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LifeWatch observatory data: Zooplankton observations in the Belgian part of the North Sea

Jonas Mortelmans  | Jolien Goossens | Luz Amadei Martínez | Klaas Deneudt |
André Cattrijsse | Francisco Hernandez

Flanders Marine Institute (VLIZ),
Oostende, Belgium

Correspondence

Jonas Mortelmans, Wandelaarkaai 7, 8400
Oostende, Belgium.

Email: jonas.mortelmans@vliz.be

Luz Amadei Martínez, Protistology &
Aquatic Ecology (PAE), Ghent University,
Ghent, Belgium

Present Address

Jolien Goossens, Marine Biology Research
Group (MARBIOL), Ghent University,
Ghent, Belgium

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Funding for the data collection and
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European Research Infrastructure within
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operates an E-Science Infrastructure for
Biodiversity and Ecosystem Research and
consists of biodiversity observatories, data
systems, web services, and modelling tools.

Abstract

Through regular sampling surveys, the Flanders Marine Institute (VLIZ) is generating a long-term data series for the Belgian coastal water and sandbank system, a designated site in the Long Term Ecological Research (LTER) network. The data series is built from sampling activities initiated in 2012 in the framework of the LifeWatch marine observatory. Nine nearshore stations are sampled monthly, with an additional eight offshore stations sampled seasonally. This paper presents the generated data series for zooplankton densities and size measurements, analysed using a ZooScan plankton imaging device together with the ZooProcess and Plankton Identifier software packages. To date 673.017 biological particles have been collected and identified. The collection and processing of the 2012–2018 dataset is described, along with its data curation and quality control. Yearly versions of the data are published in a standardized format together with environmental parameters, accompanied by an extensive metadata description and labelled with digital identifiers for traceability. The data are published under a CC-BY 4.0 license, allowing use of the data under the condition of providing the reference to the original source.

KEYWORDS

Belgian part of the North Sea, LifeWatch Belgium, marine, zooplankton

Dataset

The LifeWatch observatory data on zooplankton comes as yearly updates. To date, three versions are available, of which this is the most recent:

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1 | INTRODUCTION

Zooplankton is ubiquitous in aquatic environments, and comprises a wide range of heterotrophic organisms. Due to their limited locomotion, these organisms are mainly carried by currents along water bodies (Lenz, 2000). Because of the association with certain water masses, zooplankton is an ideal subject for examining potential, physical and biological interactions (Bonnet and Frid, 2004; Eisner *et al.*, 2013; Pinchuk and Eisner, 2017). Furthermore, many species have a rapid life cycle, a fast growth rate (Hirst and Forster, 2013; Kiørboe and Hirst, 2014), and are highly susceptible to environmental change (Castellani and Edwards, 2017). As they are at the base of the marine food chain, these communities can regulate the growth of high-level trophic organisms (Castellani and Edwards, 2017), and since zooplankton is hardly fished by man, they are independent of fishing intensity (Omori, 1978). These key features enable zooplankton to play a critically important role in an ecosystem, delivering important information on the state and dynamics of the pelagic ecosystem and food web functioning (Edwards and Richardson, 2004; O'Brien, Wiebe and Hay, 2011; O'Brien, Wiebe and Falkenhaus, 2013; Castellani and Edwards, 2017).

The Belgian part of the North Sea (BPNS) is part of the Southern Bight of the North Sea and covers 3447 square kilometres. The entire area is characterized by shallow depths (up to 40 m) and a series of subtidal sandbank systems (Figure 1). The biotic and abiotic descriptors in the BPNS are influenced by freshwater river discharges from Yzer, Scheldt, Meus, Seine; and saline water from the Channel originating from the Atlantic (Nihoul and Hecq, 1984; Lacroix *et al.*, 2004). Zooplankton is ubiquitous in the BPNS, and is found

in every season with densities up to 40.000 ind.m⁻³ (Van Ginderdeuren, 2013), although average densities are much lower at around 1–400 ind.m⁻³ (Van Ginderdeuren, 2013). Although the pelagic ecosystem is huge and of significant importance, it is scarcely studied in Belgian waters: recent studies on marine zooplankton in the BPNS are increasing (Van Ginderdeuren, Fiers, *et al.*, 2012; Van Ginderdeuren, Hostens, *et al.*, 2012; Van Ginderdeuren, 2013; Deschutter *et al.*, 2017,2018; Mortelmans *et al.*, 2017). In contrast, the Belgian estuarine zooplankton has received more attention throughout the time (Bakker and De Pauw, 1975; Soetaert and Van Rijswijk, 1993; Appeltans *et al.*, 2003; Tackx *et al.*, 2004; Mialet *et al.*, 2011).

A typical method to study zooplankton includes taxonomic analysis by microscope: a time-consuming step, and extremely dependent on the identifier's expertise and fatigue (Castellani and Edwards, 2017). The International Council for the Exploration of the Sea (ICES) Zooplankton Status Reports mostly do not use high-level taxonomic groups, but only keep track of low-level taxonomic groups (O'Brien, Wiebe and Hay, 2011; O'Brien, Wiebe and Falkenhaus, 2013). Novel high-frequency techniques based on image recognition (e.g. ZooScan, Video Plankton Recorder, Underwater Vision Profiler, FlowCam, Flowcytometer) along with the accompanying software to enable (semi) automated image analysis, densities, size measurements and biomass estimations are becoming exceedingly important (e.g. Visual Plankton Davis *et al.*, 2005), ZooProcess with Plankton Identifier (Gorsky *et al.*, 2010), ZooImage (Bell and Hopcroft, 2008), or Ecotaxa (Picheral, Colin and Irisson, 2017). ZooScan data series has the potential to provide zooplankton indicators for the EU Marine Strategy Framework Directive (Uusitalo *et al.*, 2016). However, such an assessment is currently not applied to Belgium waters.

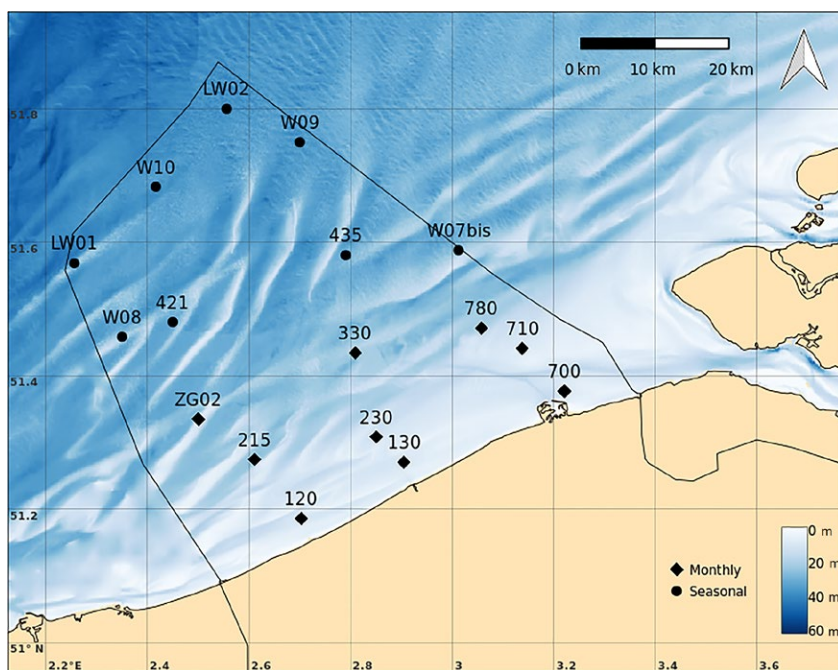


FIGURE 1 Study sites on the Belgian part of the North Sea (BPNS). Nine stations onshore, visited monthly: 130 (2.90535, 51.27055); 780 (3.057283, 51.471367); 330 (2.809083, 51.434117); 230 (2.85035, 51.308683); 710 (3.138283, 51.441217); 215 (2.61075, 51.274867); ZG02 (2.500717, 51.33515); 120 (2.702483, 51.186083); 700 (3.221017, 51.377); and eight additional offshore stations, visited seasonally: LW01 (2.256, 51.568667); LW02 (2.556, 51.8); 435 (2.790333, 51.580667); W07bis (3.012517, 51.588033); W08 (2.35, 51.458333); W09 (2.7, 51.75); W10 (2.416667, 51.683333), and 421 (2.45, 51.4805)

During the multidisciplinary sampling campaigns described here, zooplankton samples are collected along with associated biochemical and biodiversity information, and associated with the related abiotic descriptors.

2 | DESIGN DESCRIPTION

A grid of 17 stations in the BPNS is being sampled since 2012 (Figure 1). Nine stations covering the nearshore area are sampled during 1-day surveys, with a monthly frequency. Eight additional offshore stations are sampled during 2-day surveys, with a seasonal frequency (Figure 2). The locations

of these stations, more-or-less evenly distributed over the BPNS, were chosen based on the availability of historical data for those stations, as well as for reasons of complementarity with existing water-quality monitoring by other Belgian institutes. The zooplankton sampling activities were initiated in 2012 by VLIZ, in the framework of LifeWatch, which is a landmark European Research Infrastructure within the European Strategy Forum on Research (ESFRI) roadmap.

During the multidisciplinary sampling campaigns described here, all zooplankton samples were collected aboard the research vessel (RV) Simon Stevin, which is equipped to efficiently capture and store the metadata associated with samples, actions and environmental parameters. When at

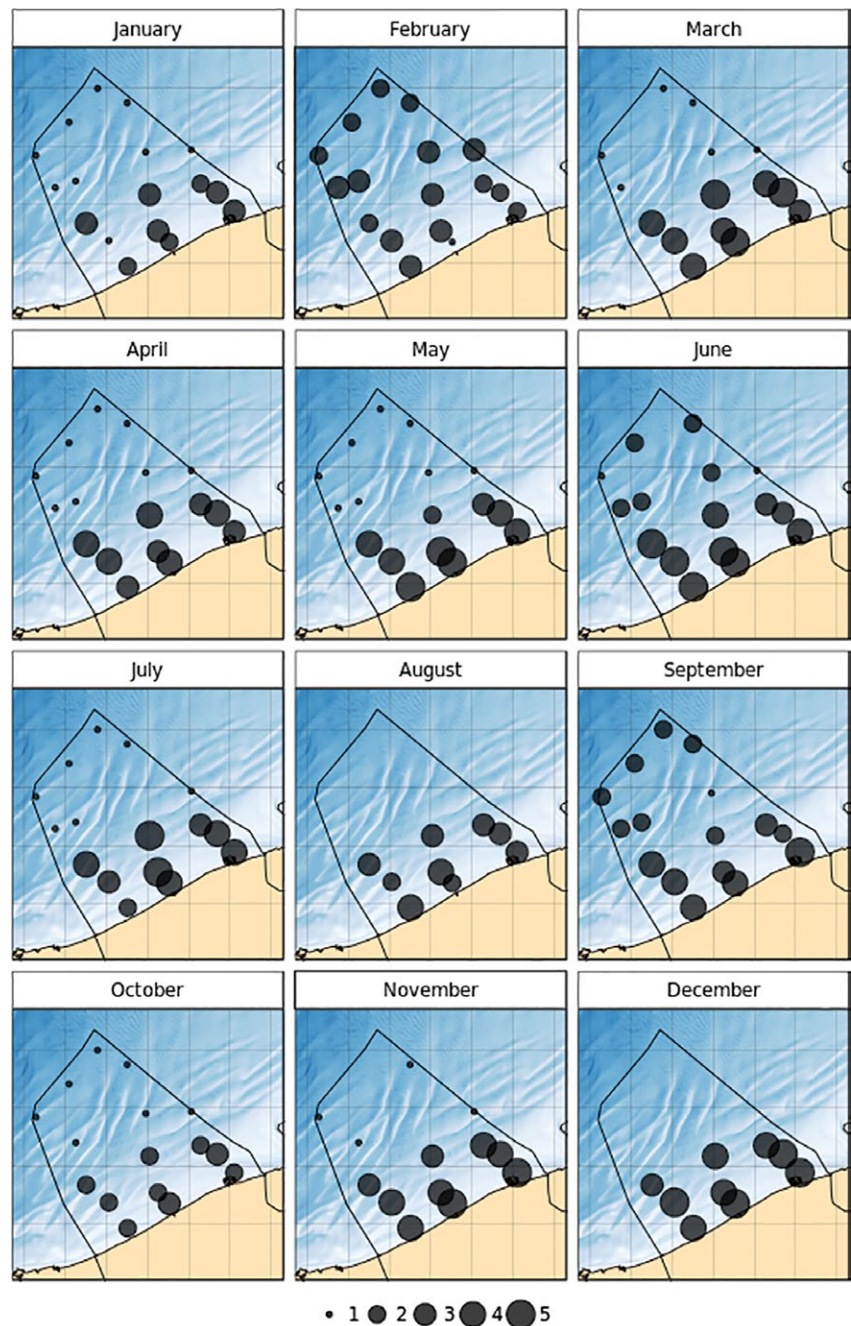


FIGURE 2 Spatio-temporal data availability in the sampled area. The dot size indicates the number of samples at the given sampling station, in that specific month. Data are originating from Flanders Marine Institute (2019) and shows data between August 2012 and August 2018

sea, the Marine Information and Data Acquisition System (MIDAS) registers the navigation data (including heading, current time, latitude, longitude, speed and course over ground, navigation depth and draught) as well as meteorological (air temperature and relative humidity, wind direction and speed) and oceanographic data (sea surface-water temperature, salinity, chlorophyll a and sound velocity). MIDAS also allows marine scientists to log their research activities during each scientific campaign. Specific actions onboard are registered on the spot and the related metadata are made available online, every 24 hours, through an automated synchronization with the VLIZ ICT network. Details on researchers, trips and cruises are stored, together with metadata from actions (e.g. time and geographical location of start and stop of the scientific activities, notes, station,

seawater through a 200 μm mesh size net and zooplankton is concentrated in a plastic net bucket. A small amount of soda water is poured into the sample to narcotize the zooplankton (Goswami, 2004). Finally, formaldehyde is added to a 6% concentration. The volume filtered is chosen by the scientist, but is limited to 18.000 L/h. The filtered volume is noted in MIDAS and in the trip report as a backup.

The second method collected surface seawater using PVC or stainless steel buckets: buckets are thrown overboard and hauled up, the contents poured through a 50 μm mesh Apstein net. The Apstein net is 1.2 m long, with a 50 μm mesh size net and a 50 cm diameter. The sample is concentrated in the plastic net bucket at the end of the net, in which water escapes through a 50 μm mesh window. In total, 50L of seawater is hauled and filtered through the Apstein net. Soda water and

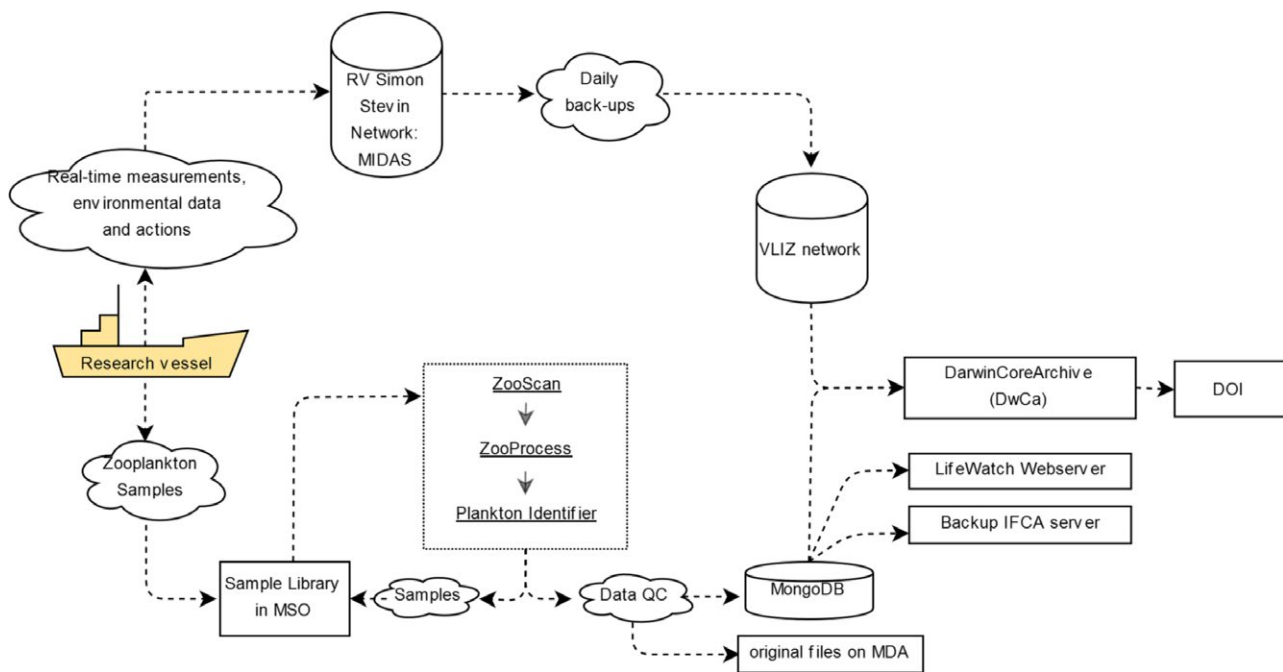


FIGURE 3 Schematic overview of the dataflow from ship to user

action type and status of deployment) (Figure 3). The system further aids in planning cruises and registering ship activities.

3 | SAMPLING METHODS

The zooplankton samples described in this dataset are collected by three distinct methods namely: the first applied between July and August 2012, the second applied between August 2012 and December 2013, the third applied from January 2014 onwards (Figure 4).

The first method collected surface seawater by means of a plankton pump, and only makes up for 19 samples from July and August 2012. The plankton pump will pump the

formaldehyde are added to a 6% formaldehyde concentration. Approximately 1 month after the original fixation in the laboratory (Marine Station Ostend, MSO), the formaldehyde is washed away and replaced with a 70% final concentration of ethanol, without further staining the collections. Washed samples are stored at a fixed and documented location in the sample library available at MSO (Flanders Marine Institute, 2018c).

Evaluation after the first sampling year showed a limitation in the sampling protocol due to the surface-water sampling, and led to the optimization of the protocol towards a more standardized and globally accepted method to collect zooplankton. This third method has been applied from January 2014 onwards. Zooplankton is collected by means of a WP2 vertical plankton net, a design proposed by

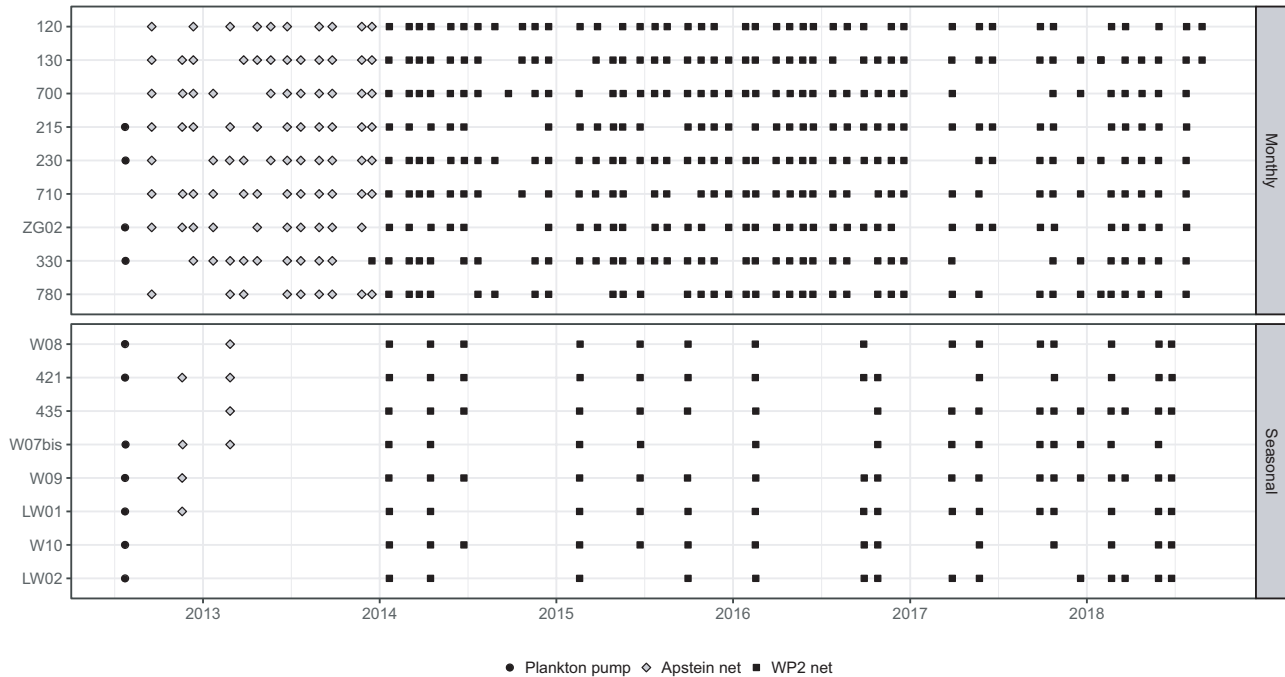


FIGURE 4 Data availability in the sampled area in the Belgian Part of the North Sea, according to the sampling protocol (as described in Sampling method: plankton pump, Apstein net, WP2 net), and station name (as described in Design description: monthly campaigns and seasonal campaigns)

the UNESCO Working Party 2 (Fraser, 1968) (Hydrobios, Kiel, Germany) (Figure 5). The WP2 vertical plankton net consists of a 57-cm diameter steel ring, a mechanical flow-meter with back-run stop attached to the steel ring, a 2.6-m long net with a 200 μm mesh size, a plastic bucket to collect the sample, and a heavyweight to prevent uplifting by currents (Figure 5). The side winch of the RV Simon Stevin lowers the complete setup down to just above sea bottom, and then slowly hauls it up again (max. speed of 1 m/s). After lifting the net from the water, the outside of the net is sprayed with a deck wash to wash the organisms into the bucket. These actions are registered in MIDAS, documenting start and stop times and the start and stop coordinates. The sample is concentrated in the plastic net bucket where zooplankton remains while water escapes through a 200 μm mesh window (Figure 5). The flow is noted in MIDAS and

in the trip report as a backup. The complete bucket is removed from the net for further processing in the wet laboratory of the RV Simon Stevin. Soda water and formaldehyde are added to a 6% formaldehyde concentration. After approximately 1 month with the original fixation in the MSO, the formaldehyde is washed away and replaced with a 70% final concentration of ethanol, without further staining the collections. Washed samples are stored at a fixed and documented location in the sample library available at MSO (Flanders Marine Institute, 2018c). The entire data series has been collected using a 200 μm mesh size, which is commonly used to collect zooplankton (Castellani and Edwards, 2017). This allows users to compare the collected data to recent zooplankton research in Belgium (e.g. Van Ginderdeuren, 2013) and yields organisms large enough to successfully scan with the ZooScan device.



FIGURE 5 From left to right: a WP2 plankton net attached to the side winch of the RV Simon Stevin, being rinsed with a hose; a detail of the WP2 collecting bucket at the bottom of the net; the ZooScanner with a sample on the scanning bed

Due to weather conditions, it was not always possible to sample according to planning, and stations have been omitted from the planning (e.g. Figures 2 and 4).

4 | ANALYSIS METHODS

4.1 | Zooscan

The ZooScan (HYDROPTIC) is a waterproof scanner which allows users to process liquid samples (Figure 5). The ZooScan generates high-resolution digital images of preserved zooplankton: it is capable of scanning a 4800 dpi images, with a pixel resolution of 10.6 μm . A plastic frame is inserted onto the scanning bed for acquisition and processing scans as a single image. After scanning, the sample can be recovered completely by flipping the entire scanning bed. The sample is then preserved again with a 70% ethanol concentration, and stored again in the sample library available at MSO (Flanders Marine Institute, 2018c).

Although it is recommended to scan zooplankton densities of 1000–2000 particles per scan (Grosjean *et al.*, 2004; Gorsky *et al.*, 2010; Vandromme *et al.*, 2011), samples from the BPNS with densities of around 3000–4000 particles per scan could be processed without problem. To reach this density, plankton samples are fractioned with a Motoda splitter (Motoda, 1959), a Plexiglas box designed for fractioning a liquid sample. Depending on the season and densities of plankton, fractions of 1/4 to 1/256 are used, this being taken into account to calculate densities at a later stage. Samples from Belgian waters are very size-uniform, therefore size-fractioning is not necessary, in contrast to samples from Atlantic waters where it is recommended to size-fraction the samples at 1 cm (Gorsky *et al.*, 2010; Vandromme *et al.*, 2011). It is essential to manually separate overlapping organisms on the scanning bed, to allow the processing software to identify individual particles (Gorsky *et al.*, 2010). The complete procedure as described above takes 20–30 minutes.

4.2 | Zooprocess

The ZooProcess software is based on the ImageJ macro language (Abràmoff, Magalhães and Ram, 2005; Rasband, 2005). It is used for image processing, especially for the extraction of regions of interest (ROI) of the scan. Attributes are linked to each ROI in a specific Plankton Identifier file (PID file). Further information and scripts have been published before (Gorsky *et al.*, 2010; Picheral, 2011, 2018). In the current version of ZooProcess, 67 attributes are linked to each ROI, the most relevant being the length of primary axis of the best fitting ellipse (major), length of the secondary axis of the best fitting ellipse (minor), shape (elongation, compactness) and apparent elliptical biovolume (EBv). Key attributes were published as an appendix (Gorsky *et al.*, 2010).

4.3 | Plankton Identifier

Plankton Identifier 1.3.4 is a free software package (Gasparini and Antajan, 2018), that allows for automatic identification of ROIs in your samples. Plankton Identifier allows the user to create a learning set for ROIs by manually assigning taxonomic names to certain ROIs. The software subsequently uses this learning set to assign taxonomic names to ROIs in the actual samples. This method provides counts of individuals of each taxonomic group. Besides biotic groups (e.g. Calanoida, Harpacticoida, Appendicularia, *Noctiluca*, Cumacea ...), micro-debris (e.g. plastics, fibres) can also be recognized and counted. The current learning file is built to recognize samples from the Southern Bight of the North Sea. After prediction by Plankton Identifier, a validation file is generated. This validation file is based on the PID file, in which an additional column will indicate the predicted name of a certain ROI.

4.4 | Quality Control

The current level of taxonomic resolution is restricted and allows data processing by both taxonomist as well as non-taxonomists. All ROIs predicted by Plankton Identifier will be validated twice: initially by non-taxonomist, ultimately by an expert on a yearly basis. This yields a solid dataset, easily comparable to other studies (e.g. the study by Van Ginderdeuren, 2013 using microscopy counts). All quality-controlled data are flagged, and validated names are automatically added to the validation file that was generated by Plankton Identifier.

5 | DATASET LOCATION AND FORMAT

The quality-controlled validation files and ROIs from the ZooScanner are archived to a network archive on the VLIZ servers, and linked with the corresponding metadata in the MIDAS system. These archives are backed-up automatically every 24 hours, and subsequently imported into a MongoDB database in JSON-language. This database allows data manipulation, further quality control and visualization without losing the link with the actual ROIs. The MongoDB database is backed-up and uploaded on-to the IFCA server (Santander, Spain). The LifeWatch data explorer (Flanders Marine Institute, 2018a) allows users to browse quality-controlled data from the MongoDB database to select on specific parameters, apply temporal and spatial filters and create exports of the resulting query.

Besides the continuous availability through the LifeWatch data explorer (Flanders Marine Institute, 2018a), these zooplankton data can contribute to further international biodiversity data initiatives such as the

Ocean Biogeographic Information System (OBIS) and the Biology portal of the European Marine Observation and Data Network (EMODnet). This contribution consists of a yearly export of linked subsets of the MongoDB (data) and the MIDAS system (metadata) which is reformatted according to the OBIS-ENV-DATA format. OBIS-ENV-DATA is an adaptation of the Darwin Core Archive (DwC-A) scheme specifically designed for sample-based marine biological data. This format allows to package taxonomic and environmental data, together with sampling related information, in a single, self-contained dataset (De Pooter *et al.*, 2017). In the OBIS-ENV-DATA standard, the DwC-A file consists of an Event core linked to two extensions namely: Occurrence extension and ExtendedMeasurementOrFact extension (eMoF). The Event core is used to store hierarchical information related to the sampling location, time and depth. Taxonomic information of the presence or absence of a biological entity is captured in the Occurrence extension. The eMoF contains biotic measurements associated with the occurrences (e.g. abundance), environmental data collected at the time of the sampling (including temperature and salinity), and information about sampling devices, and sampling protocols. Within this format, all data are linked to a domain-specific controlled vocabulary developed by the British Oceanographic Datacentre and the European SeaDataNet project. These vocabularies are accessible web services organized in collections (e.g.: P01 for identifying marine environmental and biological measurements, P06 to identify units and L22 for defining sensors and instruments).

Although the zooplankton samples were processed and analysed, it is always possible to recover the original samples from the scanner, and store them again in the sample library available at MSO (Flanders Marine Institute, 2018c). The Belgian LifeWatch project aspires to generate a sample library containing samples available for further biodiversity or ecosystem research. A well-documented and accessible sample library holds a unique potential for retrospective research, for example providing insights into historical phenomena, assessing baselines, trends or simply exploiting these historical samples with new techniques previously unavailable. Here, 24 taxa of zooplankton are distinguished, comprising 673.017 ROIs between December 2014 and July 2017, of which Calanoida, Harpacticoida, Appendicularia, and *Noctiluca* are most common.

Both the physical and the digital zooplankton collections are completely accessible and can be browsed via the LifeWatch data explorer (Flanders Marine Institute, 2018a). Since 2016, fixed versions of the database are distributed annually: a fixed dataset for 2016, 2017 and 2018 is available (Flanders Marine Institute, 2017, 2018b, 2019). Prior to 2016, exports were made upon user request.

6 | DATASET USE AND REUSE

The complete dataset has been given a Creative Commons CC-BY4.0 licence, allowing the use of the data under the condition of providing the reference to the original source. When using data, it is prescribed to acknowledge the LifeWatch program, and include the most recent reference to the dataset, currently being 'Flanders Marine Institute (VLIZ), Belgium (2019): LifeWatch observatory data: zooplankton observations in the Belgian part of the North Sea. <https://doi.org/10.14284/329>' (Flanders Marine Institute, 2019).

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ORCID

Jonas Mortelmans  <https://orcid.org/0000-0002-8781-7915>

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