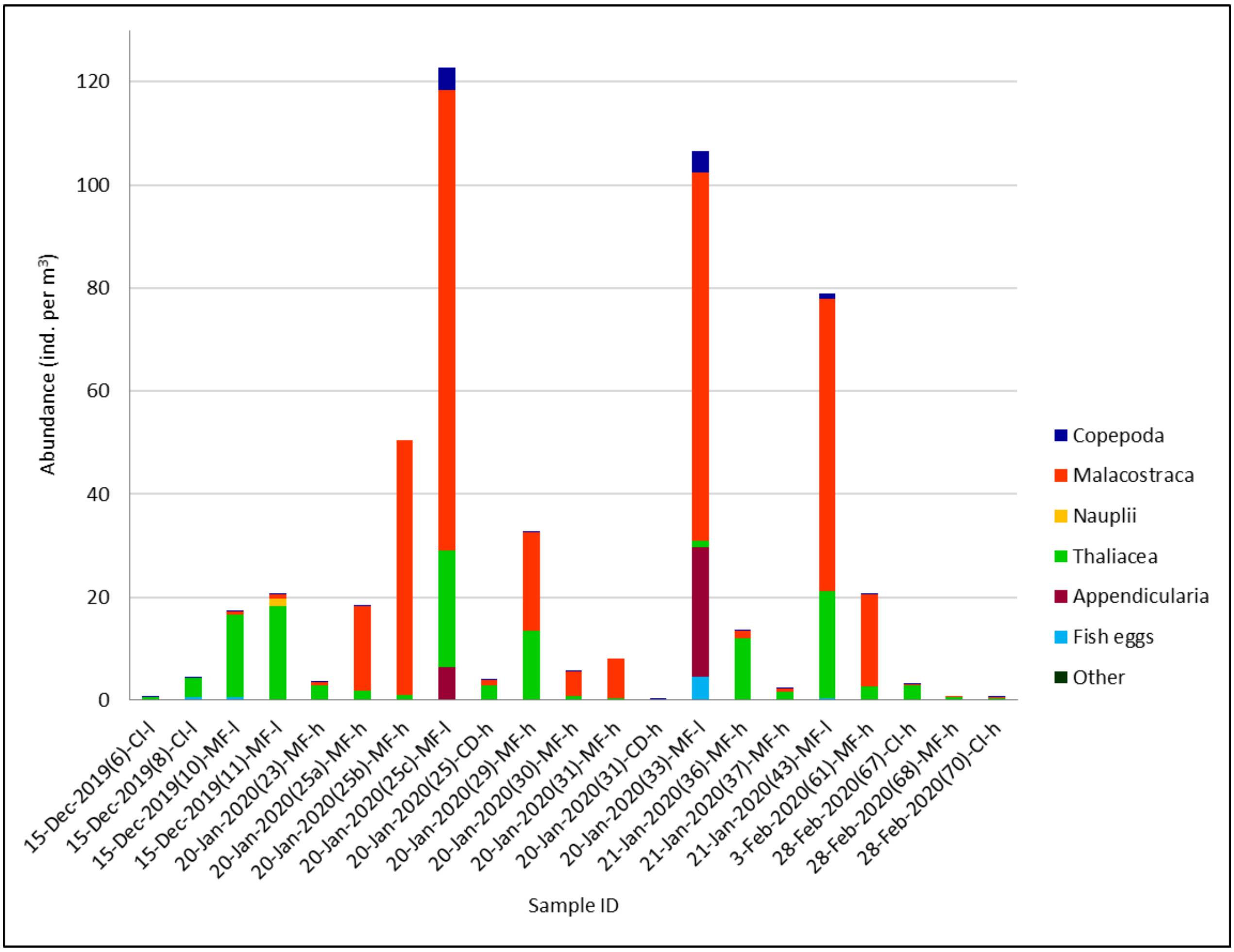


Figure 19: Abundance of zooplankton in each group for samples collected in mixed fish schools (MF) and controls (direct – CD and indirect – CI).

At four fish shoal events (1, 3, 15, 25), multiple zooplankton tows were undertaken (Figs 18, 19). All replicate tows showed broadly similar compositions but had the greatest variation in proportions/abundances of Malacostraca and Thaliacea. An exception to this were the samples taken at event 3 (NW Reef, 22/22/19) with the low-speed net. Both samples contained very high relative abundances of krill nauplii and low abundances of Malacostraca, but the Malacostraca (krill) comprised the greatest wet biomass.

At event 25, tows were conducted with both the low- (n = 1) and high-speed net (n = 2) and this enabled a direct comparison between the net types. The low-speed net captured a higher total abundance of zooplankton: 122.8 versus a mean of 34.3 ind. per m^3 with the high-speed net tows. The abundance of Malacostraca captured by the low-speed net was more than double that of the mean of the two high-speed net samples. Indirect comparisons of other low- and high-speed net samples from Mixed fish shoal events also shows generally higher abundances of Malacostraca captured by the low-speed net.

4.3.1.2 Kahawai schools

Nine zooplankton samples were taken using either the high- (n = 5), or low-speed net (n = 4) at 8 of the 10 kahawai schools encountered (Table 4, Fig. 17). Relative abundance was calculated for all samples (Fig. 20) and abundance (number of zooplankton per m^3) for 8 samples (as the sample from 22/11/19 had no flowmeter data) (Fig. 21).

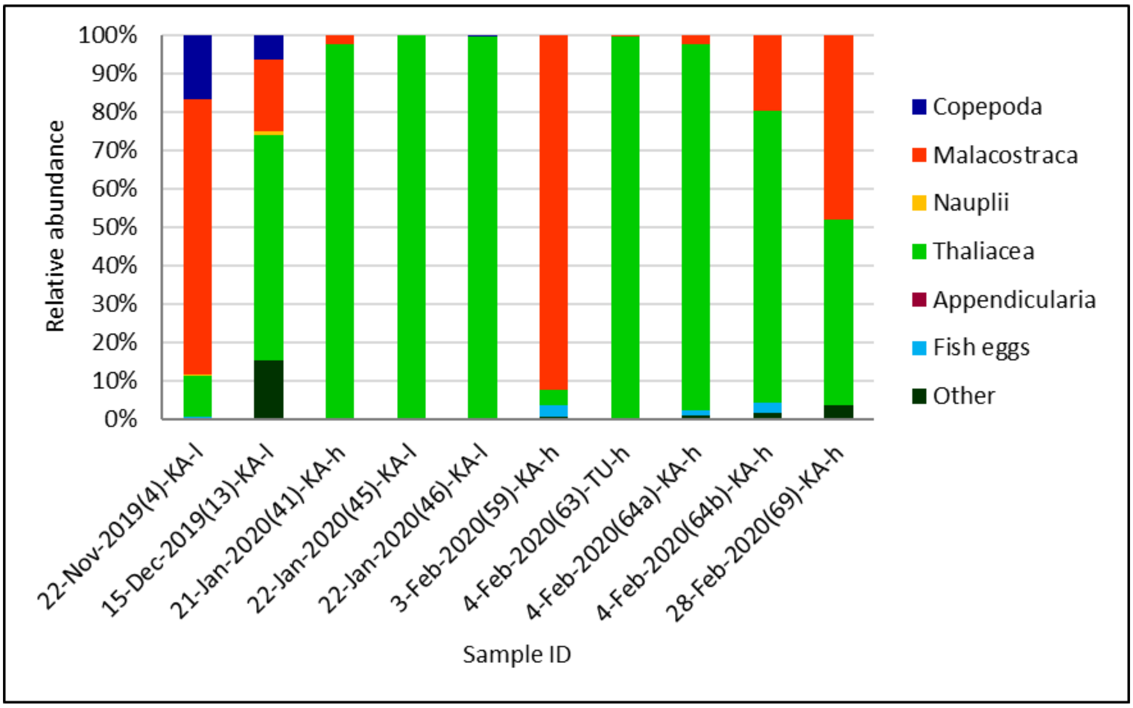


Figure 20: Relative abundance of zooplankton groups in samples taken from kahawai (KA) and tuna (TU) schools. Sampling method designated as: h – high-speed net, l – low-speed net.

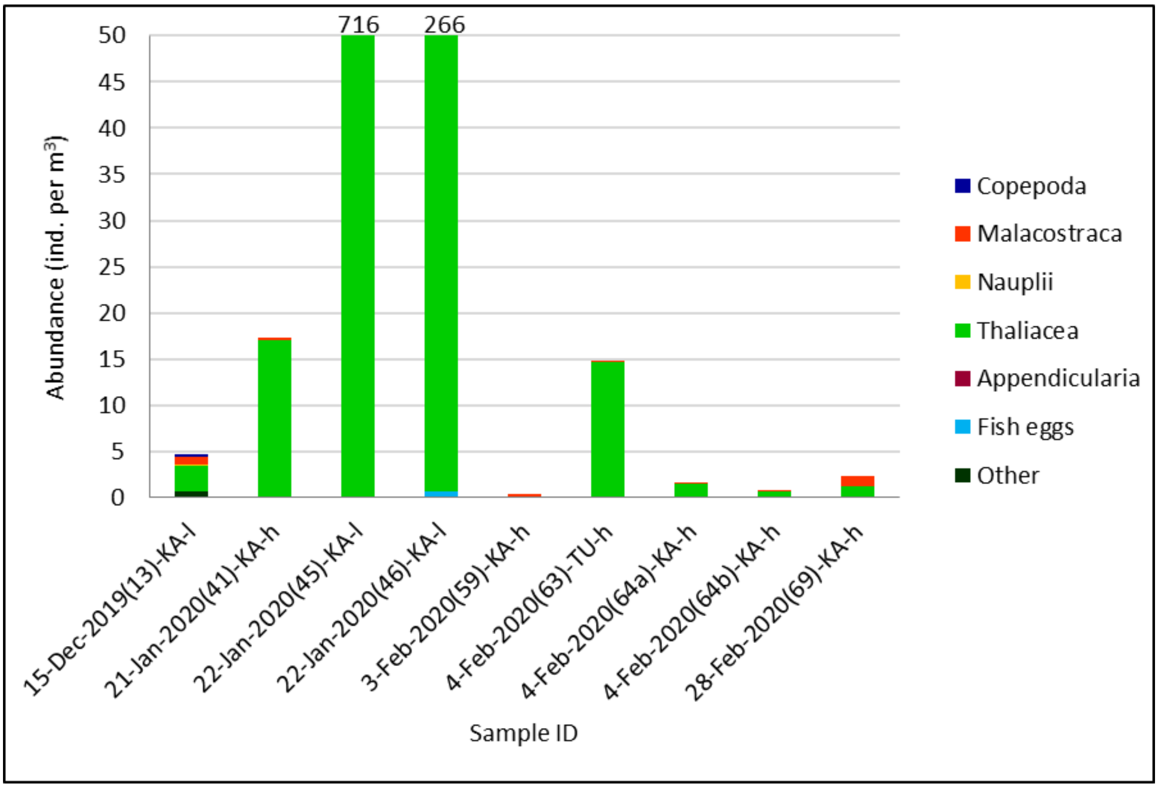


Figure 21: Abundance of zooplankton in each group for samples taken in kahawai (KA) and tuna (TU) schools. The total abundance of Thaliacea for samples 32 and 33 are given above.

Samples were generally dominated by Thaliacea and had low abundances of Malacostraca and the other zooplankton groups, except for that from event 4 (Leigh Reef, 22/11/19) that contained a large volume of krill. Comparing the performance of the two different nets shows that the low-speed net captured higher abundances of zooplankton, mostly Thaliacea.

4.3.1.3 Tuna school

One zooplankton sample was taken with the high-speed net at the single tuna school event. The sample contained mainly Thaliacea (14.64 per m^3), and a low abundance of Malacostraca (0.08 per m^3). No other zooplankton groups were present in the sample.

4.3.2 Other events

4.3.2.1 Krill patches

Eight zooplankton samples were taken at five of the six krill patch events encountered (Figs. 22, 23, 24).

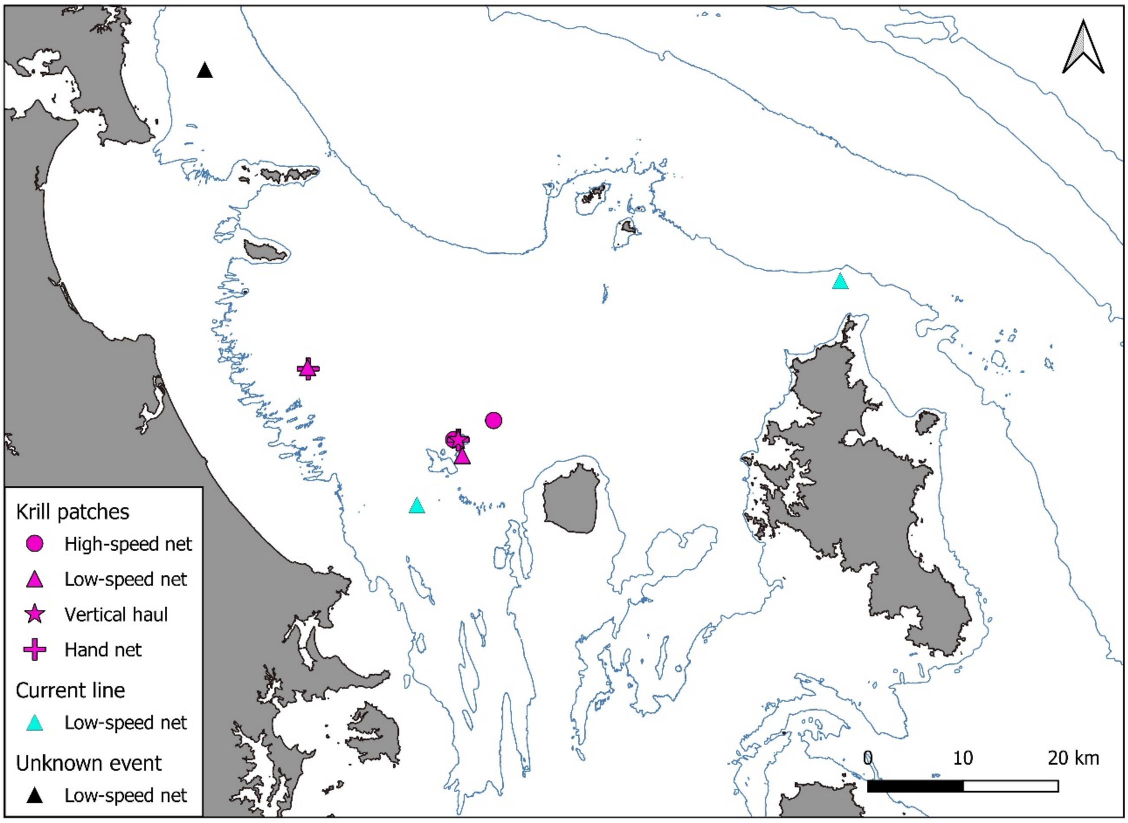


Figure 22: Location of zooplankton samples taken from non-fish shoal events with sampling method.

Of these, five were via net tows: three with the high-speed net, and two with the low-speed net. All these samples (except for the high-speed tow at E-55), had low abundances of Malacostraca ($\leq$ 0.51 per m^3) and were mainly comprised of Thaliacea. At event 55 (NW Reef region, 03/02/2020), a huge sample of Malacostraca was obtained with the high-speed net, approximately 7 liters wet volume and 1993 per m^3, predominantly krill. This was by far the greatest abundance of Malacostraca obtained during this research season. This sample also contained the highest abundance of krill nauplii – 3.08 per m^3. Two samples (35, 54a) were taken with a hand-net, scooping zooplankton directly from krill patches. Both samples were comprised predominantly of krill with one also containing a high proportion of krill nauplii. One vertical haul was undertaken using the low-speed net (NW Reef, 03/02/2020) and contained mainly Copepoda and Thaliacea.

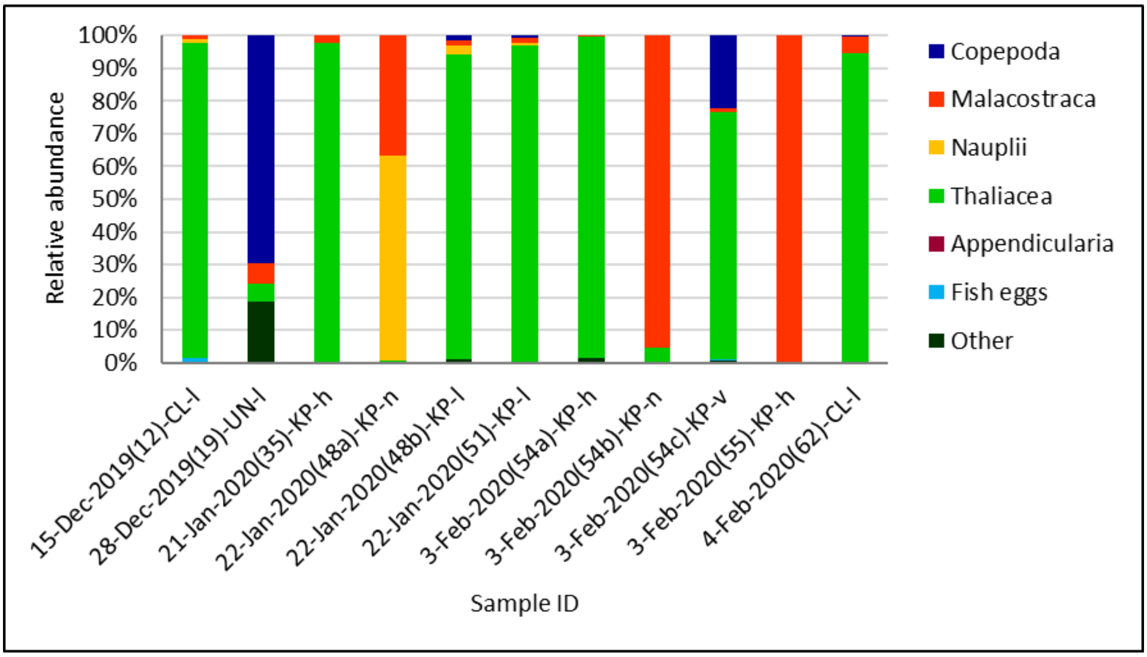


Figure 23: Relative abundance of zooplankton groups in current lines (CL), krill patches (KP) and unknown (UN) events. Sampling methods: h – high-speed net, l – low-speed net, n – hand net, v – vertical haul. Where more than one sample was taken at an event this is designated as a, b, etc.

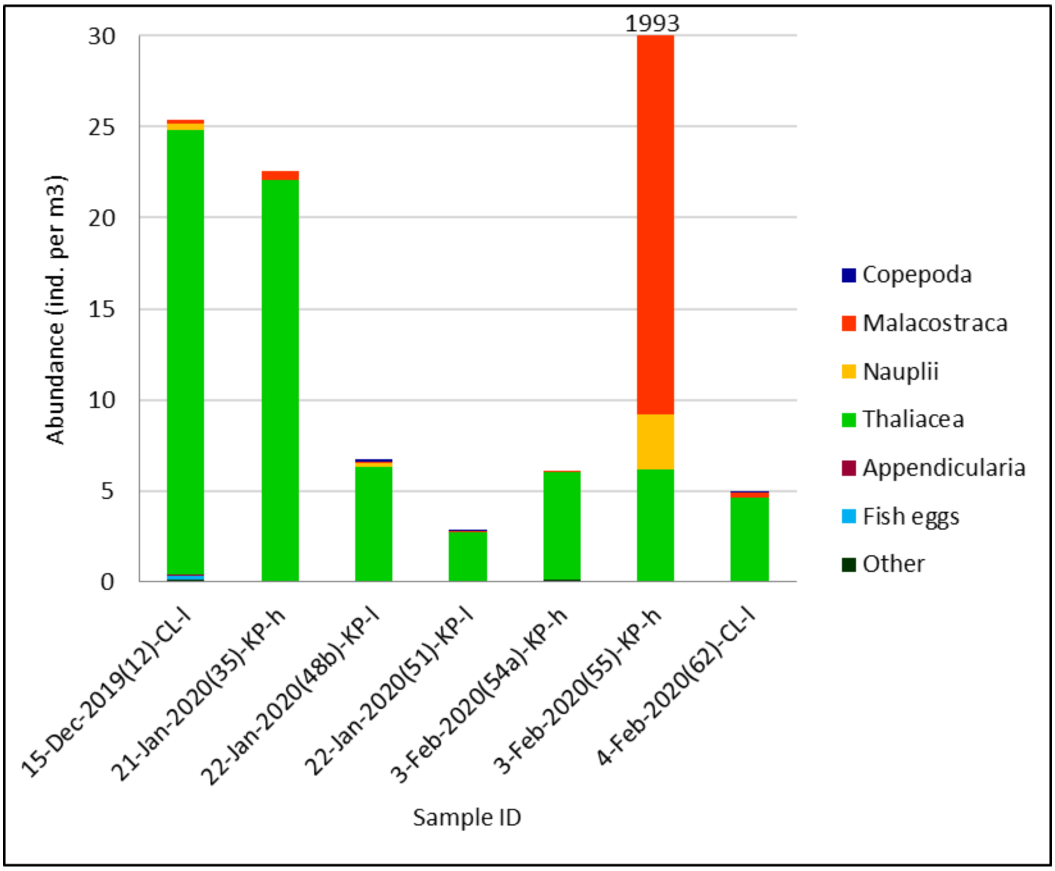


Figure 24: Abundance of zooplankton per group for samples collected in current lines (CL) and krill patches (KP). The total abundance of Malacostraca in sample 55 is given above.

4.3.2.2 Current lines

Two samples were taken with the low-speed net at two of the three current line events encountered (Fig. 22). Both samples were relatively small in terms of overall abundance of zooplankton: 25.40 and 4.90 per m^3, with Thaliacea the prominent group (Figs 23, 24).

4.3.2.3 Unknown

One sample was taken with the low-speed net from 1 of the 3 "unknown" events encountered. The sample contained a high proportion of Copepoda (69%), a relatively high proportion of Other (19%) -comprised entirely of echinoderm larvae - and low proportions of Malacostraca, Thaliacea and Fish eggs.

4.4 Categorical analysis

Among secondary bird species found at sampling sites, both BUSH and SOSH were found more frequently at sites characterised by a higher abundance of Malacostraca when compared to FFSH. WFTE were found more frequently at sites characterised by a higher abundance of Thaliacea when compared to FAPR, FFSH, FLSH and SOSH (Table 1).

Table 1: Statistically significant relationships between zooplankton groups and secondary bird species.

Zooplankton Group	Secondary Birds
Malacostraca	FFSH < BUSH
Malacostraca	FFSH < SOSH
Thaliacea	FAPR < WFTE
Thaliacea	FFSH < WFTE
Thaliacea	FLSH < WFTE
Thaliacea	SOSH < WFTE

Among primary fish species present at sampling sites, mackerel species were found more frequently at sites characterised by a higher abundance of Malacostraca when compared to trevally. Kahawai were found more frequently at sites characterised by a higher abundance of Thaliacea when compared to trevally (Table 2).

Table 2: Statistically significant relationships between zooplankton groups and primary fish species.

Zooplankton Group	Primary Fish
Malacostraca	Trevally < Mackerel spp.
Thaliacea	Trevally < Kahawai

Among secondary fish species present at sampling sites, kahawai and blue maomao were found more frequently at sites characterised by a higher abundance of Malacostraca when compared to juvenile fish species. Mackerel species were found more frequently at sites characterised by a higher abundance of Thaliacea when compared to blue maomao, juvenile fish species and kahawai (Table 3).

Table 3: Statistically significant relationships between zooplankton groups and secondary fish species.

Zooplankton Group	Secondary Fish
Malacostraca	Juvenile fish spp.< Blue maomao
Malacostraca	Juvenile fish spp. < Kahawai
Thaliacea	Blue maomao < Mackerel spp.
Thaliacea	Juvenile fish spp. < Mackerel spp.
Thaliacea	Kahawai < Mackerel spp.

Collectively, these results indicate that Malacostraca (mostly krill) and Thaliacea (salps) play a role in influencing the occurrence of some fish species and seabirds. As primary fish species present at sampling events, mackerel species were strongly associated with higher abundance of krill, while kahawai were associated with higher abundance of salps. White fronted terns were consistently present as a secondary species where salps were more abundant.

4.5 Krill length

The number of krill measured for each day of sampling varied from 10 to 100 krill and was dependent on the number of zooplankton samples taken per day that captured krill. A broad trend is seen across the field season in mean krill length which, aside from the 22 November data, shows a bell curve with date, increasing to a maximum of 10.93 mm on the 20 and 21 January and a minimum of 7.43 mm on 28 February (Fig. 25). However, there was a large variation in krill lengths across the field season overall. Krill that were < 6 mm length were present in zooplankton samples on most days, however, krill of > 14 mm length were less common in samples taken during February.

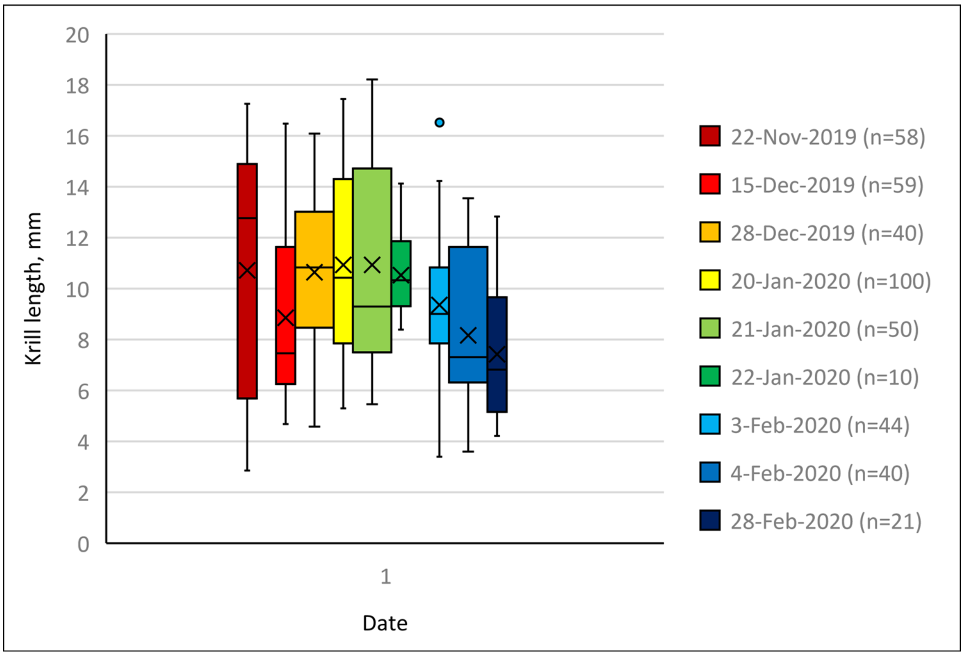


Figure 25 *(preceding page)*: Box plots of krill length grouped by sampling trip date. The number of krill measured from each day is given in brackets.

The number of krill measured at each type of sampling event varied from 10 to 211 krill and was dependent on the number of zooplankton samples taken that captured krill at each type of event (Fig. 26). Over the entire sampling season, the greatest number of zooplankton samples were taken at mixed fish shoal events and the least at tuna school and unknown events. There is a general trend for krill length to vary in relation to event type; larger krill (> 10 mm) were more often found at mixed fish shoal, tuna and unknown events. For example, on average the length of krill for all mixed fish shoal events was generally larger (mean = 10.93 mm) than for all the control events (mean = 8.52 mm). However, as with the krill length data grouped by trip date, there was large variation in krill lengths for event type categories.

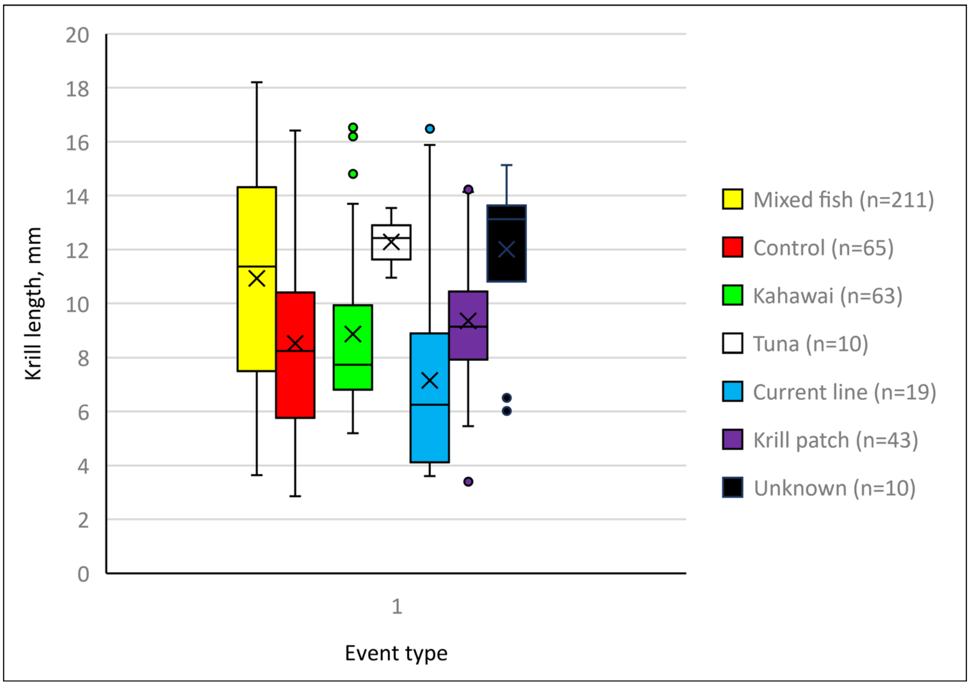


Figure 16: Box plots showing krill length grouped by event type. The number of krill measured from each event type is given in brackets.

4.6 Fish stomach contents

Seventeen fish comprising five species, were caught from four different event types (Table 5, Figs. 26, 27):

- 3 trevally *Pseudocaranx georgianus*
- 3 snapper *Chrysophrys auratus*
- 6 kahawai *Arripis trutta*
- 2 kingfish *Seriola lalandi*
- 3 albacore tuna *Tunnus alalonga*

Out of these, 16 fish were retained, and 12 stomach contents samples were obtained (four fish had empty stomachs). Ten of these stomach samples were obtained in conjunction with zooplankton samples. One fish (an under-sized kingfish) was released alive.

Table 5: Fish species caught in different event types between 20 January and 3 February 2020.

Fish ID	Event type	Fork length (mm)	Stomach contents sample?	Zooplankton samples collected at this event?
20-Jan-2020(E25)-Trev1	Mixed shoal	447	Y	Y
20-Jan-2020(E25)-Trev2	Mixed shoal	410	Y	Y
20-Jan-2020(E25)-Snap1	Mixed shoal	342	Y	Y
20-Jan-2020(E25)-Kaha1	Mixed shoal	515	Y	Y
20-Jan-2020(E25)-Kaha2	Mixed shoal	526	Y	Y
21-Jan-2020(E37)-Trev3	Mixed shoal	395	Y	N
21-Jan-2020(E42)-King1	Mixed shoal	650	N	N
21-Jan-2020(E42)-King2	Mixed shoal	960	N	N
22-Jan-2020(E46)-Kaha3	Kahawai school	320	Y	Y
22-Jan-2020(E50)-Snap2	Control	500	N	Y
3-Feb-2020(E54)-Snap3	Krill patches	450	N	Y
3-Feb-2020(E57)-Kaha4	Mixed shoal	550	Y	N
3-Feb-2020(E61)-Kaha5	Mixed shoal	500	Y	Y
4-Feb-2020(E63)-Alba1	Tuna school	500	Y	Y
4-Feb-2020(E63)-Alba2	Tuna school	490	Y	Y
4-Feb-2020(E63)-Alba3	Tuna school	500	Y	Y
4-Feb-2020(E65)-Kaha6	Kahawai school	540	N	N

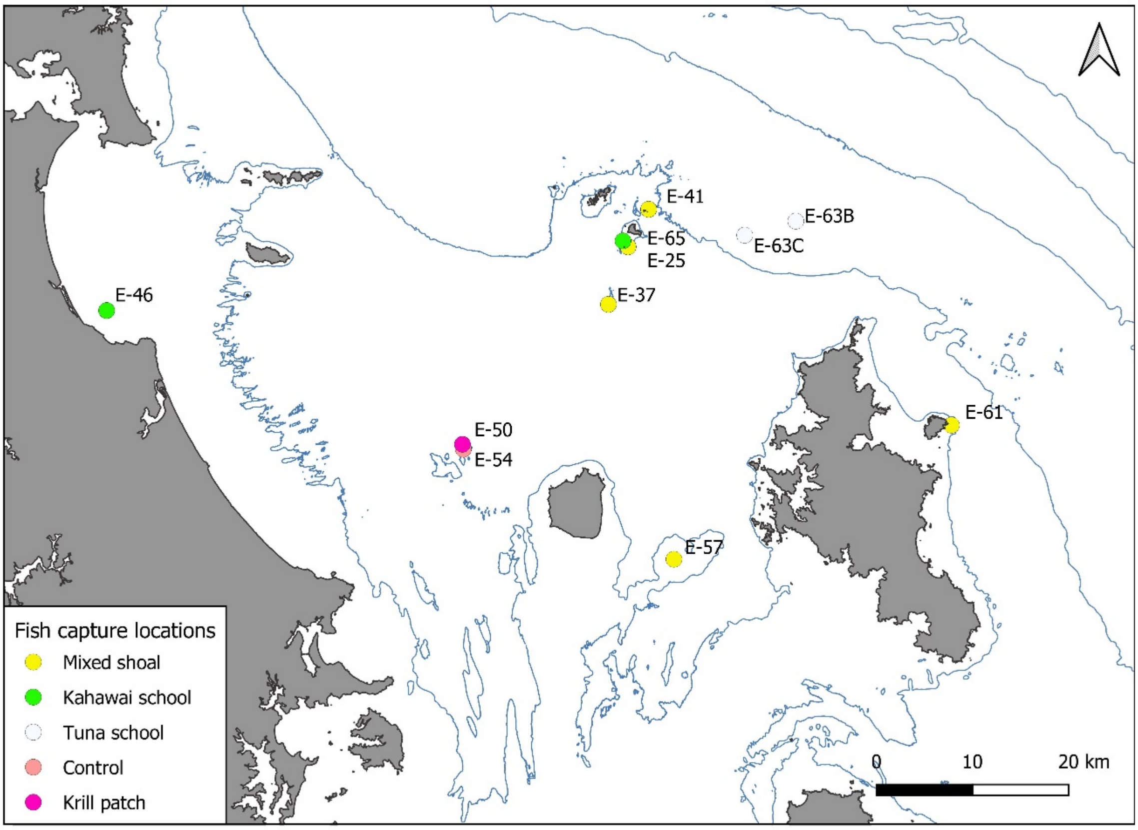


Figure 27: Location of fish captures with event type and number. Refer to Table 5 to see fish details relating to each event number.

4.7 Prey selectivity by fishes

Three trevally were caught at two separate sampling events, both categorised as mixed fish shoal events (Fig. 28). All trevally showed a negative selectivity for prey in the Copepoda, Thaliacea and Appendicularia groups and a positive selectivity for prey in the Malacostraca group (predominantly krill). Trevally 1 and 2 also had a positive selectivity for prey in the Other group (just one arrow worm found in the gut in both cases).

Four kahawai were caught at three separate sampling events, with three caught at mixed fish shoal events and one was caught at a kahawai school event, i.e. Kaha 3 (Fig. 29). At the mixed fish shoal events, the kahawai had a positive selectivity for prey in the Malacostraca group (predominantly krill). The kahawai from the kahawai school event had a strong selectivity for prey in the Other group (juvenile fish). At this particular sampling event, no juvenile fish were caught in the zooplankton sample from this site as the sample was comprised almost entirely of Thaliacea.

Three albacore tuna were caught at a single tuna school event that covered a wide area (Fig. 30). All of the albacore showed a strong positive selectivity for Malacostraca (krill and mantis shrimp larvae) and Albacore 1 also had a strong positive selectivity for prey in the Other group (juvenile fish and squid).

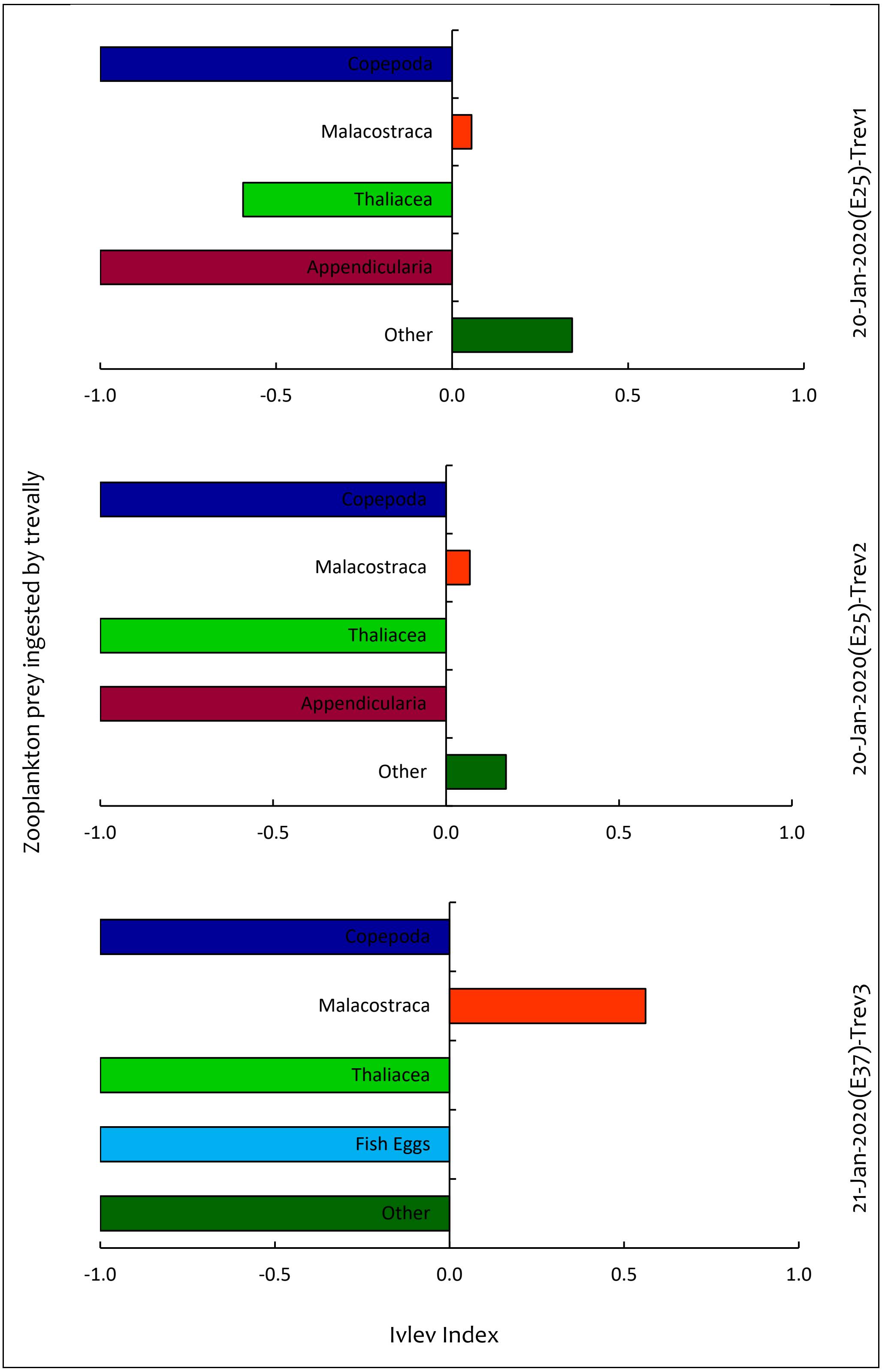


Figure 28: Ivlev Index of trevally caught in conjunction with zooplankton tow samples. The fish sample ID (at right) gives the date of capture, the event number and individual fish number.

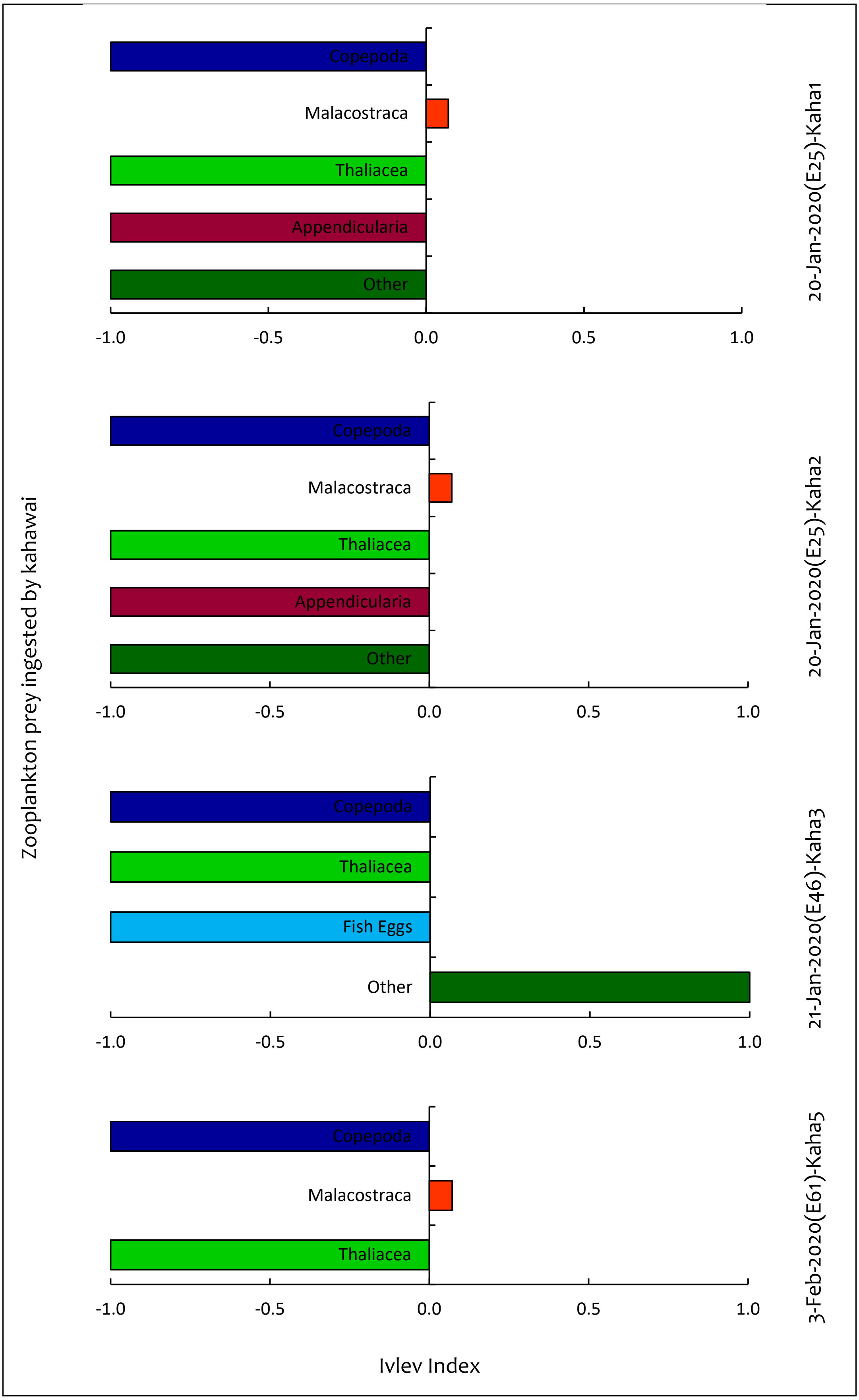

Zooplankton prey ingested by kahawai
20-Jan-2020(E25)-Kaha1
Copepoda
Malacostraca
Thaliacea
Appendicularia
Other
-1.0
-0.5
0.0
0.5
1.0
20-Jan-2020(E25)-Kaha2
Copepoda
Malacostraca
Thaliacea
Appendicularia
Other
-1.0
-0.5
0.0
0.5
1.0
21-Jan-2020(E46)-Kaha3
Copepoda
Thaliacea
Fish Eggs
Other
-1.0
-0.5
0.0
0.5
1.0
3-Feb-2020(E61)-Kaha5
Copepoda
Malacostraca
Thaliacea
-1.0
-0.5
0.0
0.5
1.0
Ivlev Index

Figure 29: Ivlev Index of kahawai caught in conjunction with zooplankton tow samples. The fish sample ID (at right) gives the date of capture, the event number and individual fish number.

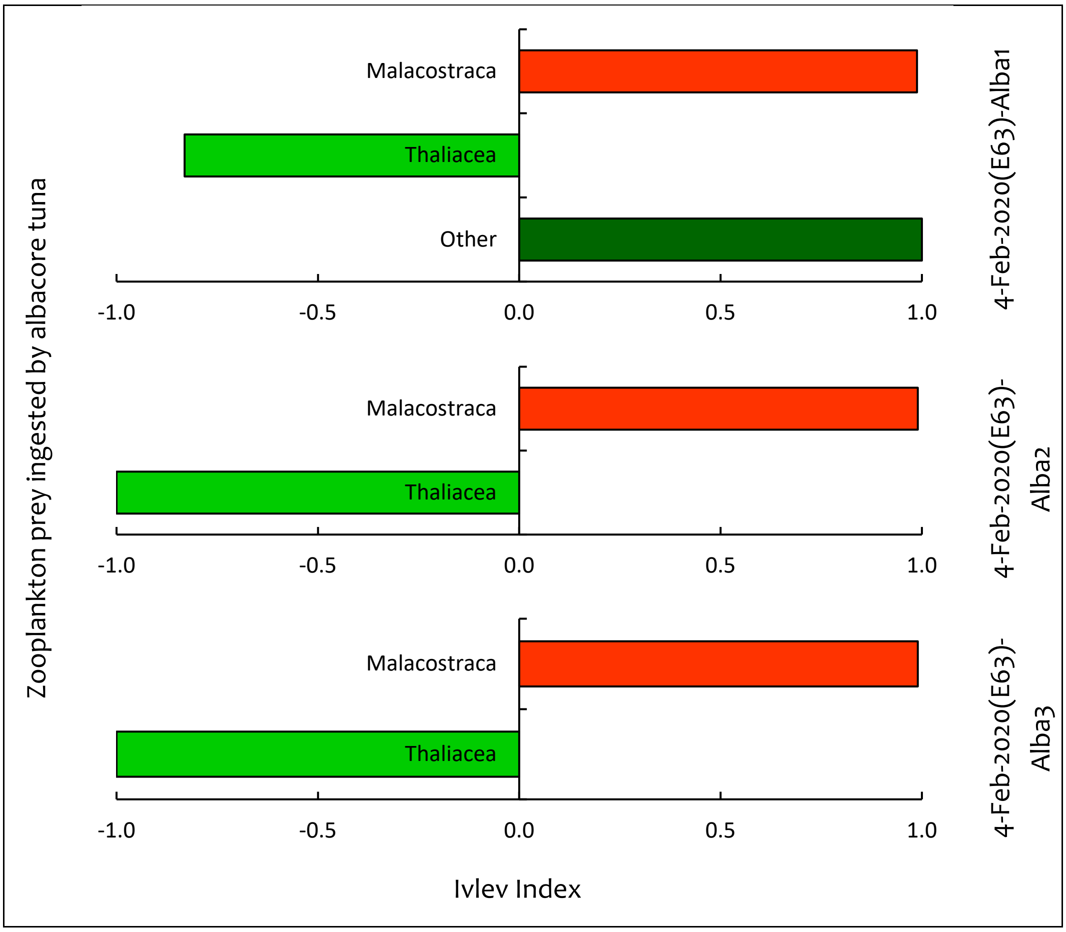


Figure 30: Ivlev Index of albacore tuna caught in conjunction with zooplankton tow samples. The fish sample ID (at right) gives the date of capture, the event number and individual fish number.

5 DISCUSSION

The general hypothesis of this study is that fish shoals drive krill and other prey to the surface making them more readily available to surface feeding seabirds. The alternative hypothesis is that krill aggregate at or near the surface in areas of upwelling or current flows which fish shoals target, providing a visual and potentially olfactory cues to seabirds.

In both cases, when fish schools come across the krill patches (in high enough concentrations) they go into 'feeding mode', massing even more tightly together and potentially further concentrating the krill; in turn their feeding activity advertises krill presence. The commotion, and potentially smell and sound of the fish feeding at the surface act as cues for seabirds that there is abundant prey available.

However, krill were found to aggregate in areas away from fish shoals but targeted by seabirds cued by other visual signs besides surface shoaling activity and potentially olfactory signs. For example, in very calm conditions, even the riffles caused by small fish attacking krill swarms from below (Fig. 14) advertise the krill presence to birds foraging in the area.

5.1 Shoal events

Of the three types of fish shoal event seen this research year (2019-2020), the highest abundances of potential seabird zooplankton prey (krill and other Malacostraca) were generally sampled from the mixed-fish shoals. These events occurred in locations where islands or underwater pinnacles rise from deeper water; key locations being the waters surrounding NW Reef and the Mokohinau Islands. Mixed-fish shoal events also tended to be the most dramatic in activity, sometimes with the shoals covering a large area, with fish breaking the surface at times and large numbers of seabirds feeding in association. While trevally tended to be the dominant fish species seen, kahawai, kingfish, and snapper were also caught from or below these shoals. Stomach contents from the trevally and kahawai were almost entirely comprised of krill. Control zooplankton tows all contained low abundances of Malacostraca, indicating that the fish shoal activity occurred at small spatial scales in relation to the presence of krill.

The kahawai schools occurred both near the mainland coast and locations affected by current flow and/or upwelling, such as around the Mokohinau Islands and Leigh Reef. They were not as commonly found as the mixed fish shoals. The kahawai appeared to be feeding on one of two prey types at these events, small fish at the events off Waipu Cove (indicated by a stomach contents sample) and likely krill at the locations in areas of current flow and/or upwelling. Fish and seabird activity were more scattered at the Waipu Cove events while at the other events the kahawai were often tightly massed, feeding near the surface with more dynamic seabird activity occurring. However, zooplankton samples taken at these events, generally contained low abundances of Malacostraca, possibly due to the net 'missing' dense areas of krill.

The tuna school event had a different type of activity to the other fish shoal events, with the tuna and seabirds scattered over a large area in deeper water (c.110m). The albacore tuna stomach contents samples were comprised of predominantly krill. However, the zooplankton tow sample only captured a small amount of zooplankton, mostly Thaliacea. This could have been due to the net missing a patch of zooplankton or due to the krill at this type of event being more dispersed. From the aggressive behaviour of the foraging seabirds (contrasting with 'pecking' behaviour at krill swarms, it is likely small fish were the prey here for both the tuna and seabirds.

5.2 Krill patches or swarms

Patches of krill (or krill swarms) at the sea surface, sometimes occurring scattered over large areas, with no shoaling fish associated, were found on several occasions associated with seabirds feeding. In calm glassy conditions, the krill activity was extenuated by small or juvenile fish attacking the swarms from below and disrupting the surface, providing visual cues for seabirds. There was also a distinct smell at these events which would provide olfactory cues for Procellariiformes (e.g. shearwaters, petrels and prions) who have a highly developed sense of smell (Nevitt, 2008). The krill species here, *N. australis*, only occurs in coastal waters of SE Australia and New Zealand and is known to be an important prey for many species of fish, seabirds, and cetaceans (Bary, 1954; O'Brien, 1988; McClatchie et al., 1989). *N. australis* is known for its daytime surface swarming activity, but the reasons for this behaviour are not clear. It has been suggested that they: congregate at the surface to feed; are driven to the surface by predators; are passively brought to surface by currents or upwelling; or they actively come to the surface to satisfy internal demands related to maturation or reproduction (Komaki 1967). Swarming in *N. australis* (and other krill species), has been found to often be highly coordinated with individuals showing parallel orientation and reacting to external stimuli (e.g. predators, stationary obstructions) as a unit; in a similar way to fish schools (O'Brien, 1988). Dense patches of krill are formed, surrounded by areas of water with no krill. This patchiness, together with their potential reactive movements to avoid vessels and sampling gear, can make adequate sampling of krill difficult.

5.3 Other types of events

As previously noted (Gaskin, 2018), seabirds feeding in association with cetaceans were observed on several occasions during this project, adding further data on this important feeding behaviour for a number of species. However, with the focus in this report on fish school dynamics, discussion of these associations is not included here. It should be noted that trials with plankton tows during POP2017-06 yielded little in terms of specimens and few clues to the exact nature of cetacean foraging other than prey, generally discards, seen at the surface.

Other events where seabirds were observed feeding were at current lines. White-faced storm petrels were the most common seabird species, 'dancing' on the sea surface and feeding on prey.

5.4 Analyses

Due to the relatively small number of sampling events this field season as a result of COVID restrictions, physical parameters were not able to be used in the categorical analyses and also limited the statistical power to detect possible differences using a three-way comparison between zooplankton, fish and seabirds. However, significant relationships were determined between the zooplankton and some secondary bird species, zooplankton and some primary fish species, and between zooplankton and some secondary fish species. The analysis may be able to be further expanded by using data from all three years of this study to deliver greater statistical power for the comparisons. Zooplankton abundance and diversity are determined predominantly by oceanographic (e.g., temperature, upwelling zones) and biological factors (e.g., primary productivity and predation) which result in a large amount of spatial and temporal variability (Zeldis & Willis, 2015). However, the detailed mechanisms of the drivers of this spatial and temporal heterogeneity in relation to availability of seabird prey in the wider Hauraki Gulf has not been modelled.

Krill are an important food source for both seabirds and fishes (Gaskin et al 2019). In this study, *N. australis* was seen swarming at the surface during the day, particularly at mixed fish shoal and krill patch events. The reason for this surface swarming behaviour during the day, which makes them highly vulnerable to predation by seabirds and fish, is not fully understood (O'Brien, 1988). It is thought that mature krill may aggregate at the surface for reproductive reasons (Mauchine & Fisher, 1969). Mature females of *N. australis* range in length from 9.8 – 17.0 mm and males from 12.0 – 16.0 mm (Barry, 1954; Brinton et al., 2000). Krill of these sizes, including females carrying eggs as well as metanauplii (i.e. the first free-swimming stage) were found most commonly at mixed fish shoal events throughout the field season. However, smaller krill occurred at these events also, indicating other reasons for surface swarming behaviour.

Mixed fish shoal events were dominated by trevally and kahawai and these shoals sometimes occurred over large areas, particularly in the vicinity of the Mokohinau Islands. The gut contents of both kahawai and trevally captured from these events were comprised predominantly of krill. From underwater video observations, krill could often be seen in dense patches near the water's surface. Fairy prions and Buller's shearwaters tended to be the most common bird species at these events. A previous study of the gut contents of these two seabirds found that, particularly for fairy prions, krill was an important prey type (Kozmian-Ledward et al., 2019).

By far, the greatest abundances of krill were found at krill patch events (in the absence of shoaling or work up activity) with the highest abundance in one zooplankton sample being 1993 krill per m^3. The predominant fish present at these events were mackerel species and juvenile fish species. When compared with a study on *N. australis* in Tasmania (O'Brien, 1988), this krill abundance is still relatively low. Krill densities of 3000 to > 450,000 individuals per m^3 were measured in Tasmania and the biomass of an individual swarm could exceed 100 kg wet weight. However, because of the highly patchy nature of krill occurrence, sampling can be hit or miss. At two krill patch sampling events, no krill at all were captured in the net tow despite the krill swarm being visible at the surface from the sampling vessel and recorded using underwater cameras.

Due to the importance of *N. australis* in the diet of various seabird and fish species (as well as baleen whales and manta rays) in the wider Hauraki Gulf region, more research is recommended on the distribution, life-cycle, behaviour, effects of environmental factors, and whether commercial fishing of krill-eating fish species has a positive or negative effect on krill abundance.

5.5 Inter-annual comparisons

In the previous years of this zooplankton research, fish shoal activity was not characterised into 'types' but instead into two broad categories: "workup" and "no workup". This, combined with the lack of quantitative data on zooplankton abundance (no flowmeter), meant that statistical differences between zooplankton composition and abundance for workup and non-workup samples were hard to determine. General observations of the data did suggest that Malacostraca were more abundant at workup events, but this is not statistically defined. This season's work has shown that there are characteristics between different types of seabird feeding events, zooplankton and fish present, and between zooplankton and some secondary fish species in terms of bathymetry and oceanographic factors which should be explored further.

Sampling methods in the previous two research years differ slightly from this year: this year, two net types were used and predominantly surface tows were conducted; in the previous years, both vertical hauls and surface tows were conducted, all with the fine mesh, low-speed net. Despite these differences, some general comparisons can be made between zooplankton samples across the years. Higher proportions and greater species diversity of Copepoda were obtained in previous years which could be due to several reasons: smaller copepods would have been less likely to retained in the high-speed net due to its coarser mesh; copepods may be more common deeper in the water column; and, copepods were generally more common in spring and autumn, seasons not sampled this year. The Malacostraca and Thalicea groups appear to occur in generally similar relative proportions throughout the years. However, without the abundance data in the previous years this is not quantifiable. For the Nauplii group, last year, barnacle nauplii were included in this group and were common in samples taken in May. This year, krill nauplii only were included in this group. Given their small size (< 0.6 mm), nauplii would have not been readily retained by the 1.32 mm mesh of the high-speed net. However, in one sample taken with the high-speed net at a krill patch event, a high abundance of nauplii was retained, possibly due to the extremely high numbers of krill captured here. Appendicularia were not common in samples this season compared to the previous seasons. In the 2017-2018 research season, Appendicularia were present in 93% of samples, compared to 8% this season. This could be due again to greater numbers being taken by vertical hauls. There were no samples dominated by fish eggs this year as there had been in previous years. Egg size range measured from last year was 0.78 – 1.38 mm (n = 11), mainly smaller than the high-speed net mesh. Inter-annual differences in zooplankton sample composition could also be due to climatic variability between years

This study reinforces observations made during previous research (INT2016-04 and POP2017-06) that seabirds adopt a range of feeding associations with respect to prey, and importantly the way prey is made available. Seabird science continually emphasises the role of seabirds as indicator species for marine ecosystem health (Furness & Camphuysen, 1997, Tasker et al., 2000, Wagner & Boersma, 2011). Fisheries can reduce the abundance of forage fish and may also change the community structure of fish schools resulting in smaller and less frequent workups reducing food availability. Depending on the level of dependence of seabirds on these foraging opportunities, this could result in impacts to populations of seabirds. Taking an ecosystem approach is required to understand this dynamic system (Hebshi et al., 2008, Maxwell & Morgan, 2013). Our research has focussed on a suite of species that we have identified as key for the study of fish schools/shoaling fish in north-eastern North Island waters and potential indirect adverse effects (Gaskin, 2017, Gaskin et al., 2019).

There is the need to continue to develop our multi-disciplinary approach necessary to fully investigate indirect effects of fisheries on seabirds through the study of these species, complemented by ongoing investigation into fish school dynamics and seabird diet, foraging distribution and behaviour utilising GPS or satellite tracking, and breeding success.

6 RECOMMENDATIONS

6.1 General

- Zooplankton sampling timed to link to seabird breeding cycles is required over multiple years and across each full season (September to May) to cover multiple species.

- This year's study demonstrated the significant advantages of using a high-speed dedicated research vessel for sampling, enabling large areas to be covered and multiple seabird feeding events to be sampled much more efficiently during periods of good weather. While much more effective, the use of such research vessel comes with significantly more cost.

6.2 Complementary research

- Connect at-sea sampling with areas of sea identified by GPS tracking of seabird species as important feeding grounds. Despite relatively small sample sizes, preliminary GPS tracking of four key indicator species (Buller's and fluttering shearwaters, fairy prions and Australasian gannets) undertaken separately from this project, have confirmed at-sea observations of occurrence around key bathymetric features and highlighted other important foraging locations within the wider Hauraki Gulf region.
- A comprehensive integrated tracking programme using remote GPS loggers downloading to base stations set up in colonies is recommended for multiple years starting with the four indicator species we have identified. Additional species could include: flesh-footed shearwater, black petrel, little penguin and northern diving.
- Furthermore, tracking of flesh-footed shearwaters (Kirk 2017) and also black petrels (Bell et al in prep) together with observations of this species feeding in association with cetaceans highlights the need to examine those relationships more closely.
- Stable isotope analyses from blood and feather samples, and opportunistic diet sampling collected through all key stages of their respective breeding cycles for all species studied to detect any annual changes in prey and foraging area.

6.3 Event sampling

- In general, a full suite of data for biological variables should be made at each event to allow for full comparisons of all variables.
- The floating camera rig should be deployed at all sampling locations to ground-truth topside observations of fishes. Ideally, additional GoPro's to be mounted, one at the top of the rig above water to film topside activity of seabirds and fishes, and one at the bottom of the rig pointing straight down into the water to record any fish activity beneath the rig.
- Oceanographic data recording – measurements of SST, salinity, water clarity and chlorophyll-*a* to be taken at all sampling events. Ideally have dedicated YSI meter that is known to be calibrated correctly for each trip. A more efficient method of filtering seawater for the chlorophyll-*a* samples is required than the very slow syringe method used to date. For example, a portable vacuum filtration unit.
- The use of the flowmeter is invaluable for standardizing sampling and needs to be retained.

- Seabird data collection to be standardised to include primary species, secondary species, abundance, and behaviours.
- Control zooplankton tows to be undertaken with more frequency.

6.4 Fish captures

- Increase fish sampling at different types of event and for different species to determine how fish diet varies with zooplankton composition and with fish species. Stomach contents show what is in the water and may include things that have avoided the net such as small fish. They also indicate fish are being highly selective so there should be more fish sampling in different types of event and for different species to determine how fish diet varies with zooplankton composition and with fish species.
- Develop an effective technique for the capture of bait fishes that can be integrated into the sampling programme because no bait fish samples were obtained through the sampling this season either through fishing efforts, or in the zooplankton net.

6.5 Captures of birds at sea

- Capture of key indicator Procellariform species to collect regurgitations to establish direct links of seabird diets to the zooplankton. Net guns have been developed as an effective tool for capturing seabirds at sea for research purposes (Gaskin in prep).

6.6 Zooplankton lab analysis

- With greater knowledge of key dietary items for seabirds, the categories for zooplankton sampling should be revised to reflect their relative importance to seabird diet.
- Continue to expand the macro photography of specimens and work towards a zooplankton identification guide for northern North Island region.

7 ACKNOWLEDGEMENTS

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