Qualitative analysis of zooplankton samples from the Fin Whale Project’s study area

Edmaktub Association 2016

Xavier Fernández Sala
Content

1. Introduction .................................................................................................................. 3

2. Materials and methods .................................................................................................. 6
   2.1. Zooplankton sample collection ........................................................................... 6
   2.2. Zooplankton taxonomy and identification ......................................................... 6

3. Results ............................................................................................................................ 7
   3.1. Taxonomy ............................................................................................................. 7
   3.2. Photo-identification ............................................................................................ 8

4. Discussion ....................................................................................................................... 17

5. Acknowledgements ......................................................................................................... 18

6. References ..................................................................................................................... 19
   6.1. General references ............................................................................................. 19
   6.2. Taxonomy and identification references ............................................................ 20

Annex I ............................................................................................................................... 22
   i. Zooplankton sample collection .......................................................................... 22
   ii. Taxonomy and identification of plankton organisms .......................................... 23
Qualitative analysis of zooplankton samples from the Fin Whale Project’s study area

1. Introduction

The Edmaktub association has been carrying out the Fin Whale Project (FWP) for the last 3 years. The main goal of it is to achieve as much information as possible regarding the ecology and the biology of fin whales (Balaenoptera physalus), taking advantage of their presence in Catalan waters (Spain) during their migration in spring months. The FWP has a wide study area in the Garraf coast, which spans from the Vallcara cove (Sitges) to Cunit and until almost 8 nautical miles out to sea, covering an area of 840 km² (Fig. 1).

The area of study has several bathymetric features that might help to decipher the reason why some fin whales would leave their migrating path to get closer to the coast. One important characteristic is the presence of a submarine canyon called the Foix canyon. Canyons have a key role in some coastal ecosystems, enhancing species richness and biological productivity (Hickey 1995) by generating ascendant currents filled with nutrients from the seafloor. Besides, the channelling of organic matter from the shelf to deep water through submarine canyons gives rise to high biomass levels and production rates in the plankton and benthos in such canyons (Vetter 1994; Vetter & Dayton 1998). Another characteristic is that further north out of the study area there is the mouth of the river Llobregat. When reaching the sea its waters increase the total amount of nutrients found in their surroundings, including the study area. All this favours the phytoplankton bloom that happens every year in winter-spring months (Bosc et al., 2004) (Fig. 2) which leads to the growth of the zooplankton community in the area.
The reason why whales visit these waters is not clear but a plausible cause could be to meet their feeding needs. Edmaktub has reported several whales feeding in the area (Fig. 3) and many showing foraging behaviour. What’s more, some of these whales were seen twice or more times during the same season, which means that the animals stayed in the area for few days or even for some weeks. Moreover, fin whale’s faeces were seen during the FWP surveys (not only in this year’s surveys but also in 2014 and 2015), which are another indication that whales might have been feeding in the area.

Mediterranean Sea encompasses pelagic areas of remarkable primary productivity (Jacques 1990). These areas can sustain large zooplankton (notably euphasiid) biomass and thus a viable population of fin whales (Goy & Thiriot 1976). Fin whales are predators of a wide spectrum of marine organisms, ranging from copepods to euphasiids, small schooling fish such as capelin (Mallotus villosus) and herring (Clupea harengus), anchovy (Engraulis encrasicolus), sand lance (Ammodytes spp.) and probably sardine (Sardina pilchardus) (Gambell 1985; Notarbartolo-Di-Sciara et al., 2003). Despite this, very little is known about whether Mediterranean fin whales feed solely on krill, about the availability of krill to whales in other seasons than spring and whether or not fin whales feed at other times of the year than spring.

Figure 2 Annual changes in chlorophyll concentration in the Mediterranean Sea (Bosc, et al., 2004). Winter and spring are the seasons in which the chlorophyll concentration is higher in the northwestern Mediterranean Sea.
Studies about fin whale’s diet, based primarily on the inspection of stranded whales’ stomachs in the Ligurian-Corsican-Povençal area, suggest *Meganyctiphanes norvegica* as the only known prey in this portion of the Mediterranean during summer (Orsi Relini & Giordano 1992). The same result was found when fin whale’s faeces collected in the western Mediterranean Sea were analysed. In 2006, Canese et al. provided the first ever documented cases of fin whales feeding on other organisms than Northern Krill. The animals were seen and recorded by professional cameramen feeding on *Nyctiphanes couchii* (euphasiid) near the Coast of Lampedusa Island. It was the first ever documented winter-feeding of fin whales in the Mediterranean Sea.

According to Edmaktub fin whales are present and feed in Garraf waters during spring (February-May). No literature regarding fin whales feeding in these waters could be found so Edmaktub would be the first association that documents this scenario. No description of the zooplankton community of the study area has been done so far either, so no information about what can whales be feeding on is known. Garraf coast might be a small feeding area for fin whales during their migration towards the Ligurian Sea with all the ecological impacts that this would imply for the management and conservation of this endangered species.

Plankton analysis is a complex task, especially when done by non-specialized people. Here, a **preliminary report that describes part of the zooplankton community** of the FWP’s area of study from a qualitative perspective is presented.
2. Materials and methods

2.1 Zooplankton sample collection
During the FWP surveys a 50cm diameter and 130cm length conic net with a 200µm mesh was deployed for 15 minutes every time that whales were seen feeding in the study area or displaying foraging behaviours. A diving computer was attached to the net to monitor the depth. The vessel was set to travel at an approximately constant speed of 3 knots. Once the time was over the net was collected and the sample was placed in a small and clean bucket and the net rinsed to make sure that no sample was left in it. Then the sample was filtered with a cloth filter and put in a properly labelled container filled with 70% alcohol until the sample had a volume of 100ml. The sample was kept in a portable fridge with ice until it was analysed.

2.2 Zooplankton taxonomy and identification
Once in the laboratory each sample was examined under a binocular microscope and splitted in several subsamples according to the taxon present in it such as copepods, amphipods, euphasiids, tunicates, jellyfishes, fishes, molluses, larvae and eggs. For that, a 9ml subsample taken from the main sample was examined for 10 minutes, looking for organisms belonging to a specific group. The same procedure was applied for every group. When considered necessary to represent as much biodiversity as possible a subsample was examined for more than 10 minutes.

To read the whole protocol see Annex I.
3. Results

3.1 Taxonomy

The organisms found in the samples are currently classified in the following clades:

→ Phylum Cnidaria; Class Hydrozoa; Order Siphonophorae; Family Diphyidae.
→ Phylum Cnidaria; Order Anthoathecata; Family Porpitidae; Genus Velella; Species: \textit{V. velella}.
→ Phylum Annelida; Class Polichaeta.
→ Phylum Arthropoda; Class Maxillopoda; Subclase Copepoda.
→ Phylum Arthropoda; Class Malacostraca; Orden Decapoda (Larvae).
→ Phylum Arthropoda; Class Malacostraca; Orden Euphausiacea.
→ Phylum Arthropoda; Class Malacostraca (Larvae; first stages).
→ Phylum Arthropoda; Class Malacostraca; Orden Amphipoda; Familia Hyperiidae.
→ Phylum Arthropoda; Class Malacostraca; Orden Amphipoda; Familia Phronimidae.
→ Phylum Arthropoda; Class Malacostraca; Orden Amphipoda.
→ Phylum Chaetognatha; Class Sagittoidea.
→ Phylum Chordata; Subphylum Tunicata; Class Thaliacea.
→ Phylum Chordata; Class Actinopterygii.
→ Elongated egg and rounded egg.

All pictures have a scale bar that represents $2\text{mm}$. 
3.2. Photo-identification

In the following pictures all the organisms that could be identified after analysing the samples can be seen.

**A:** Phylum Cnidaria; Class: Hydrozoa; Order: Siphonophorae; Family: Diphyidae.

**B:** Phylum Cnidaria; Order: Anthoathecata; Family: Porpitidae; Genus: Vellela; Species: *V. velella.*

**C & D:** Phylum Annelida: Class Polichaeta (*C and D are the same individuals*).

**E - M:** Phylum Arthropoda; Class Maxillopoda; Subclass Copepoda (*I and J are the same individuals.*)

*Notice how L carries eggs (arrow).*
**N - P:** Phylum Arthropoda; Class Malacostraca; Order Decapoda (Larvae).

**Q - V:** Phylum Arthropoda; Class Malacostraca; Order Euphausiacea
(Q, R and S and U and V are the same individual. Notice the gills in S and in V (arrow)).
W - AE: Phylum Arthropoda; Class Malacostraca (Larvae; first stages).
W and X are zoea larva from brachyuran crabs.
AF - AH: Phylum Arthropoda; Class Malacostraca; Order Amphipoda; Family Hyperiidae.
AI - AM: Phylum Arthropoda; Class Malacostraca; Order Amphipoda; Family Phronimidae

(AI and AK are the same individual).
AN - AS: Phylum Arthropoda; Class Malacostraca; Order Amphipoda.
AT - AV: Phylum Chaetognatha; Class Sagittoidea.

AW - AY: Phylum Chordata; Subphylum Tunicata; Class: Thaliacea

(*AW and AX are the same individual*).
Other taxon found: Apart from the organisms listed above, individuals belonging to the **Pandeidae** family (*Phylum Cinadria; Class Hydrozoa; Order Anthoathecata*), to the **Abylidae** family (*Phylum Cinadria; Classe Hydrozoa; Orden Siphonophorae*), sea angels of the **Gymnosomata** taxon (*Phylum Mollusca; Class Gastropoda*) and other **Gastropods**.

**AZ**: Phylum Chordata; Class Actinopterygii.

**AAA**: Elongated egg & rounded egg.
4. Discussion

This is the first qualitative analysis of the zooplankton community performed in the Fin Whale Project’s (FWP) study area where Edmaktub has reported several fin whales feeding and foraging. All the taxon found in the samples are described in the literature as part of the common biodiversity of the Mediterranean zooplankton community (Gil 2011; Vieitez 2004; San Martin 2003; Ruffo 1998; Hayward & Ryland 1995; Ruffo, 1993; Ruffo 1989; Riedl 1986; Ruffo 1982; Kensley 1978; Fauchald 1977 and Tattersall 1951).

Fishermen have reported fin whales in the area for decades during the February-May period as well as that euphasiids get entangled in big quantities in their nets, especially at the beginning of spring. Krill is known as the main prey for fin whales so apparently whales could come and take advantage of this scenario and feed on it. In the Ligurian sea Tarling et al. (1999) monitored an euphasiid community during their vertical migration along the water column and it sank until 175m. In this study, all the samples were collected at a mean depth of 15.64m±3.96. This depth can be easily overpassed by fin whales, which can dive to more than 100m (Croll et al. 2001). During their dives the animals stay from 2 to 10 minutes underwater and no information about the depths they reached during the study are available. Panigada et al. (2006) tagged 15 fin whales from 1998-2002 in the Ligurian sea and the results showed that the animals performed deep dives to more than 400m depth, presumably to feed. In the Garraf coast Edmaktub has recorded some animals feeding in shallow waters although most of them were seen diving in deep waters.

In the future, plankton analyses should be done with more accurate instruments that register the exact depth at which the samples are being collected as well as with devices that allow quantitative analyses of the sample. Besides, the use of tags to monitor the animals while diving would be of great interest to bring some light in their ecology and behaviour during their presence in Catalan waters. With the available data in this report it cannot be assumed that fin whales fed on the organisms listed before even though a few individuals were seen feeding near the surface.

More research is needed to go further in the description of the zooplankton community present in the study area as well as to understand why fin whales visit the area every year.
5. Acknowledgements

I want to thank the Edmaktub association for bringing me the opportunity of being part of the Fin Whale Project during all these months. I also want to thank Dr. Berta Caballero from the Natural Sciences Museum of Barcelona, Paoletta Satta from the Centre d’Estudis Avançats de Blanes and Dr. Albert Calbet from the Oceanographic and Marine Biology department of the Marine Sciences Institute (CSIC) for all their help with plankton taxonomy and identification.
6. References

6.1 General references


physalus, in the Liguro-Provençal Basin. European Research on Cetaceans, 6, 138–141.


6.2 Taxonomy and identification references

Fauchald, K. (1977). The polychaete worms, definitions and keys to the orders, families and genera. Natural History Museum of Los Angeles County: Los Angeles, CA (USA), Science Series. 28: 1-188.


Annex I

This protocol was developed during the Fin Whale Project carried out by Edmaktub in the 2016 surveys.

i. Zooplankton sample collection

a. Material:
   i) Plankton net.
   ii) Rope (1x 60m & 1x 1m).
   iii) 3kg lead.
   iv) Diving computer.
   v) Cable tie.
   vi) Small bucket (x1).
   vii) 50ml syringe (x1).
   viii) 70% alcohol.
   ix) 100ml containers.
   x) Fine permanent marker.
   xi) Filter to filter the sample.
   xii) Filtered seawater.
   xiii) Portable fridge.
   xiv) Photographic Camera

b. Protocol:
   I. **Previously**, decide which is the most appropriate time to deploy the plankton net during the surveys.
   II. Attach a diving computer to a 3kg lead. Use a cable tie.
   III. Attach the lead with the diving computer to the net using the 1m rope (bowline).
   IV. Use the 60m rope to maintain the plankton net attached to the boat (bowline).
   V. Make sure that the lid from the bottom part of the net is properly screwed.
   VI. With the engine in neutral and the sails not hoisted deploy the net from the stern of the boat carefully. The net must be deployed with the lead in its down side.
   VII. Make sure that there is no entanglement with the cables of the net during the first metres of deployment.
   VIII. Keep leaving rope until desirable and make a cleat hitch knot to the mooring cleat.
   IX. Once the net is completely deployed leave it under the water for 15 minutes.
X. Maintain the boat at a constant speed of 3 knots (approximately).

XI. **Register the initial latitude and longitude** (also register the speed of the boat, the strength and direction of the wind, the cloudiness, the swell, the visibility, if there are whales around and their behaviour if possible).

XII. After 15 minutes reduce the speed until neutral.

XIII. Start collecting the net and then **register the final latitude and longitude**.

XIV. Once the net is on board carefully drain as much water as possible using the own filter of the net.

XV. Take the bottom lid out making sure that all the water with the plankton sample ends up in a small and clean bucket. Rinse the lateral filter and collect the sample stuck in it as well.

XVI. Take as many pictures as desirable of the plankton sample.

XVII. If required, take a fresh plankton sample with a 50ml syringe and keep it with seawater in a container. Label the container and keep it in the portable fridge.

XVIII. Filter the rest of the sample with a mesh small enough to retain the planktonic organisms (e.g. 200μm nylon mesh).

XIX. Put the plankton sample in a container.

XX. Use filtered seawater to collect possible rests of the sample that might stay in the mesh.

Use as less filtered seawater as possible (it will decrease the % of the alcohol).

XXI. Add 70% alcohol to the container with the sample until **100ml**.

XXII. Label the container and keep it in the portable fridge.

---

**ii. Taxonomy and identification of the planktonic organisms**

a. Material:

i) Binocular microscope.

ii) Very fine tip forceps (x2).

iii) 50ml containers.

iv) 70% alcohol.

v) Camera attachable to the binocular microscope and to a PC.

vi) Pasteur pipettes.

vii) Timer.

viii) Petri plate.

ix) Fine permanent marker.

x) Adjustable height stool.
b. Protocol:

- Step 1: General view of the sample
  I. Attach the camera to the binocular microscope and open the software to take pictures with it.
  II. Take one container with a plankton sample. You are going to work with the same sample until the contrary is specified.
  III. Open the container and use a Pasteur pipette to softly homogenise the sample by taking in and out alcohol from the sample several times. Organisms will be sucked in and out.
  IV. Once homogenised collect 9ml with a Pasteur pipette.
  V. Put them in a Petri plate and this under the binocular microscope.
  VI. Have a general look of the sample with the lowest magnification and take a picture of it. Take more than one if required to represent the diversity of organisms.
  VII. Put the 9ml of the sample back into the container.

- Step 2: Separate the organisms by taxa
  The purpose of this step is to make a first separation of the animals so that it will be easier to identify them later. The separation will be based on their general anatomy and as many groups (taxon) as deemed necessary can be created.
  I. Based on what it is seen in the general inspection of the sample decide how many groups/taxon should be created.
  II. Fill several containers with 30ml of 70% alcohol (as many as groups/taxon have been created). Label them with a name that identifies the taxa and the date in which the sample was collected (e.g. Copepod mmddyy). Suggestion: use the following groups: Copepods, Amphipods, Prawns, Worms & Fishes, Larvae & Eggs & Snails, Jellyfish, Unknown. List them if needed (e.g. Copepod 1, Copepod 2).

  THE MAIN CHARACTERISTICS OF EACH TAXON SHOULD BE KNOWN FROM THIS STEP ON

  III. Take one container that has one plankton SAMPLE (the one collected on the boat).
  IV. Homogenise the plankton sample the same way as in “Step 1 III”.
  V. Once homogenised collect 9ml of the plankton and put them in a Petri plate and this under the binocular microscope.
  VI. Set the timer to 10 minutes.
VII. Using a very fin tip forceps **focus on one group of organisms** (e.g. *Copepod 1*).

VIII. Put the timer on and start separating the organisms of the taxa of interest (e.g. *Copepod 1*) from the rest in one of the containers with 30ml of 70% alcohol.

IX. Once the time is gone stop collecting individuals. Close the container with the separated individuals. Take another container with 30ml of 70% alcohol.

X. Set the timer again and decide in which group you will focus (e.g. *Copepod 2, Amphipod, …*).

XI. Start the timer and do the same as in “**Step 2 VII, VIII & IX**”.

XII. Repeat the same procedure for all the taxon. Once all the taxa have been separated move to the next **SAMPLE** (those taken in the boat during the surveys).

- **Step 3: Identification of the different taxa**
  
  I. Take the containers with the organisms separated by taxa.
  
  II. Look at the organisms under the binocular microscope and take pictures of them.
  
  III. Look for anatomical differences and characteristics among them to go further in the taxonomical analyses.
  
  IV. Use a scale bar to state the length of the individuals.
  
  V. Separate the individuals in new containers with 70% alcohol by order, family, genus or species if needed.