The validation case on invasive crustaceans of the LifeWatch ERIC Internal Joint Initiative: State of the art and next steps forward

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LifeWatch ERIC, the e-Science European infrastructure for biodiversity and ecosystem research, launched an Internal Joint Initiative on Non-indigenous Species and Invasive Alien Species (NIS-IAS) as they are considered one of the major drivers of biodiversity and ecosystem change. Here, the case study focused on the trophic biogeography of invasive crustaceans is presented, describing the procedures, resources, and analytical web services implemented to investigate the trophic habits of these taxa by using carbon and nitrogen stable isotope data. The case study offers a number of analytical tools to determine the variability of the trophic position of invasive crustaceans in a spatially-explicit context and to model it as a function of relevant environmental predictors. Literature-based stable isotope data of the Atlantic blue crab Callinectes sapidus and of the Louisiana crayfish Procambarus clarkii have been used to evaluate the functionalities and outcomes of the workflow. The Tesseract Virtual Research Environment integrates all the analytical services offered by LifeWatch ERIC, including the ones developed for this case study, by means of a user-friendly interface. The analytical functions implemented for the crustacean workflow provide a proof of concept for future open e-science platforms focusing on NIS-IAS. The workflow conceptual structure can be adapted to a wide range of species, and can be further improved to support researchers in monitoring and predicting trophic-related impacts of NIS-IAS. In addition, it can support policymakers and stakeholders in the implementation of effective management and control measures to limit the negative effects of bioinvaders in recipient environments.

KEYWORDS

virtual research environment, invasive species, carbon 13, nitrogen 15, Callinectes sapidus, Procambarus clarkii, trophic position, ecological impact
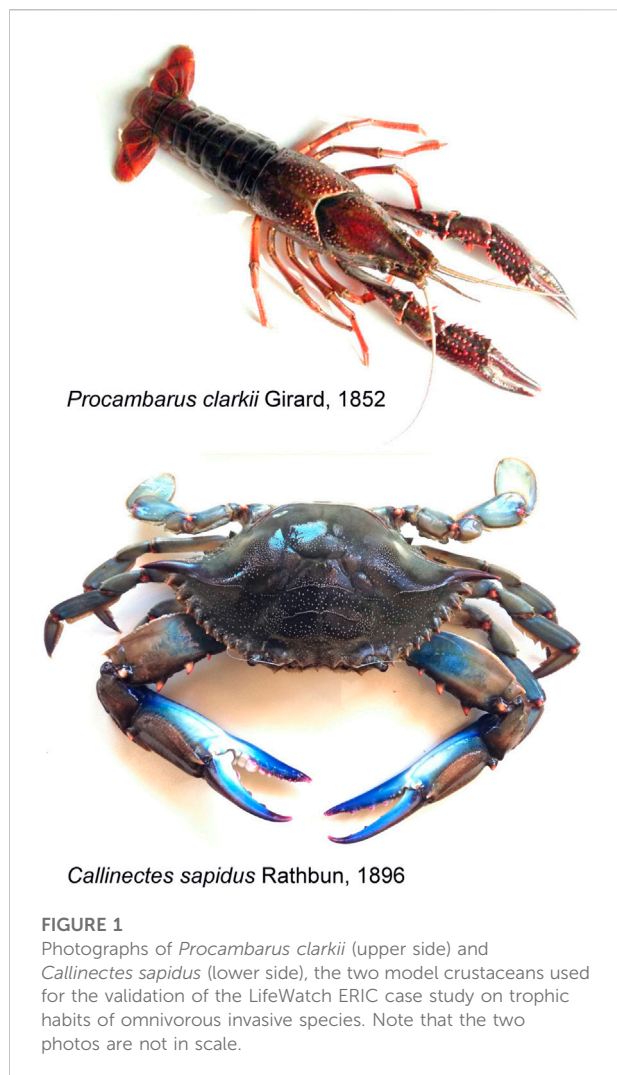
1 Introduction

The modern era of globalization is characterized by an intensification of human activities, such as the transport of people and goods and the creation of artificial corridors, facilitating the introduction of species outside their native geographic ranges (Banks et al., 2015). The European Union has recognized that Non-Indigenous Species (NIS hereafter) require immediate consideration and adequate management and control actions, and has addressed this issue in Regulation 1143/2014 (European Commission, 2014) and in the Biodiversity Strategy to 2030 (European Commission, 2020). The former requires member states to provide lists of NIS that could determine significant negative impacts on biodiversity and on related ecosystem services. However, the availability of comprehensive and measurable impact estimates is still limited (see e.g., Katsanevakis et al., 2014; Tsirintanis et al., 2022 for the Mediterranean Sea), partly due to the scarce information on NIS ecology in nature and on the type and strength of the interactions they establish with the native biota (Cardeccia et al., 2018; Katsanevakis and Moustakas, 2018). The need for a thorough assessment of NIS impacts is particularly imperative when they cause adverse ecological and economic effects and reach the "invasive" status (after UNEP, 1992; Invasive Alien Species–IAS: Russell and Blackburn, 2017).

To date, the impacts of IAS have been mostly evaluated by using qualitative evidence-based knowledge of experts rather than quantitative evidence (Corrales et al., 2020; Essl et al., 2020). In addition, it is difficult to infer common patterns from the observed impacts, as this evidence is generally context-dependent and limited in space and time (Courchamp et al., 2017; Torchin et al., 2021; Watkins et al., 2021). A number of recent regional and global scale investigations, focusing on the impact of bioinvaders, have demonstrated that meta-analytical approaches can overcome the paucity and diversity of available information, and can identify and test the primary factors affecting the context-dependency of IAS impacts. Moreover, they have indicated relatively straightforward ecological rules to locally predict the threat of biological invasions on native communities (Thomsen et al., 2014; Gallardo et al., 2016; Mollot et al., 2017; Anton et al., 2019; Bradley et al., 2019). These studies have shown that the impact of invaders on the biodiversity and functioning of recipient ecosystems depend critically on their trophic habits. For example, Thomsen et al. (2014), Mollot et al. (2017), and Anton et al. (2019) indicated that the trophic position of aquatic invaders controls their adverse effects on biodiversity, a result confirmed by a global meta-analysis on community responses to invasion performed by Bradley et al. (2019). Additionally, Gallardo et al. (2016) indicated that introduced predators and omnivores are the trophic groups that exert the most negative effects on the lowest levels of benthic food webs in aquatic ecosystems as also acknowledged by other studies

(Vazquez, 2006; Romanuk et al., 2009; Havel et al., 2015; Mancinelli et al., 2017b; Médoc et al., 2018).

Building on this evidence, a validation case focused on the trophic habits of crustacean IAS and their impact on invaded benthic food webs was conceived. The case study has been developed by LifeWatch ERIC (https://www.lifewatch. eu/), the e-Science European infrastructure offering e-science tools and facilities to the scientific community committed to biodiversity and ecosystems research. Nowadays, a large variety of projects/initiatives provides datasets, databases, and data analysis tools to tackle different emerging challenges in the field of biodiversity and ecosystem conservation (Escribano et al., 2018; Stephenson and Stengel, 2020). However, the fragmentation of available data often hinders the capacity to reveal key ecological patterns and hampers analytical efforts aiming at integrating such a heterogeneous body of information (Bingham et al., 2017). To overcome the existing limitations in data accessibility, discoverability, and interoperability, LifeWatch ERIC has devoted its efforts to collate and harmonize biodiversity and ecosystems-related data according to the FAIR principles (Findable, Accessible, Interoperable, Reusable: Wilkinson et al., 2016). Most importantly, LifeWatch ERIC provides a diverse range of data and analytical web services, arranged in purpose-built pipelines of work or workflows, and properly structured in Virtual Research Environments (VREs). The latter are web-based, community-oriented, comprehensive, flexible, and secure working environments, allowing users to perform and complete their analyses in biodiversity and ecosystems research (Basset and Los, 2012; see also Enke et al., 2012; Candela et al., 2013). Given the strict association between biological invasions on one hand and the conservation and management of biodiversity in natural ecosystems on the other, in 2019 the executive board of LifeWatch ERIC launched an Internal Joint Initiative (https://www. lifewatch.eu/internal-joint-initiative/). Through the identification of demonstrative case studies on NIS-IAS, the aim of the initiative was to implement validation cases providing data and e-tools to the scientific community to develop evidence-based knowledge on key ecological topics in different research areas of invasion ecology. They included the prediction of dispersion scenarios of keystone NIS in terrestrial ecosystems, the early-detection and monitoring of NIS spread in aquatic habitats through conventional ecological monitoring procedures and DNA metabarcoding, the EUNIS (European Nature Information System, https://eunis.eea.europa.eu/) habitat and ecosystem vulnerability to NIS invasion, and the evaluation of the spatial variability and predictability of trophic-related ecological impacts of invasive crustaceans.

Here the latter validation case is presented, describing the spatially-explicit trophic variations of two study species, i.e., the

FIGURE 1
Photographs of *Procambarus clarkii* (upper side) and
Callinectes sapidus (lower side), the two model crustaceans used
for the validation of the LifeWatch ERIC case study on trophic
habits of omnivorous invasive species. Note that the two
photos are not in scale.

Louisiana crayfish *Procambarus clarkii* and the Atlantic blue crab
Callinectes sapidus, two omnivorous invasive species in European
freshwaters and coastal waters. The possibility that climatic
drivers acting at large spatial scales may influence the trophic
behavior of omnivorous species, independently from their native
or non-indigenous nature, remains to date unexplored. This
occurs notwithstanding the increasing interest in the linkage
between species' trophic-related traits and the ongoing global
climate change, since global warming has the potential to
determine trophic shifts and variations in the trophic niche of
aquatic organisms, ultimately altering the structure and functions
of both freshwater and marine food webs (Bartley et al., 2019;
Wallingford et al., 2020; Henry and Sorte, 2022).

Specifically, here an effort was made to describe: 1) the
procedures used to conceive, design, and define the structure
and the tools needed to assess the trophic habits of invasive
crustaceans using stable isotope data; 2) the methodological
approaches adopted to implement the validation case and the

workflow design, and 3) the structure of the datasets on which the
analytical workflow relies.

Moreover, two preliminary examples focused on the analysis
of stable isotope datasets collated for the validation case and
resolved at both population- and individual-scale are provided,
illustrating the potential outcomes expected from the advanced
implementation of the analytical workflow at the core of the
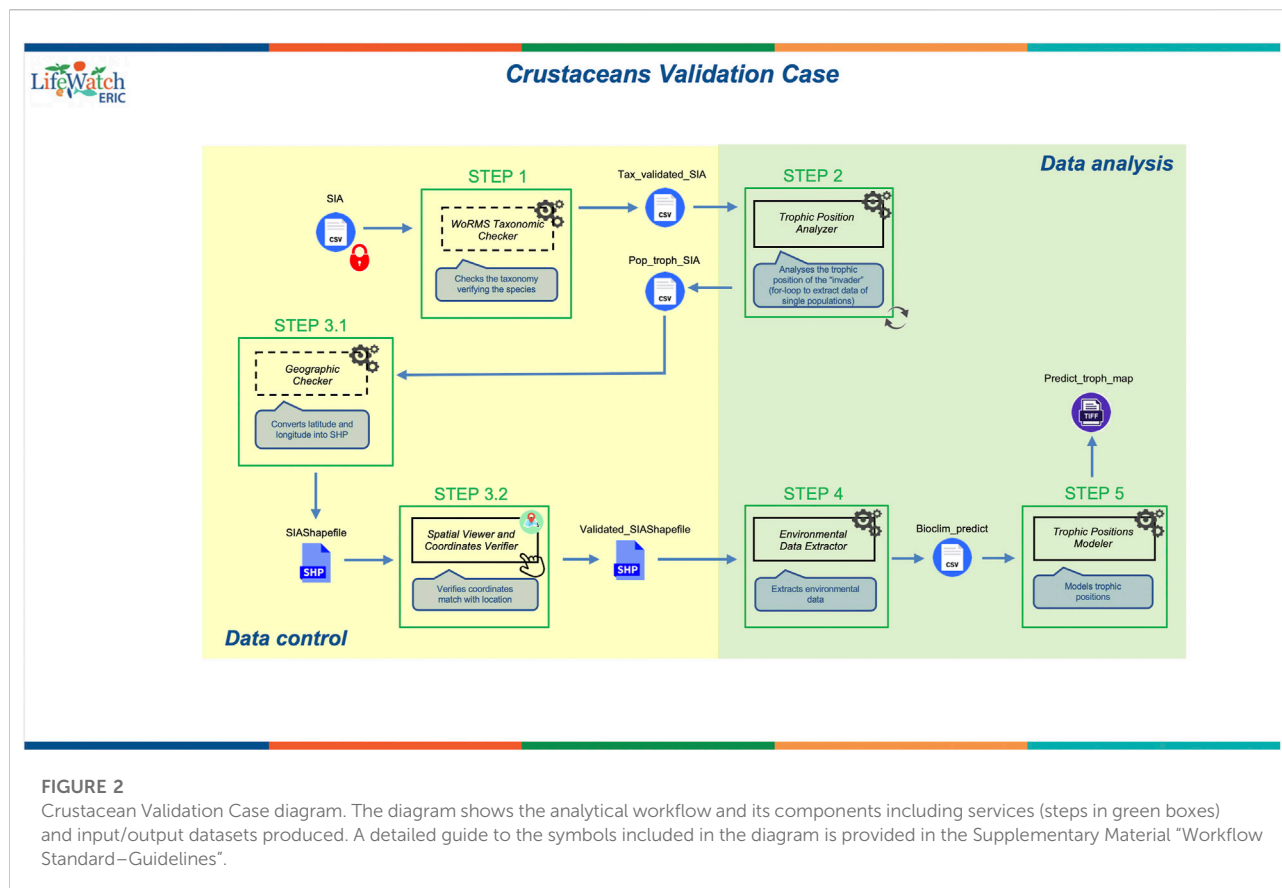validation case.

2 Methods

2.1 Study species

The validation case focused on two widely distributed species
of invasive omnivorous crustaceans, i.e., the Louisiana crayfish *P.
clarkii* and the Atlantic blue crab *C. sapidus* (Figure 1).

Procambarus clarkii (Girard, 1852) is native to the southern
United States and northern Mexico, but it has been introduced
widely in all continents, with the exception of Australia and
Antarctica (Loureiro et al., 2015), for economic purposes,
i.e., aquaculture and fishing activities (Hobbs et al., 1989;
Oficialdegui et al., 2019) and for the biological control of
schistosomes and of other snail-transmitted parasites, as
occurred in a number of African countries (Hofkin et al.,
1991; Sulieman et al., 2013). *Procambarus clarkii* is included
in the European list of IAS of greatest concern (European
Commission, 2008). Due to its biological and ecological
characteristics (Hänfling et al., 2011), this species causes
serious harm to the biodiversity and functions of the invaded
environments as well as to ecosystem services and human
infrastructures (e.g. irrigation canals and rice crops:
Twardochleb et al., 2013; Souty-Grosset et al., 2016).

The origin of the Atlantic blue crab *C. sapidus* (Rathbun,
1896) is the western Atlantic coast and its native range spans
from New England to Uruguay (Millikin and Williams, 1984).
This omnivorous crab was introduced in Europe in 1901 and
first recorded in the Mediterranean basin in 1947, although it
has been suggested that it had arrived a decade earlier
(Nehring, 2011). In the last decade, *C. sapidus* has spread
almost ubiquitously in the Black Sea and in the eastern and
central Mediterranean Sea, with the western and southern
sectors of the basin as fronts of the most recent range
expansions (Mancinelli et al., 2021). Since 2006, *C. sapidus*
has been recognized as an invasive species in the
Mediterranean Sea (Streftaris and Zenetos, 2006), however,
a review published in 2017 (Mancinelli et al., 2017a) and a risk
assessment performed in 2021 for the EU (Rabitsch et al.,
2022), highlighted the rather scant information on its actual
impact on recipient communities. Clavero et al. (2022)
provided the first quantitative assessment of the adverse
effects exerted by the blue crab on the biodiversity of
recipient communities.

FIGURE 2
Crustacean Validation Case diagram. The diagram shows the analytical workflow and its components including services (steps in green boxes) and input/output datasets produced. A detailed guide to the symbols included in the diagram is provided in the Supplementary Material "Workflow Standard–Guidelines".

2.2 Data collection

Stable isotopic data were collated from the literature to provide extensive, standardized, and spatially-explicit information on the trophic habits of the study species at different spatial scales. Stable isotopes analysis has gained huge popularity for the study of aquatic food webs in the last decades, allowing for robust comparisons of species dietary habits at a local as well as regional and global scale (e.g., Mancinelli, 2012; Annabi et al., 2018; Pethybridge et al., 2018; Mancini et al., 2021; Mancini et al., 2022). This methodology is currently recognized as a key tool to deepen the understanding of ecosystem processes and functioning and to evaluate the response of aquatic ecosystems to anthropogenic impacts, including global climate change (Mancinelli and Vizzini, 2015; Gautam and Lee, 2016).

An extensive literature search was performed to collate geo-referenced stable isotope data resolved at individual- and population-scale. Extensive details on the adopted procedures are provided in Di Muri et al. (2022a); Di Muri et al. (2022b). In brief, the online databases ISI Web of Science and Scopus were searched by using a multiple search criterion and the term "*Procambarus clarkii*" or

"*Callinectes sapidus*" in conjunction with "stable isotopes". The results were complemented with those obtained from queries on Google Scholar (https://scholar.google.com/) using identical keywords. The entries obtained from the literature search had their titles and abstracts screened in order to remove manipulative studies and reviews. The full text of the remaining studies was examined in detail to select those where the occurrence of the two species was reported explicitly, together with information on the country, latitude and longitude, the year of the record, and at least the δ^{15}N value of the sampled specimens. Publications where the sampling locations were reported in maps were also included in the selection and Google Earth was used to extract the geographic coordinates. In addition, the literature sources to be eligible had to include isotopic information on potential vegetal or animal prey. The publications meeting the aforementioned criteria were eventually selected and δ^{15}N and δ^{13}C values were extracted from tables and figures. Figures were digitized after a fivefold enlargement and converted to numerical form using the graph capture freeware WebPlotDigitizer (ver. 4.5; https://automeris.io/WebPlotDigitizer/). In the dataset resolved at individual scale, δ^{15}N and δ^{13}C values refer to single specimens, whereas in the population-scale dataset, mean values of carbon and nitrogen
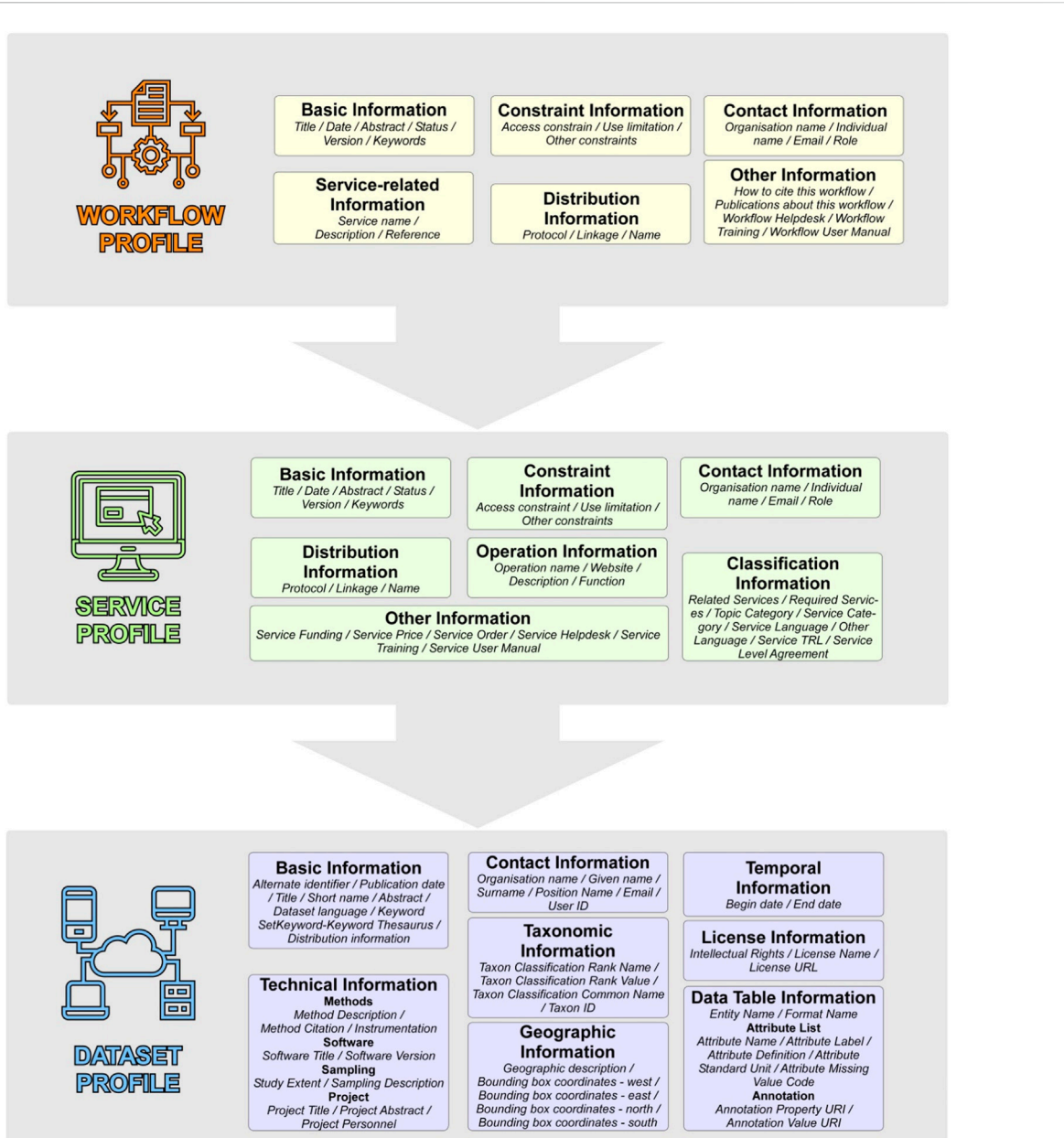
FIGURE 3
LifeWatch ERIC metadata profile for workflows (yellow), services (green), and datasets (blue). The asterisks (*) indicate the mandatory attributes. A complete description of the LifeWatch ERIC Application profiles is available in Vaira et al. (2022).

stable isotopes were extracted from the literature along with their standard deviations and sample sizes if available. Lastly, the authors of the selected publications were contacted directly to obtain additional unpublished information and raw data, hence, the collated datasets include published data from peer-reviewed articles and grey literature as well as data gathered from unpublished sources, ultimately offering visibility to previously unavailable information.

2.3 The workflow

The datasets are conceived and designed to be analyzed by means of a number of sequential analytical web services included in a workflow. The structure of the workflow is presented as a diagram in Figure 2 and was developed using the LifeWatch ERIC guidelines as a general reference and through a collaborative approach between scientists and ICT

TABLE 1 List of climatic variables and related databases and layer names used for the multiple correlation analysis.

Database	Variable name	Layer name
MARSPEC	Mean annual SST	biogeo13
MARSPEC	SST of the coldest ice-free month	biogeo14
MARSPEC	SST of the warmest ice-free month	biogeo15
MARSPEC	Annual Range in SST	biogeo16
MARSPEC	Annual Variance in SST	biogeo17
MERRAclim	Annual Mean Temperature	BIO1
MERRAclim	Mean Diurnal Range	BIO2
MERRAclim	Isothermality	BIO3
MERRAclim	Temperature Seasonality	BIO4
MERRAclim	Max Temperature of Warmest Month	BIO5
MERRAclim	Min Temperature of Coldest Month	BIO6
MERRAclim	Temperature Annual Range	BIO7
MERRAclim	Mean Temperature of Wettest Quarter	BIO8
MERRAclim	Mean Temperature of Driest Quarter	BIO9
MERRAclim	Mean Temperature of Warmest Quarter	BIO10
MERRAclim	Mean Temperature of Coldest Quarter	BIO11

*SST, sea surface temperature.

experts. The overall goal of the workflow is to analyze the trophic geography of the two crustacean species under analysis, assuming their trophic position as an indirect indicator of their ecological impact on recipient aquatic food webs. This is performed by first running a spatially-explicit quantitative assessment of the trophic position of the two species and then by finding potential correlations between the invaders' trophic position and climatic drivers that are expected to change in the future (e.g., water temperature or salinity for marine ecosystems and air temperature for terrestrial ecosystems).

Specifically, the workflow was designed to include three functional analytical services for calculating the impact of the invaders (data analysis, in green in Figure 2) and three additional services to check the quality of the data entered by the users (data control, in yellow in Figure 2) and allowing for automatic corrections and adjustments if required.

The key data analysis steps and services included therein consist of: 1) the calculation of the trophic position of the invasive species through a Bayesian approach (Trophic Position Analyzer); 2) the extraction of environmental predictors from cloud-based repositories (Environmental Data Extractor); this service fetches and downloads from available repositories oceanographic and bioclimatic raster layers for the geographic coordinates included in the input dataset; and 3) the modelling of

the invaders' trophic position as a function of the environmental variables using a Generalized Additive Model (Trophic Positions Modeler). The data control steps allow the user to: 1) check the scientific names of the species included in the input dataset (WoRMS Taxonomic Checker); 2) create a shapefile using the geographic coordinates included in the input dataset (Geographic Checker); and 3) verify the accuracy of the geographic coordinates (Spatial Viewer and Coordinates Verifier) by matching the latitude and the longitude with the corresponding locations and countries included in the input dataset, and by using the shapefile created within the previous service (i.e., Geographic Checker).

The resources created for the workflow (i.e., workflow, services, and datasets) are described using the LifeWatch ERIC application profiles and are published on the LifeWatch ERIC Metadata Catalogue (https://metadatacatalogue.lifewatch.eu). The metadata records of the workflow and services are documented with the ISO19139 standard and include respectively 24 and 34 metadata attributes (Figure 3). Dataset metadata records are documented with EML2.2.0 standard and the metadata schema includes 77 attributes (Figure 3). A complete description of the LifeWatch ERIC Application profiles is available in Vaira et al. (2022).

Links to metadata resources within the LifeWatch ERIC Metadata Catalogue and used for this case study are provided in the Supplementary Material.
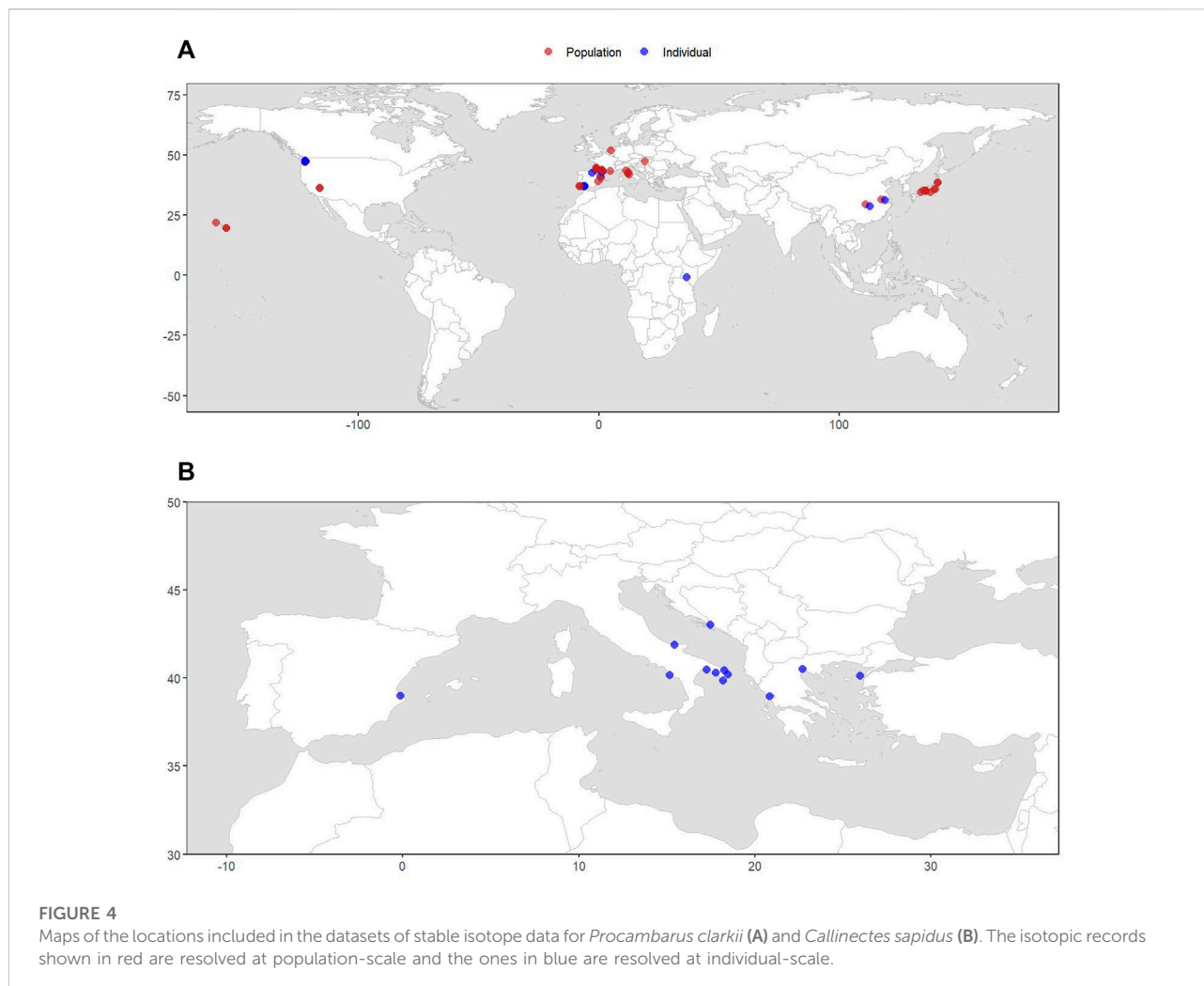
2.4 Data analysis

The analyses are implemented within the workflow through the R statistical environment (R Development Core Team, 2022). Specifically, the trophic position of the invasive species (Trophic Position Analyzer, Figure 2) is calculated using the *tRophicPosition* package (version 0.7.7; Quezada-Romegialli et al., 2018; Quezada-Romegialli et al., 2019). Trophic position estimations are conventionally performed using:

$$TP_{\delta^{15}N} = \left(\delta^{15}N_{Consumer} - \delta^{15}N_{Baseline}\right)\Big/\Delta^{15}N + \lambda$$

This equation is a generalization of that presented in Vander Zanden et al. (1997), where $\delta^{15}N_{Consumer}$ is the nitrogen isotopic signature of the invasive species under investigation (i.e., *P. clarkii* or *C. sapidus*), $\Delta^{15}N$ is the trophic discrimination factor of $\delta^{15}N$ (TDF hereafter), and $\delta^{15}N_{Baseline}$ and λ are, respectively, the nitrogen isotopic signature and the trophic level of the baseline species.

In the *tRophicPosition* package, the trophic position is estimated using a Bayesian approach whereby nitrogen values of consumers and baselines as well as the TDF are modelled as random variables, each having a prior normal distribution on their means and a uniform prior distribution on their standard deviations, and the trophic position is treated as a random
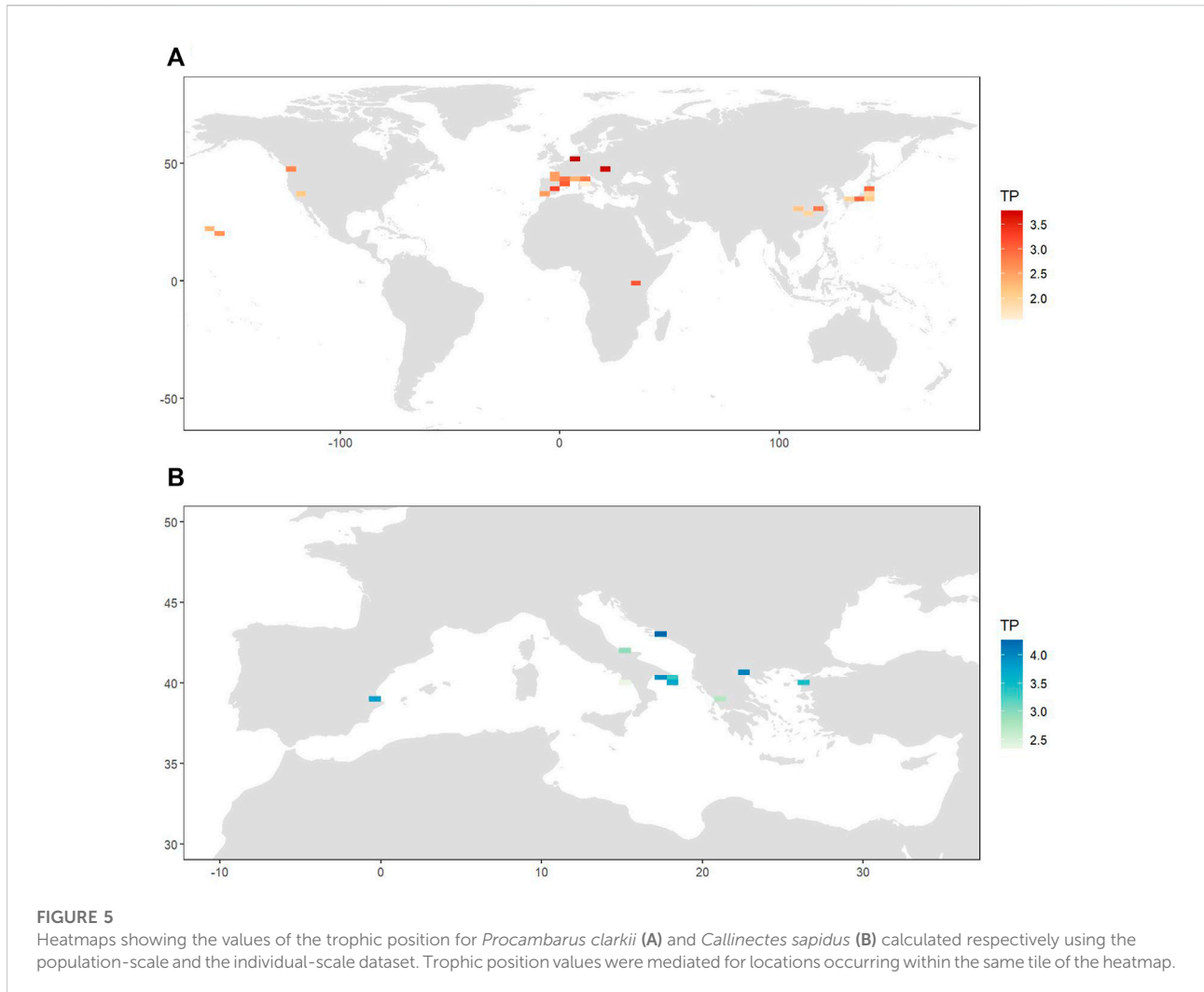
FIGURE 4
Maps of the locations included in the datasets of stable isotope data for *Procambarus clarkii* **(A)** and *Callinectes sapidus* **(B)**. The isotopic records shown in red are resolved at population-scale and the ones in blue are resolved at individual-scale.

parameter. The trophic position is also modelled as having a uniform prior distribution whereas λ is fixed. Given the omnivorous trophic habits of the species considered in this case study, the values of the trophic positions are estimated adopting a TDF of 0.27 ± 2.15 for carbon and a TDF of 2.58 ± 1.96 for nitrogen (mean ± 1SD, n = 49 and 91, respectively), calculated as the average of published enrichment factors for aquatic crustaceans (including *C. sapidus* and the crayfish *Orconectes viridis* and *Cherax destructor*) feeding on both vegetal and animal items (De Giorgi et al., 2022), and more consistent with other literature syntheses focused on crustaceans (Vanderklift and Ponsard, 2003; Carrozzo et al., 2014). The complete list of species selected as baselines and their assigned trophic levels can be found in Mancinelli and Di Muri. (2022a); Mancinelli and Di Muri. (2022b).

The Environmental Data Extractor service (Figure 2) performs the selection of a suite of oceanographic and climatic data from Bio-Oracle v2.0 (Tyberghein et al., 2012; Assis et al., 2018 for *C. sapidus*) and from WorldClim 2 (Fick

and Hijmans, 2017 for *P. clarkii*). Bio-Oracle is a high-resolution GIS database of oceanic climate layers obtained from remotely sensed and *in situ* oceanographic observations with a common spatial resolution of five arcmin. The database additionally includes future projections produced for 2040–2050 and for 2090–2100 by averaging data from distinct Atmosphere/Ocean Global Climate Models (AOGCMs). WorldClim is a dataset with a global coverage including satellite-based climatic layers that have been built for the period 1970–2000 with a 30 arc-seconds spatial resolution and including downscaled future climate projections. The bioclimatic variables from these repositories are retrieved in R using the package *sdmpredictors* (v. 0.2.12; Bosch et al., 2022).

In the Trophic Position Modeler service (Figure 2), a generalized additive mixed model (GAMM; Wood, 2017) is used to examine relationships between the values of the trophic position and the suite of selected environmental predictors. GAMMs are built in R using the *gamm4*

FIGURE 5
Heatmaps showing the values of the trophic position for *Procambarus clarkii* **(A)** and *Callinectes sapidus* **(B)** calculated respectively using the population-scale and the individual-scale dataset. Trophic position values were mediated for locations occurring within the same tile of the heatmap.

package (Wood and Scheipl, 2020) with a Gaussian error distribution and an identity link function; smoothing splines are used to examine non-linear fits for all marginal terms considered in the model. Exclusive economic zones and countries are incorporated as random effects to account for intra-group correlations and over-dispersion frequently arising in spatial analyses (Wood, 2017), whereas a scale-invariant tensor product is used to model the covariates for the geographical locations. Model selection is then performed manually using a backward elimination approach (starting with all candidate variables), and the best model fit is selected using the Akaike Information Criterion for small sample sizes (AICc).

Here, for illustrative purposes, the analyses presented focus on the population-scale dataset of *P. clarkii* (Mancinelli and Di Muri, 2022b) and on the individual-scale dataset of *C. sapidus* (Mancinelli and Di Muri, 2022a) using temperature-related continuous predictors for present conditions. The geo-

referenced trophic position values were estimated according to Vander Zanden et al. (1997) and temperature-related oceanographic and climatic high-resolution layers were selected from MERRAclim (Carrete Vega et al., 2017 for *P. clarkii*) and MARSPEC (Sbrocco and Barber, 2013 for *C. sapidus*) (Table 1). Sea surface temperatures were used for *C. sapidus*, whereas air temperatures were used as a proxy of inland water temperatures for *P. clarkii* due to the unavailability of detailed raster layers for surface water temperatures in freshwater habitats (Piccolroaz et al., 2018; Table. 1). Subsequently, a simplified procedure based on a multiple correlation approach was performed with the aim of identifying the most adequate temperature-related variables to predict the variation in the trophic positions observed within the two datasets. Climatic variables and trophic position values were log-transformed and mean-centered for the analysis. The identification of the Minimum Adequate Model (MAM hereafter; Whittingham et al., 2006) was based on the heuristic generation of

TABLE 2 Bayesian Information Criterion (BIC) values and Bayes factors (B) estimated by the heuristic procedure implemented for Minimum Adequate Model (MAM) selection for *Procambarus clarkii*. Only the 10 models showing the lowest BIC values are reported. See Table 1 for layer acronyms.

Layers included in the model	BIC	B
BIO8	3.66	
BIO1	4.43	1.47
BIO10	5.12	2.08
BIO6	5.21	2.17
BIO9	5.53	2.55
BIO6-BIO8	6.43	3.99
BIO5-BIO8	6.73	4.64
BIO8-BIO10	6.74	4.66
BIO7-BIO8	6.93	5.13
BIO2-BIO8	7.28	6.11

alternative regression models. Model selection was performed by adopting an information theoretic criterion (Burnham and Anderson, 2002; Hegyi and Garamszegi, 2011) based on the estimation of the Bayesian Information Criterion (BIC; Schwarz, 1978) calculated for each combination of n explanatory variables. For model comparisons, approximate Bayes factors $B_{1,2}$ were calculated as:

$$B_{1,2} = exp\left\{\frac{-1}{2}\Delta_{1,2}\right\}$$

where $B_{1,2} = BIC(k_1) - BIC(k_2)$, and $BIC(k_n)$ are the BIC values of models k_1 and k_2. If $B_{1,2} > 1$, then model k_1 is the model selected by the procedure (Neath and Cavanaugh, 2012) and identified as the best MAM. Model building and statistical analyses were performed following Fox and Weisberg. (2011).

Further analyses, not included in the current version of the workflow, were performed to provide an example of metrics that could be inferred by using stable isotope data, as well as to present the possible future implementations of the workflow. Using the individual-resolved data of *C. sapidus* six quantitative population metrics, originally described by Layman et al. (2007), were estimated for each location including the mean distance to centroid (CD) as a measure of population trophic diversity, nitrogen range (NR) and carbon range (CR) as indicators of the total nitrogen and carbon range exploited by each population, the standard deviation of nearest neighbor distance (SDNND) as an index of population trophic evenness, the total area (TA) as a measure of each population niche area estimated using a convex hull drawn around the most extreme data points on an isotope bi-plot. As TA is expected to increase with sample size (Jackson et al., 2011), additional estimations of niche areas were performed using the corrected standard ellipse
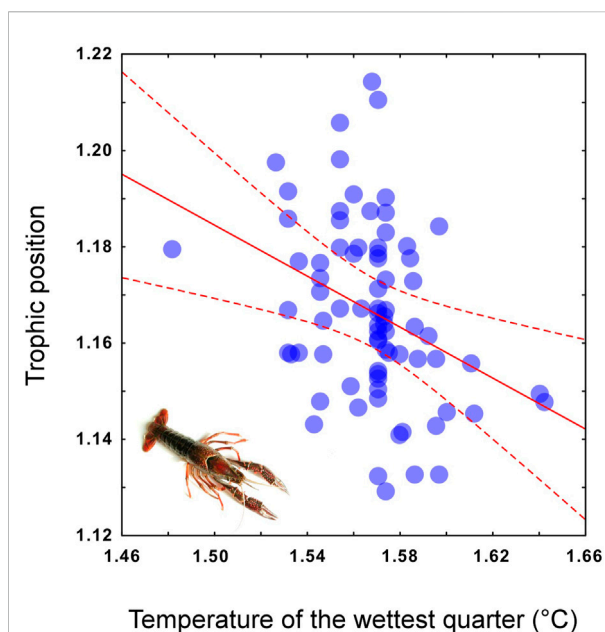
FIGURE 6
Trophic position of *Procambarus clarkii* calculated from the population-scale dataset as predicted by local mean temperature of the wettest quarter values. For the regression curve (solid line) 95% confidence intervals are included (dashed line). Log-transformed and mean-centered data are used in the scatterplot.

area (SEAc) as a measure of each population niche area estimated using a standard ellipse calculated from the variance and covariance of $\delta^{13}C$ and $\delta^{15}N$ values and corrected for small sample sizes. All metrics were calculated using the package *SIBER* (Jackson and Parnell, 2021) in the R statistical computing environment (R Development Core Team, 2022).

3 Results

Two datasets for *P. clarkii* and one dataset for *C. sapidus* were collated and included carbon and nitrogen isotopic values of the species and of their potential prey, and used as baselines for the analyses, within invaded habitats Mancinelli and Di Muri. (2022a); Mancinelli and Di Muri. (2022b). An additional dataset with stable isotope values of *C. sapidus* collected within the native area is currently under completion.

The literature search on *P. clarkii* identified 41 studies, performed between 2005 and 2021 within the global invaded range of the species, including geo-referenced stable isotope information. A population-scale dataset with mean and standard deviation values of 160 carbon and nitrogen isotopic records for *P. clarkii* and its potential prey was collated. In addition, a dataset resolved at the individual scale and

TABLE 3 Sample number (n), trophic position (TP), mean distance to centroid (CD), nitrogen range (NR), carbon range (CR), standard deviation of nearest neighbor distance (SDNND), total area (TA), and corrected standard ellipse area (SEAc) of the 12 *Callinectes sapidus* populations included in the individual-scale dataset.

Location	n	TP	CD	NR	CR	SDNND	TA	SEAc
Acquatina	20	3.35	2.06	3.24	8.63	0.4	15.96	6.52
Gandia	19	3.8	2.91	7.55	9.22	0.78	35.46	15.14
Lesina	24	2.96	0.85	2.65	3.31	0.25	4.83	1.4
Loudias	15	4.04	2.52	6.57	6.97	0.83	24.91	12.17
Pogonitsa	19	2.73	1.77	5.18	4.78	0.51	14.96	6.32
Parila	15	4.26	1.12	2.78	3.37	0.19	5	2.59
Alimini	7	3.34	1.29	3.29	3.14	1.1	5.23	4.46
Mar Piccolo	13	4.49	3.63	4.93	8.34	0.26	20.87	17.57
Torre Colimena	48	3.4	2.61	8.76	7.85	0.43	34.9	11.95
Spunderati	16	3.74	3.16	2.04	11.57	0.25	9.53	4.41
Alento River	10	2.34	0.88	2.51	2.19	0.28	2.44	1.5
Gokceada	30	3.46	0.74	3.2	2	0.24	2.9	0.82

consisting of 1,168 isotopic records was produced. The isotopic values included within the two datasets were gathered from 39 locations and 10 countries across Europe, Asia, Africa, and North America and, therefore, including all continents where *P. clarkii* has been found (Figure 4A).

The average trophic position of *P. clarkii* calculated from the population-scale isotopic dataset was 2.7 ± 0.07 (mean ± SE); estimations varied considerably among locations and ranged between values close to 2, indicating fully herbivorous trophic habits, and values >4, suggesting a carnivorous diet including other predatory species (Figure 5).

The multiple regression and the subsequent heuristic search for the MAM performed using the trophic positions of *P. clarkii* as the response variable and the 11 temperature-related MERRAclim variables as predictors, indicated that the best MAM was a single-variable model including the mean temperature of the wettest quarter as the only significant predictor (Table 2: BIC; Figure 6) and a significant negative relationship was observed (Pearson correlation: $r = -0.35$; $p = 0.002$; Figure 6). Noticeably, a significant inverse correlation was observed also with the mean annual temperature (Pearson correlation: $r = -0.24$, $p = 0.03$).
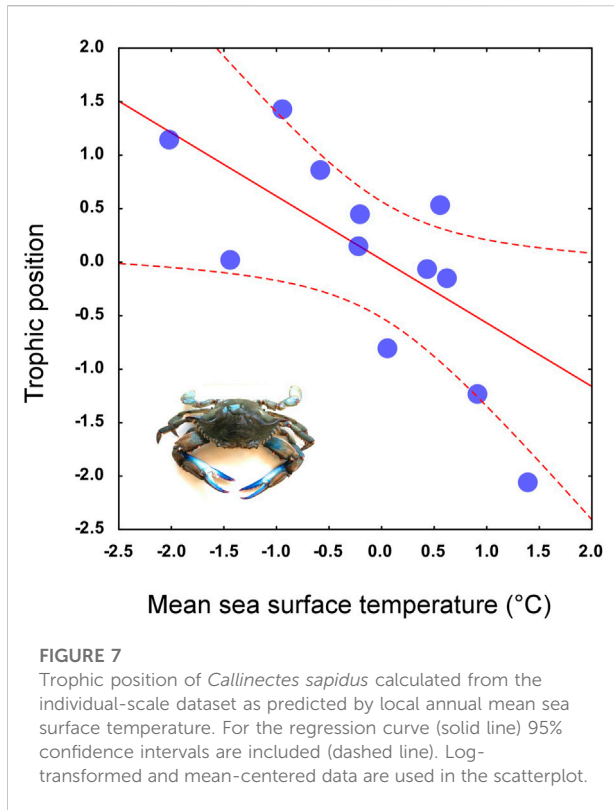
For *C. sapidus*, an individual-scale dataset was collated including 360 isotopic records gathered from 12 Mediterranean locations where *C. sapidus* has been introduced and is currently considered invasive.

The mean trophic position of *C. sapidus* was 3.5 ± 0.2 (mean ± SE), with values showing a two-fold variation between 2.3 and 4.5 (Table 3; Figure 5). The multiple regression and MAM identification procedure performed to find relations between the trophic positions of *C.*

TABLE 4 Bayesian Information Criterion (BIC) values and Bayes factors (B) estimated by the heuristic procedure implemented for Minimum Adequate Model (MAM) selection for *Callinectes sapidus*. Only the 10 models showing the lowest BIC values are reported. See Table 1 for layer acronyms.

Layers included in the model	BIC	B
biogeo13	−0.1	
biogeo15	3.31	5.5
biogeo14	4.42	9.58
biogeo16	4.94	12.43
biogeo17	4.97	12.62
biogeo13-biogeo15	0.78	1.55
biogeo13-biogeo14	1.84	2.64
biogeo13-biogeo17	2.35	3.4
biogeo13-biogeo16	2.35	3.4
biogeo15-biogeo16	5.56	16.95

sapidus and five MARSPEC temperature-related variables indicated that the best predictive MAM was provided by the mean annual sea surface temperature (Table 4; Figure 7). Specifically, a significant negative relationship was observed (Figure 7; Pearson correlation: $r = -0.59$, $p = 0.04$), indicating that the highest trophic positions were related with the lowest sea surface temperatures. The isotopic niche metrics estimated for the 12 populations of *C. sapidus* included in the individual-scale dataset are reported in Table 3. None of them was correlated with sample size or trophic position estimations (Table 5); furthermore, total

FIGURE 7
Trophic position of *Callinectes sapidus* calculated from the individual-scale dataset as predicted by local annual mean sea surface temperature. For the regression curve (solid line) 95% confidence intervals are included (dashed line). Log-transformed and mean-centered data are used in the scatterplot.

TABLE 5 Correlation matrix (Pearson r coefficient) among sample number (n), trophic position (TP), mean distance to centroid (CD), nitrogen range (NR), carbon range (CR), standard deviation of nearest neighbor distance (SDNND), total area (TA), and corrected standard ellipse area (SEAc) of the 12 *Callinectes sapidus* populations included in the individual-scale dataset. All metrics log-transformed and mean-centered before analysis. Significant correlations with $p < 0.05$ are highlighted in bold.

	TP	CD	NR	CR	SDNND	TA	SEAc
N	0.01	0.07	0.40	0.19	-0.34	0.33	0.04
TP		0.55	0.25	0.49	0.04	0.43	0.51
CD			0.53	**0.94**	0.21	**0.86**	**0.90**
NR				0.42	0.48	**0.84**	**0.78**
CR					0.14	**0.84**	**0.80**
SDNND						0.38	0.46
TA							**0.94**

area (TA), and corrected standard ellipse area (SEAc) estimations resulted highly correlated with each other and with mean distance to centroid (CD), nitrogen range (NR), and carbon range (CR) values. No significant bivariate relationships were observed between isotopic niche metrics and temperature-related MARSPEC predictors (Table 6), even though total area (TA) and corrected standard ellipse area (SEAc) values showed a positive relationship with the sea surface temperature of the warmest ice-free month ($p < 0.09$; Table 5).

4 Discussion

This paper described the procedures employed to collect data and design the workflow at the basis of the validation case focused on the two invasive crustaceans, i.e., *C. sapidus* and *P. clarkii*, and developed within the LifeWatch ERIC Internal Joint Initiative on non-indigenous and invasive species. The functionalities of the workflow have been illustrated by carrying out a preliminary analysis of the data performed both at population- and individual-scale. Independently from the resolution of the datasets, the results revealed the existence of significant relationships between temperature-related variables and the trophic positions of the two invasive crustaceans.

Ultimately, the outcomes showed the ability of the workflow to identify environmental-driven trophic patterns at different spatio-temporal scales by using stable isotope analysis.

The availability of stable isotope information is crucial to investigate drivers of changes in the trophic structure within different ecosystems; however, such studies are often hampered by the availability of stable isotope data. For example, within this case study, an extensive and labour-intensive literature search was performed to gather stable isotope information across a variety of sources. Isotopic data collected by researchers worldwide are currently spread over a variety of sources, including peer-reviewed articles, gray literature, datasets stored in different repositories, or other resources which are not accessible to users (Woo and Scheipl, 2020). The lack of a unique and standardized data repository for stable isotopes hinders the possibility of casting these data into wide, continental or global, spatial contexts and of using them beyond the scope for which they were originally generated (Pauli et al., 2017). Therefore, potential drivers of trophic variations acting at large geographical scales may remain undetected and the possibility of estimating the role of global environmental pressures, such as climate warming, in species trophic habits may be overlooked. It follows that this precious body of information deserves to be properly standardized, managed and disseminated, and made available in the long-term in a stable, reliable, and organized repository. Scientists have long acknowledged the need to build centralized databases for stable isotope data obtained from ecological studies in order to facilitate the integration and re-usability of such data across multiple scientific disciplines (Pauli et al., 2017; Eftimov et al., 2019; Woo et al., 2021). Such a centralized repository of stable isotope data would prevent the time-consuming efforts of data collection from different sources. In

TABLE 6 Correlation matrix (Pearson r coefficient) between MARSPEC temperature-related predictors (see Table 1 for acronyms) and sample number (n), mean distance to centroid (CD), nitrogen range (NR), carbon range (CR), standard deviation of nearest neighbor distance (SDNND), total area (TA), and corrected standard ellipse area (SEAc) of the 12 *Callinectes sapidus* populations included in the individual-scale dataset. All metrics log-transformed and mean-centered before analysis.

	biogeo13	biogeo14	biogeo15	biogeo16	biogeo17
n	−0.20	−0.24	−0.12	0.20	0.23
CD	0.20	0.24	0.46	0.03	0.03
NR	0.27	0.15	0.40	0.08	0.13
CR	0.14	0.13	0.42	0.13	0.13
SDNND	0.40	0.15	0.55	0.16	0.22
TA	0.24	0.13	0.49	0.16	0.20
SEAc	0.29	0.27	0.49	0.01	0.05

addition, it would facilitate data integration, standardization, harmonization, and ensure data interoperability by making each dataset accessible in a both human and machine-readable format through the use of controlled metadata and defined ontologies (Eftimov et al., 2019). In this context, the LifeWatch e-Infrastructure with its distributed nodes and facilities offers suitable features to guarantee the long-term storage of data, metadata, and semantic resources, such as those of the LifeWatch Italy Data Portal (https://dataportal.lifewatchitaly.eu), the LifeWatch ERIC Metadata Catalogue (https://metadatacatalogue.lifewatch.eu), and EcoPortal (http://ecoportal.lifewatch.eu/). The e-Infrastructure provides stable and reliable web-based solutions to share data and analytical tools and to make them findable, accessible, interoperable, and reusable according to the FAIR principles (Wilkinson et al., 2016), and it would, therefore, represent an ideal repository of stable isotope data for both ecological and environmental studies.

The validation of our analytical workflow on the population-scale dataset for *P. clarkii* and on the individual-scale dataset for *C. sapidus* indicated that the collection of geo-referenced stable isotope information on invasive species and on their potential prey can be used to explore ecologically-meaningful relationships between environmental variables and the trophic positions of the invaders and, ultimately, develop scenarios for future trends. The preliminary analytical outcomes presented here are supported by other studies where such associations were also observed. For example, the use of a global meta-analysis of stable isotope data demonstrated that changes in the trophic positions of three tuna species were associated with latitudinal and oxygen gradients (Pethybridge et al., 2018). Using a similar analytical approach, this study revealed that temperature-related variables may be associated with the variations in the trophic positions of *P. clarkii* and *C.*

sapidus within invaded aquatic food webs (Figures 6,7). It follows that, independently from ecological constraints related to the structure of the recipient communities in terms of e.g., resource availability, species composition, or local environmental conditions, the trophic role played by an invader within a food web is related with large-scale environmental drivers ultimately influenced by climate change. Specifically, the results of this study indicated that at relatively high temperatures omnivorous invaders occupy higher trophic levels in food webs, whereas at lower temperatures they may shift to lower positions within the trophic network. To provide an in-depth analysis of the factors involved in the observed relationships goes beyond the scope of the present study; however, as it is acknowledged that the whole-body metabolic costs increase with higher external temperatures (Brown et al., 2004; Arim et al., 2007; Bonnaffé et al., 2021), the observed shifts in the trophic positions of *P. clarkii* and *C. sapidus* may be explained by changes in the metabolic responses of the species as a result of their trophic plasticity. How and to what extent the observed trophic shifts affect the ecological impact of omnivorous invasive species on recipient communities is an open question which requires additional investigation. Yet it is likely the variation from omnivorous to primarily carnivorous trophic habits may increase their overall impact as previously suggested (Thomsen et al., 2014; Gallardo et al., 2016; Mollot et al., 2017; Anton et al., 2019; Bradley et al., 2019). The observed relationships between external temperature and trophic position additionally indicate that future changes in the climatic conditions experienced by the two species may affect their trophic positions and, in turn, their impact in invaded food webs. To date, while a considerable effort has been made to evaluate the effects of climate warming on the frequency of introduction and invasiveness of NIS-IAS (Bellard et al., 2018; Ricciardi et al., 2021), less consideration has been given to the

study of climatic variations and on their influence on the impact of established invaders. This study clearly shows that aquatic predators and their functional role in food webs may be particularly vulnerable to climate change and that global warming may play a major role in determining future functional characteristics of the species, and, in the case of bioinvaders, their trophic impact on recipient communities and ecosystems.

To provide higher flexibility to the end-users and to expand the current scope of the workflow it would be essential to widen its analytical features by, for instance, adding trophic niche metrics calculation and accounting for all possible combinations of climatic variables that could shape the trophic habits of invasive species. The future implementations will allow for the testing of bioclimatic predictors of trophic positions of different species, native and non-indigenous, in potentially all types of environments, including aquatic and terrestrial. The possibility of querying and extracting environmental variables of all kinds from different repositories, online platforms, and databases, beyond the ones used within the case study, will allow for greater customization by end-users and make the potentiality of this workflow unlimited.

In conclusion, the present work clearly demonstrated how collaboration between scientific and ICT communities for the development of cutting-edge e-tools could greatly facilitate scientists in finding evidence on rapid responses of biodiversity and ecosystems to the most critical issues for conservation and inform environmental managers to take prompt action. The LifeWatch ERIC Internal Joint Initiative on invasive species and the specific case study described herein focuses on evaluating and predicting the trophic geography and, indirectly, trophic-related impacts of large invasive omnivorous crustaceans in invaded food webs. Such types of workflows could be implemented and deployed to address a variety of key questions in ecology including biodiversity and ecosystem responses to climate change or to other anthropogenic pressures acting at global scales.

Data availability statement

The datasets generated and analyzed for this study can be found in the LifeWatch Italy Data Portal (https://dataportal. lifewatchitaly.eu/view/urn%3Auuid%3Ae869c2cc-2011-461d-99cc-8d6d642b1624; https://dataportal.lifewatchitaly.eu/view/ urn%3Auuid%3A18a8256c-0e31-4800-af5b-958ea88faf34).

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Author contributions

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fenvs.2022. 1038635/full#supplementary-material

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