Remote Sensing in Ecology and Conservation
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RESEARCH ARTICLE

Use of an unmanned aerial-aquatic vehicle for acoustic sensing in freshwater ecosystems

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Keywords
Biodiversity monitoring, freshwater ecosystem, passive acoustic monitoring, robotics, unmanned aerial-aquatic vehicle

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Funding Information
This work was funded by the Engineering and Physical Sciences Research Council (EP/R009953/1, EP/N018494/1, EP/R026173/1, EP/S031464/1), and the EU H2020 AeroTwin project (grant ID 810321), by the National Environmental Research Council (NERC) (grant number NE/R012229/1), and carried out within the framework of the EUROfusion Consortium, funded by the European Union *via* the Euratom Research and Training Programme (Grant Agreement No 101052200—EUROfusion). The work of Mirko Kovac is supported by the Royal Society Wolfson fellowship (RSWF/R1/18003) as well as the Imperial-Empa partnership.

Editor: Vincent Lecours
Associate Editor: Gwilym Rowlands

Received: 12 October 2022; Revised: 30 October 2023; Accepted: 2 November 2023

doi: 10.1002/rse2.373

Remote Sensing in Ecology and Conservation 2024;**10** (3):343–359

Abstract

Freshwater ecosystems are endangered, underfunded and understudied, making new methods such as passive acoustic monitoring (PAM) essential for improving the efficiency and effectiveness of data collection. However, many challenges are still to be addressed with PAM: difficulty in accessing research sites, the logistics of implementing large-scale studies and the invasiveness of data collection. When combined with PAM and other sensing strategies, mobile robotics are a promising solution to directly address these challenges. In this paper, we integrate water surface and underwater acoustic monitoring equipment onto a prototype unmanned aerial-aquatic vehicle (UAAV) capable of sailing and flight (SailMAV). Twelve autonomous sailing missions were run on Lake Vrana, Croatia, during which acoustic data were collected, and the ability of the UAAV to facilitate the collection of acoustic data demonstrated. Data were simultaneously collected using standard recording methods on buoys and banksides to provide a comparative approach. Acoustic indices were used to analyse the soundscape of underwater acoustic data and BirdNET (a deep artificial neural network) was used on water surface datasets to determine bird species composition. Results show higher species richness and call abundance from UAAV surveys and high site dissimilarity owing to turnover between stationary and UAAV methods. This highlights the success of the UAAV in detecting biodiversity and the complementarity of these methods in providing a broad picture of the biodiversity of freshwater ecosystems. Increased bird diversity and underwater acoustic activity in protected areas demonstrate the benefits of protecting freshwater ecosystems; however, site dissimilarity driven by turnover highlights the importance of protecting the entire ecosystem. We show how, by integrating PAM and a UAAV, we can overcome some of the current challenges in freshwater biodiversity monitoring, improving accessibility, increasing spatial scale and coverage, and reducing invasiveness.

Introduction

Freshwater ecosystems and environmental monitoring

Freshwater ecosystems provide essential ecosystem services including food and clean water, and despite covering just 1% of the earth surface, they support over 10% of all species on earth (Darwall et al., 2018). Biodiversity in these ecosystems is in crisis across a range of taxa, including birds (Darwall et al., 2018; Dudgeon et al., 2006), with an average 84% decline in species abundance and one in three species at risk of extinction (WWF, 2020). This is driven by an unsustainable and dramatic increase in the use of natural resources, which has led to pollution from agriculture and industry, habitat loss and the spread of invasive species (Darwall et al., 2018; Desjonquères et al., 2020; Linke et al., 2018).

Monitoring and evaluating the response of ecosystems to anthropogenic change and understanding the effectiveness of current strategies are essential for effective management and improved decision making (Bennett et al., 2018; Dixon et al., 2019; Gibb et al., 2019), yet freshwater research is consistently under prioritized and underfunded (Darwall et al., 2018; Maasri et al., 2022).

Current challenges in freshwater methods

Traditional methods of environmental monitoring can be expensive, time-consuming and practically challenging (Campos et al., 2021; Gibb et al., 2019; Marvin et al., 2016). Monitoring in aquatic ecosystems can be particularly challenging as changes can be hard to see and access limited (Desjonquères et al., 2020; Gottesman et al., 2020; Linke et al., 2018). Common methods to monitor species include netting and electrofishing, both highly invasive that can distress or kill focal or other species, introduce bias into studies, do not allow continuous monitoring and require extensive person-power (Desjonquères et al., 2020; Linke et al., 2018; Radinger et al., 2019). Fifteen priorities were recently established for the advancement of freshwater biodiversity research, which included the development of new and innovative methods for biodiversity monitoring to overcome the limitations of current methods (Maasri et al., 2022).

How can conservation technology tackle these challenges?

Technology can facilitate environmental sensing by increasing the quantity, quality and efficiency of data collection, providing access to new data sources, real-time information and reducing data processing times (Hahn et al., 2022; Lahoz-Monfort et al., 2019). Integrating multiple technologies can prove even more effective for overcoming spatial and temporal limitations of ecological data collection (Marvin et al., 2016; Wich et al., 2021). New methods currently being utilized for freshwater monitoring include underwater cameras, snorkel surveys and environmental eDNA (Castañeda et al., 2020) and whilst acoustic sampling has been used for many years in marine ecosystems, it is now gaining traction in freshwater monitoring (Desjonquères et al., 2020).

The emerging field of passive acoustic monitoring (PAM) is helping biologists to collect data on vocal species at greater spatiotemporal scales, whilst reducing invasiveness and required person-power (Browning et al., 2017; Desjonquères et al., 2020; Gibb et al., 2019; Linke et al., 2018; Penar et al., 2020; Sugai et al., 2019). PAM has been shown to be effective in the study of species across a range of taxa, including birds (Celis-Murillo et al., 2012; Williams et al., 2018), mammals (Kalan et al., 2015; Wrege et al., 2017) and anurans (Willacy et al., 2015). Multiple taxa (birds, anurans, fish, insects and crustaceans) produce sounds in freshwater environments, from calling, to air expulsion and stridulation (Desjonquères et al., 2020; Linke et al., 2018), making PAM an appropriate method for freshwater ecosystems. PAM can be particularly useful in low-visibility aquatic environments because sound propagation is more effective than visual approaches (Desjonquères et al., 2020).

One limitation of PAM in freshwater ecosystems is related to the installation of recording devices, which are generally either installed on the banks of ponds, lakes or rivers, or for larger areas, on floating devices in open water (Desjonquères et al., 2020). Access to the bankside of freshwater ecosystems can be restricted, and/or can cause environmental damage, especially where fragile habitats such as reed beds or marshlands exist. Installing sensors on buoys in open water is limited and can also lead to disturbance. By combining PAM with autonomous robotic platforms, it is possible to overcome some of these limitations, reducing invasiveness and increasing spatial scale, coverage and accessibility.

Previous use of robotics in environmental sensing

Unmanned vehicles fall into four main categories, aerial, surface, underwater and ground vehicles (Pajares, 2015). Due to their ability to reduce person-power requirements and increase the spatial scale of data collection, unmanned aerial vehicles (UAVs) are now widely used in aquatic research for mapping habitats, such as the seabed or waterways, or detecting pollution, such as tracking oil spills (Pajares, 2015). Monitoring aquatic fauna using

UAVs is becoming increasingly common; however, this has been mainly limited to visual surveillance of larger marine mammals such as dugongs (Hodgson et al., 2013) and whales (Christiansen et al., 2016; Schoonmaker et al., 2008) or coastal bird communities (Chabot & Bird, 2012).

Unmanned surface vehicles (USVs) and unmanned underwater vehicles (UUVs) are the primary method used for monitoring aquatic ecosystems due to their ability to manoeuvre under and on the water's surface. To date, USVs and UUVs have focused on conducting water sampling and habitat mapping (Athar et al., 2020; Atif Mehdi et al., 2021; Ferri et al., 2015; Fornai et al., 2012; Hitz et al., 2013; Ishikawa et al., 2005; Kumagai et al., 2002; Madeo et al., 2020; Naeem et al., 2008; Paez et al., 2018; Švec et al., 2013), and whilst some USVs have been used to monitoring biodiversity, this is limited to marine ecosystems (de Robertis et al., 2021; Hill et al., 2014; Ling et al., 2016; Morris et al., 2014; Smale et al., 2012), or to monitor algal blooms in freshwater lakes (Ishikawa et al., 2005).

A new class of aerial-aquatic robots, which can transition from air to water, have demonstrated the ability to land on and then float or sail on the water surface, whilst also being able to collect water samples (Debruyn et al., 2020; Zufferey et al., 2019). SailMAV is an unmanned aerial-aquatic vehicle (UAAV) that can sail and fly autonomously. It has been developed with an adaptive morphology, which allows it switch between two configurations: one used for flight and one used for sailing. This ability to transition from air to water solves the issue of access to the water body, whilst also allowing for interaction with the environment (Zufferey et al., 2019).

The use of PAM is facilitating monitoring of freshwater ecosystems; however, challenges remain around increasing spatial scale, coverage and accessibility and reducing invasiveness. Here, we attempt to overcome these challenges by integrating water surface and underwater acoustic sensors onto a UAAV to improve acoustic data collection in a fragile freshwater ecosystem. We ask the following questions. (1) Can a UAAV facilitate acoustic data collection in freshwater ecosystems? (2) We provide a detailed comparison of data collected using both standard stationary approaches and the new sailing method using the UAAV, SailMAV and (3) Can the resulting data be used to answer questions of ecological interest?

Materials and Methods

Study site

This study took place on Lake Vrana, the largest freshwater lake in Croatia. Lake Vrana covers an area of 31.1 km^2 and varies in depth from 0.03–2.25 m (average 0.82 m). The area is at risk from human disturbance, especially nutrient run-off from intense agricultural use (Rubinić et al., 2014). The lake and surrounding area have been protected as a nature park since 1999 and in 2013 were designated as a RAMSAR Wetland site (Fig. 1; Rubinić et al., 2014). Whilst the whole lake is under protection, an 8.8 km^2 section benefits from additional protections as a ornithological reserve. No commercial or tourist activity is allowed (Fig. 1), and only boardwalks in the reserve section receive tourist activity. The ornithological reserve is covered with reed beds with many small canals and tributaries (Rubinić et al., 2014).

Equipment

SailMAV

SailMAV, shown in Figures 2 and 3, is an unmanned aerial-aquatic vehicle (UAAV) that can sail and fly autonomously, first proposed in Zufferey et al. (2019). It is composed of two hulls connected by a central wing in a catamaran configuration, one is used for flight/sailing control and navigation, whilst the other encloses sensors used for sensing the local wind and other environmental variables such as water temperature, air temperature, humidity and atmospheric pressure.

At the root of its multimodal (flight and sailing) locomotion is the dual use of aerodynamic surfaces and actuators. Figure 3 shows how both sails can be used in a horizontal configuration for flight and in a vertical configuration for sailing. A custom actuator embedded in the sail allows for its roll rotation in flight and sailing. Whilst in flight, these actuators rotate the sails by a few degrees (~5°) to vary lift forces and control the roll degree of freedom, and in sailing, the same actuators rotate the sails through a wider range of angles (±60°), so that these can effectively catch the wind and propel the robot forward. The tail encloses the rudder used in both flight and sailing for yaw control, and an elevator used for pitch control in flight. Both hulls are shaped as to permit take-off from the water surface whilst maintaining acceptable sailing performance and are equipped with propulsion units used for flight and to take-off from the water surface.

SailMAV sails in a fully autonomous mode which is based on preprogrammed missions of GPS coordinate waypoints. During these missions, propulsion is fully provided by the sails, whilst the rudder, mounted on the tail of the platform, corrects the heading direction. The robot autonomously adapts to changing wind conditions as it can directly measure the local wind direction. It also automatically switches between a direct locomotion mode and a tacking strategy that allows it to zigzag upwind, if

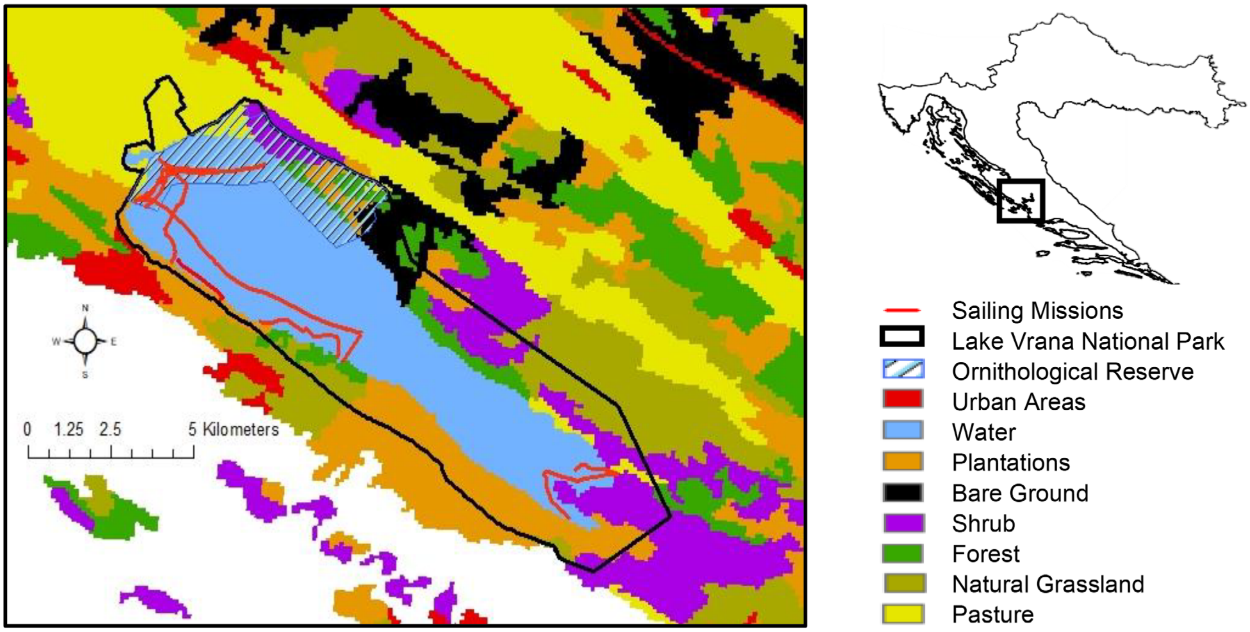

Figure 1. Land-use map of Lake Vrana. The Copernicus vegetation map is created at a scale of 100 × 100 m using the PROBA-V satellite series (Buchhorn et al., 2020). Twenty-seven land-use categories were merged into eight broader categories. Red lines represent sailing missions where acoustic data were collected. The Lake Vrana protected area is outlined in black with the highly protected ornithological reserve marked separately.

Figure 2. SailMAV in sailing mode (left) and flying mode (right).

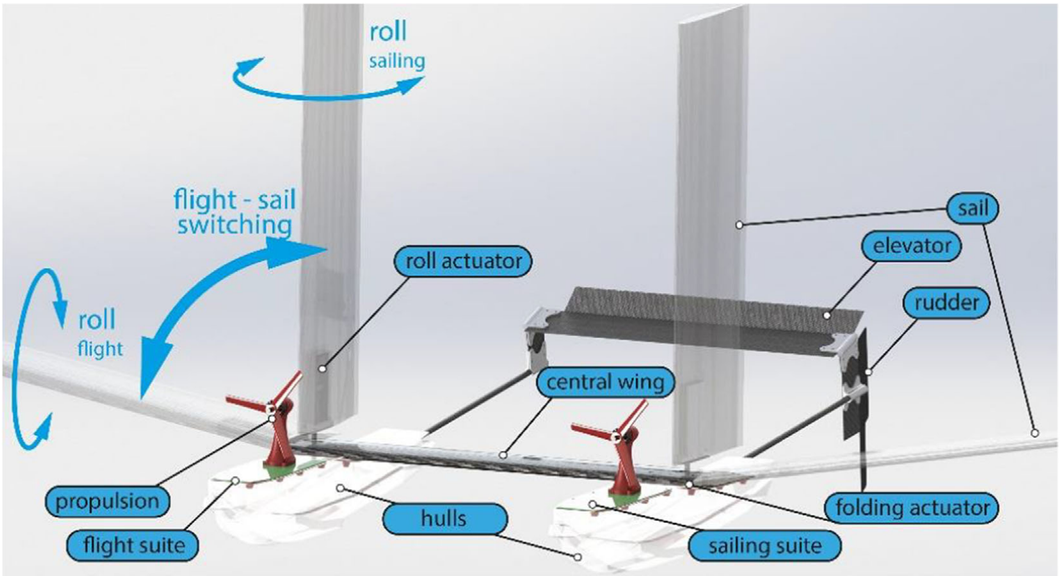

Figure 3. Diagram of SailMAV prototype used in this study, detailing actuators, aerodynamic surfaces and other principal components. Sails are overlaid in both flight and sailing model.

necessary. For flight, the sails can then be folded down allowing the robot to execute a take-off manoeuvre, using motors and propellers for propulsion. All the custom software is implemented within the PX4 framework (Meier et al., 2015) and can thus inherit all the usual UAV flight modules such as stabilized flight control, GPS-based navigation and base station communication. A full list of features is outside the scope of this paper, however, details on mission operations, wildlife intrusiveness and key performance metrics can be found in Supporting Information S2.

Acoustic recorders

Acoustic recording equipment were integrated into SailMAV and placed simultaneously at stationary locations on buoys for underwater recordings and at banksides for surface recordings. Recordings were obtained using Audio Moth devices (Open Acoustics Devices, Southampton. UK). We recorded constantly during sailing missions and at stationary locations at a sample rate of 48 000 kHz based on best practice guidelines (Bradfer-Lawrence et al., 2019). Data were stored on 32 GB U3 microSDHC cards. Two different Audio Moth devices were used, and on SailMAV, both were mounted on the stern of the left hull of the robot in a splashproof casing made from vacuum-formed PVC with acoustic vents to allow noise to pass through:

1. *Underwater recordings*: **The Audio Moth Dev was powered by a separate one-cell LiPo battery and an external hydrophone (Aquarian Audio H3). Gain settings were set to 'low' to reduce sounds from water movement over the microphone without compromising biotic sounds, as determined from field tests. The hydrophone was suspended from SailMAV at a depth of 30 cm under the water. 30 cm was chosen as any lower would have increased the drag in the water, impacting sailing performance.**
2. *Water surface recordings*: **We used the Audio Moth *u*Moth, a small, lightweight version of the standard Audio Moth with an integrated microphone and the same external power supply as the Dev. Gain was set to medium for standard terrestrial recordings.**

Sampling design

Data were collected over a period of 8 days in February and June 2022. Each day SailMAV was programmed to complete one mission during the morning and afternoon. To provide a comparison of data collected using the UAAV and the standard stationary approach, during winter, for the same time period as SailMAV missions, acoustic recorders were installed on banksides at the starting point of each mission to capture surface recordings and on buoys to capture underwater recordings. Field tests began each day at 05:00 with the aim of capturing the dawn chorus and in the afternoon at 15:00 to capture the dusk chorus. To facilitate comparisons between methods, we only compared recordings taken at the same time of the day. During each mission, we aimed to demonstrate the following features of SailMAV:

1. **To autonomously complete predefined missions between waypoints, facilitating the spatial scale and coverage of data collection and removing the need for manual operation or to maintain a visual line of sight.**
2. **To tack, defined as the ability of a vessel to change direction through oncoming wind. This reduces the effect of wind direction on sampling design, allowing areas to be sampled regardless of wind direction.**
3. **To test the effects of SailMAV on water birds, missions were planned to navigate near to flocks of waterbirds to aid our understanding of how the robot would affect the behaviour of wildlife.**
4. **To land and take-off from the water surface, which facilitates data collection on inaccessible or partially accessible water bodies.**
5. **To navigate around areas of reed beds and canals within the protected ornithological reserve. SailMAV's ability to navigate more enclosed and complex areas will determine which types of freshwater ecosystems can be sampled using this methodology.**

Daily missions varied in duration between 60 min and 3 h and between 1.52 and 6.68 km, depending on environmental conditions and the target area of interest, with a total travelled distance of 32.2 km. The weather was dry during the duration of the study period, though the wind varied from periods of calmness to 30 knots, averaging 10 knots from the Northwest direction. In total SailMAV completed 12 successful missions on the lake where audio data were recorded, seven in the ornithological reserve and seven in the remaining area of the lake, eight during winter and six during summer (some missions crossed between the reserve and disturbed areas and were therefore separated) (Fig. 1; Table S2). Two days during the summer testing period were also used to test landing and take-off, where acoustic data were not recorded.

Analysis

Soundscape analysis

We used soundscape analysis to analyse patterns of acoustic activity in underwater recordings between protected and disturbed regions and between survey methodologies. Soundscape ecology uses indices of acoustic diversity to determine measures of acoustic diversity across space and time (Pijanowski, Farina, et al., 2011; Sueur et al., 2014). Each index measures a different aspect of the soundscape, including pitch, saturation and amplitude across time and frequency bands, with most indices being sensitive to the characteristics of biophony (Bradfer-Lawrence et al., 2019; Pijanowski, Villanueva-Rivera, et al., 2011; Sueur et al., 2008), thereby providing measures of richness, evenness and heterogeneity of biotic sounds (Sueur et al., 2014). Acoustic indices have been shown to be correlated to the number of biological sounds in a recording, measures of species richness and abundance of freshwater species (Decker et al., 2020; Desjonquéres et al., 2015; Gottesman et al., 2018; Greenhalgh et al., 2021). Despite this, acoustic indices can be affected by anthropogenic and abiotic sounds (Pijanowski, Farina, et al., 2011; Sánchez-Giraldo et al., 2020), which are present in our recordings; therefore, in this study we use these indices to measure acoustic activity and do not attempt to specifically link this to measures of biodiversity. For more information on the use of acoustic indices and how they relate to traditional measures of diversity, see Supporting Information S1.3.

To extract acoustic information from the raw data, we used a suite of eight complementary acoustic indices, which, depending on study design, can be used together to provide a comprehensive picture of the soundscape (Towsey, 2018). These indices were chosen as together, they provide a good representation of the spatial–temporal variation that exists across the soundscape (Towsey, 2018). Additionally, using multiple indices that represent different parts of the soundscape helps to avoid incorrect interpretations that may occur due to competing explanations or sensitivities in different environments for a particular index value (Bradfer-Lawrence et al., 2019). We provide a brief description of each index in the Supporting Information (Table S3), for a full description of how indices are calculated refer to (Towsey, 2018).

Preprocessing: Soundscape analysis

For underwater recordings made using SailMAV, water movement over the hydrophone was present; however, it was not possible to filter out these sounds as they spanned several frequency bands (1–11 kHz). We therefore generated Long-Duration False Colour Spectrograms (LDFC Spectrograms) using Analysis Programmes software developed by Towsey (2018). This process combines the spectral data from five indices, ACI, EVN, ENT, BGN and PMN, to visually summarize the content of 24 h of audio recording, allowing repetitive sounds to be identified visually (Towsey et al., 2020). From viewing spectrograms and listening to recordings, we classified level of disturbance from water movement into 'present' or 'absent' and used these classifications as a random effect in the statistical models to account for any effects. Water movement appeared to affect recordings when the wind was stronger and SailMAV was moving quicker through the water.

Using Windows PowerShell 6.0, all data were processed using Analysis Programmes software and a value calculated for each of the eight indices for each 1-min file (Towsey et al., 2020). These data were then averaged across each mission to give one value for each of the eight indices. Whilst we recognize that fine-scale temporal analysis is important due to the changes in biotic activity that can occur across the diel cycle, we only used 1 h of data from missions and colocated stationary surveys to limit the variation that may be present.

Principal component analysis

All statistical analysis were carried out in R 3.6.0 (R Core Team, 2020). We used principal component analysis (PCA) to reduce the dimensionality of the eight acoustic indices using the *vegan* package (Oksanen et al., 2019), to find the best summary of the data in the principal components (PCs). Eigenvalues were extracted to determine the correct number of PCs to retain. PCs are retained until >70% cumulative variance is captured and if they have an eigenvalue of >1 (James et al., 2013).

To determine which combinations of indices are represented across the PCs, we used the loadings values for each index, with higher values indicating a larger effect from the index on that particular PC. PC scores, which represented a mean index value for each mission, were then extracted for use in regression analyses.

Species composition

Species accumulation curves for bird diversity data between seasons and protection level did not reach their asymptote, suggesting sampling effort was not sufficient and may affect the results (Figure S1). To account for differences in sampling effort between seasons and protected areas, we excluded one mission (182 min, mission 2) during winter and one during summer (98 min, mission 9) in the protected ornithological reserve; therefore, in total we analysed 1054 of surface water recordings, 543 in the winter and 511 in summer, 541 min during missions in disturbed areas and 513 min in the ornithological reserves. For comparisons between SailMAV and bankside recordings, we analysed the same 543 min from data collected during winter.

We used BirdNET to determine the presence of bird species within surface recordings between protected and disturbed regions and between survey methodologies. BirdNET was developed by the Cornell Lab of Ornithology and is a deep artificial neural network trained on almost 1000 species of bird across North America and Europe (Kahl et al., 2021). The system first converts raw audio data into 3-s spectrograms through a process called short-time Fourier transformation and then passes the spectrograms through a convolutional neural network to assign probabilities of species occurrence within each 3-s clip. The classifier achieves high mean average precision during testing on single-species recordings (0.791) (Kahl et al., 2021). BirdNET outputs call abundance of all bird species detected within a 1-min audio file, with a call start and end time and confidence scores. For a full description of BirdNET, see Kahl et al. (2021).

BirdNET model validation

To help ensure that the results from BirdNET were accurate, we first restricted analysis to those species found on the known species list provided by the national park service. We then validated the results from BirdNET by listening to 39 min of recordings taken during winter taken on the bankside (stationary) and the same 39 min from a sailing mission (moving). We calculated a confusion matrix using the true-positive, false-positive, true-negative and false-negative values for each set of recordings (Table S4), and then calculated accuracy, precision, recall and F1 scores for each dataset (Table S4). Results showed that the model performed better on the stationary dataset, with an F1 score of 0.75 for stationary recordings and 0.6 for moving recordings. This is likely due to interference from the mechanical noise from SailMAV (Table S5).

Alpha (α) and beta (β) diversity

Using outputs from BirdNET analysis, we created a species composition matrix showing the abundance of calls detected per species from the restricted species list during each recording period. From this, we calculated measures of α-diversity (species richness, call abundance and Simpson's index) in *vegan* package (Oksanen et al., 2019).

Using only presence data, we then calculated β-diversity using the Sørensen dissimilarity index, which measures compositional variation between communities. The resulting patterns of β-diversity were then separated into measures of turnover and nestedness. β-diversity was calculated in package *Betapart* (Baselga & Orme, 2012). We ran Nonmetric Multidimensional Scaling (NMDS) to quantify and visualize site dissimilarity.

Linear mixed models

We used linear mixed models to determine the effects of protection, season and methodology on PC1, (including water movement as a random effect) and on measures of α-diversity. We fitted models in the *nlme* (Pinheiro et al., 2020) and *lme4* package (Bates et al., 2015). Model performance was evaluated using Akaike Information Criteria and the likelihood ratio test (Zuur et al., 2009). We tested the effect of seasonality on protection; however, we found no significant differences, and this interaction term was therefore not included in the model.

Results

During 12 successful missions with SailMAV across 8 days, we collected 2263 min of acoustic recordings, 929 of underwater recordings (563 in winter 366 in summer) and 1334 of surface water recordings (725 in the winter and 609 in summer). Of this, 956 min were collected during missions in disturbed areas and 1307 min in the ornithological reserves. There are more surface than underwater recordings due to AudioMoth malfunction. SailMAV was able to successfully navigate and reach predetermined waypoints, take-off and landing from the water surface was demonstrated, as was tacking, reducing the effect of wind direction and strength on sampling.

Here, we present results comparing the difference in α- and β-diversity and acoustic diversity between recordings made on SailMAV (moving) and recordings made simultaneously on the bankside and buoys. In the Supporting Information, we present results comparing species composition and acoustic diversity across levels of protection and season, to show how this novel approach with a UAAV can be used to answer questions of ecological interest (Supporting Information S2).

Species diversity analysis

We quantified α-diversity and β-diversity between data collected at stationary locations (bankside) and on SailMAV missions (moving). Of the 250 total bird species recorded at Lake Vrana, BirdNET detected 87 species, 35% of recorded species over just 8 days of sampling.

Species richness was not significantly higher on moving surveys versus bankside recordings ($P = 0.43$), with an average of 22 species per mission compared to 16 species on bankside surveys (32% decrease) (Fig. 4A). Call abundance was not significantly higher on moving surveys compared to bankside recordings ($P = 0.66$), with an average of 217 calls per mission versus 162 calls on bankside surveys (29% decrease) (Fig. 4B). Simpson's diversity was not significantly higher on the bankside compared to moving surveys (0.77 vs. 0.60, $P = 0.19$) (Fig. 4C).

β-diversity analysis showed that dissimilarity between survey types was mainly due to turnover (0.7–1). Survey type was a strong predictor of dissimilarity between sites ($r^2 = 0.65$, $P < 0.05$), and NMDS analysis highlights compositional dissimilarity between recordings taken on SailMAV (moving) and bankside recordings (Fig. 5). Several species were controlling the differences between survey type; the great egret (*Ardea alba*) and mute swan (*Cygnus olor*) were exclusively found on moving surveys and the dunnock (*Prunella modularis*), little grebe (*Tachybaptus ruficollis*) and water rail (*Rallus aquaticus*) found exclusively in bankside surveys.

Underwater soundscape analysis

The variable correlation plot (Fig. 6) shows correlation between acoustic indices, confirming that PCA is suitable for reducing the dimensionality of the eight acoustic indices. Eigenvalues indicate that we should retain PC1, since it accounts for almost 80% of variance across analysis (Table S6). All acoustic indices are contributing equality to PC1, showing that they are all important in driving the results (Table S7). Further investigation of other PCs would be of little benefit since they contain little variation. PC1 can generally be considered a measure of acoustic activity spanning the lower–upper (0 11 kHz) frequency bands. 95% confidence ellipses show high separation between survey types (Fig. 6).

Linear model results

Model results suggest that there is no significant difference in PC1 between recordings taken on the buoy *versus* recordings taken from SailMAV (moving) (mean = 0.001 vs. −0.77, $P = 0.70$) (Fig. 7).

Discussion

SailMAV was able to take-off and land on a water body, tack, sail autonomously between waypoints, through complex environments and in proximity to wildlife, demonstrating how combining robotics and PAM can increase accessibility, improve spatial scale and coverage and reduce invasiveness of data collection. Comparative analysis of stationary versus moving methods highlights differences in both α- and β-diversity and hence the complementarity of these approaches. Soundscape and species composition analysis were able to provide insights on aquatic biodiversity, highlighting the benefits of additional protections in the ornithological reserve.

Disturbance in freshwater ecosystems

Soundscape analysis applied to underwater recordings showed some separation of the data and, on average, more acoustic activity in the protected ornithological reserve than in disturbed areas (Supporting Information S1.7), demonstrating that the soundscapes in these areas differ. Greenhalgh et al. (2021) found similar results, showing higher acoustic activity in less disturbed environments, which correlated with richness and abundance of arthropod stridulation. Our results suggest that

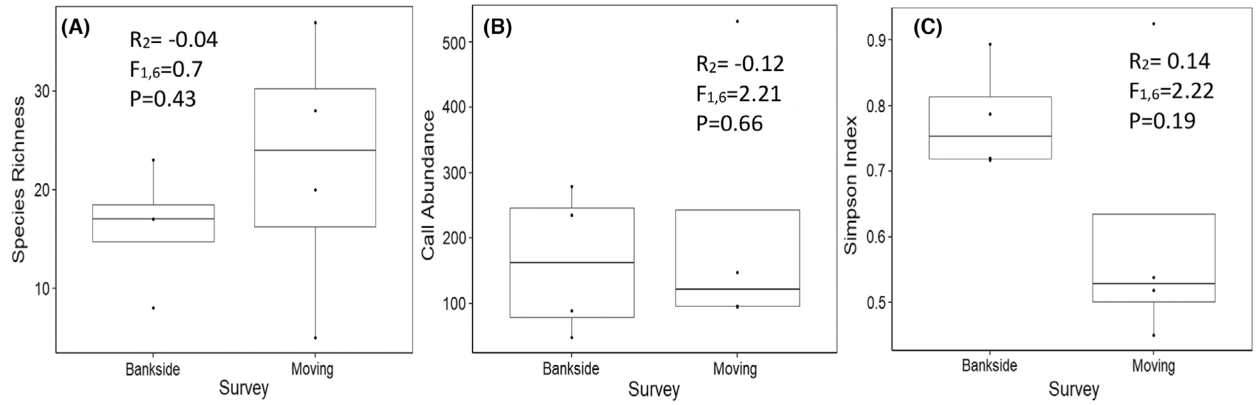

Figure 4. Boxplots showing the difference in (A) species richness, (B) call abundance and (C) Simpson's Index between surveys completed on SailMAV (moving) versus surveys from stationary bankside locations R^2, F and P values for each model are marked on the graphs. Points represent raw data values.

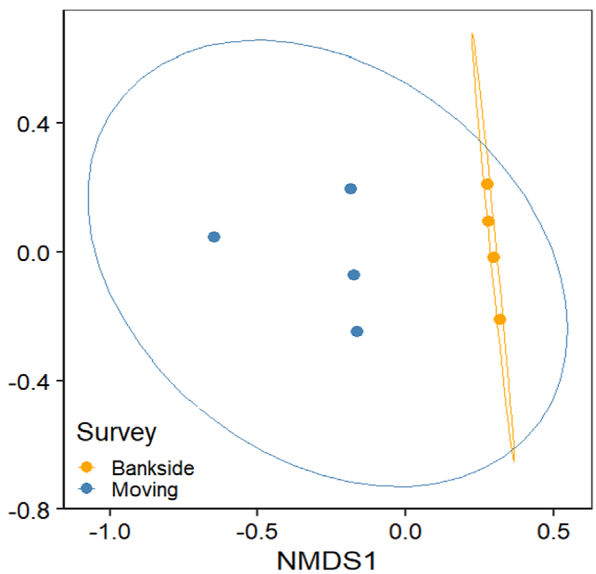

Figure 5. Nonmetric Multidimensional Scaling (NMDS) plot for site dissimilarity. NMDS Plot showing separation between survey types: stationary (bankside) and moving (SailMAV).

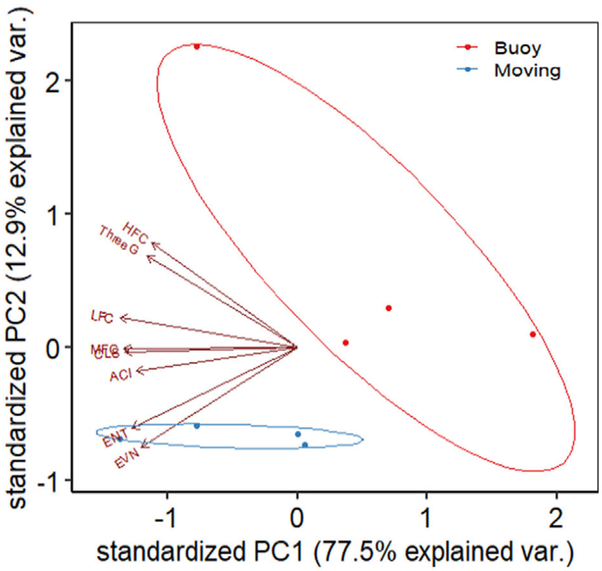

Figure 6. Variable correlation plot. Showing the relationship between the indices and principal component axis. Closer vectors are more highly correlated, and indices with longer vectors indicate increased strength of an index on that PC. The x-axis represents PC1, and y-axis PC2, with % of variance explained. 95% confidence ellipses show the degree of separation in the data based on survey type, buoy (stationary) and moving (SailMAV).

the additional protections applied to the ornithological reserve, including a ban of tourist activity and fishing, may be facilitating increased acoustic activity. However, as acoustic indices can be sensitive to background noise from anthrophony or geophony (Desjonquéres et al., 2015), we must be cautious in our interpretation of the results.

Bird species richness was higher in the ornithological reserve, suggesting that additional protections are improving bird diversity. Protected areas have been shown to improve water bird diversity (Sun et al., 2020; Wauchope et al., 2022; Zhang et al., 2015), if well managed (de Lima et al., 2013; Fijn et al., 2014; Wauchope et al., 2022; Wood et al., 2013). Dissimilarity between missions was high and is attributed mainly to species turnover, suggesting that species are being replaced between missions rather than being nested versions of each other. Dissimilarity due to turnover is driven by niche differentiation and dispersal processes, not species loss resulting from local extinction (Medeiros et al., 2016;

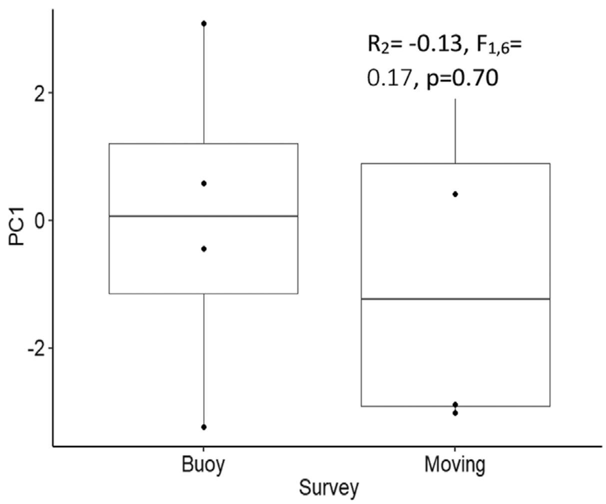

Figure 7. Boxplot showing the difference in PC1 between survey types, buoy and moving (SailMAV). R^2, F and P values for each model are marked. Circles represent raw data values.

Si et al., 2015). Dissimilarity was being driven mainly by differences in protection, which may be due to reduced anthropogenic disturbance or a difference in habitats, namely the presence of reed beds in the ornithological reserve, allowing more specialist species to thrive.

The underlying causes of dissimilarity can influence management action, and in the case of species turnover, it is suggested that all areas should be prioritized for conservation (Si et al., 2015). Lake Vrana is already protected as a RAMSAR wetland site, with the 8.8 km^2 ornithological reserve benefiting from additional protections (Rubinić et al., 2014). These additional protections may be driving differences in species composition and diversity and our results suggest that the entire ecosystem may benefit from equal protections.

Moving versus stationary surveys

Here, we provide a comparative analysis of our approach using SailMAV versus standard stationary methods of collecting PAM data, to understand the pros and cons of each method and understand how methods might be used in surveys.

Spatial scale, coverage, accessibility and invasiveness

Freshwater data collection methods are currently restricted in spatiotemporal scale and coverage (Abrahams et al., 2021), by remoteness and inaccessibility of sampling sites (Dafforn et al., 2016; Desjonquères et al., 2020) and risk environmental damage to fragile ecosystems. SailMAV has a demonstrated ability to: (1) sail autonomously between predefined waypoints; (2) to navigate through fragile reedbeds; (3) to modify its design according to the medium in which it operates, transitioning from air to water. These features improved site accessibility and increased the spatial scale and coverage of sampling, whilst reducing disturbance, when compared to stationary methods. Whilst the approach with SailMAV increases spatial scale and coverage, this does come at the expense of collecting more data in one area, and unless missions can be exactly replicated, we may lose precise repeatability. SailMAV can move between set waypoints, making repeatability possible; however, this would rely on similar weather patterns across sampling days.

Wind speed and direction

The ability to tack is a common feature amongst sailing vessels, without which they would only be able to travel with the direction of the wind, reducing autonomy in data collection and disrupting potential sampling regimes. SailMAV's ability to navigate upwind directions drastically improves sampling efficiency and design and ability to reach critical points on the water surface; however, sailing against the wind and tacking increases the time on the water in different areas. It is possible to account for this by subsampling survey data to ensure an equal number of recordings are analysed for each habitat; however, it does reduce repeatability and may increase variance in the recordings. The only way to currently account for this is to choose survey routes based on wind speed and direction and aim to follow specified routes on days of similar conditions.

Temporal sampling

The temporal scale of sampling with moving and stationary approaches is considerably different. Stationary surveys allow for one area to be surveyed continuously for an extended period of days or weeks, providing repeatability. It also facilitates recording over a full 24-h period, something which is important in revealing differences in acoustic activity between habitats (Lawson et al., 2022). Moving surveys, whilst increasing spatial scale and coverage, do not allow for the same temporal scale, potentially obscuring differences between variables and compromising repeatability. Biotic activity varies significantly across the diel cycle, and it is important to record at the same times across surveys. This can be challenging with surveys using a UAAV, for example, if there are technical difficulties as was experienced on some days. This standardization can be complicated to achieve and should always be considered during analysis. For this reason, we believe these methods are complementary, or the choice of method will depend on the question at hand. For example, if the aim is to create a species list and determine measures such as presence or species richness, then the moving method using SailMAV may be more appropriate, where as if the aim is to determine finer-scale differences between habitats, then a stationary approach might be more useful. If stationary and moving surveys were used as complementary approaches, it is essential to ensure that they are conducted, not only at the same time but also at the same time across environmental variables of interest to avoid biases from measuring different areas at different times during the diel cycle.

Noise

Underwater recordings from SailMAV contained noise from water splashing over the hydrophone if SailMAV was moving quickly, and surface recordings contained some mechanical noise from SailMAV, although it was low enough that bird sounds could be heard over it. Acoustic indices are affected by abiotic sounds, such as water movement, to account for this in the analysis we chose to classify recordings based on the level of abiotic sound from water movement. Results showed that water movement was affecting results and is likely contributing to the separation of the data during PCA analysis of moving versus stationary approaches. Acoustic indices are also affected by anthropogenic sounds such as the noise from SailMAV, which was consistent across surface recordings, spanned frequency bands from 0 to 11 500 kHz and would have biased soundscape analysis. Until this noise can be reduced or eliminated, either on the device or using postprocessing methods, this type of analysis will remain a challenge.

To assess whether the level of biotic and anthropogenic noise was affecting BirdNET analysis from surface-level recordings, we validated the functioning of the BirdNET algorithm on recordings from the stationary bankside approach and from recordings taken on SailMAV. The algorithm did not work as well on recordings taken from SailMAV (F1 score = 0.6) versus stationary recordings (F1 score = 0.75), and therefore, until this noise can be eliminated this should be taken into consideration when interpreting the results.

Findings

Data collection at stationary points, similar to point counts, compared to our approach with SailMAV, which is comparable to line transects, will impact the data and results. Here, we found higher species richness and call abundance in moving surveys compared to stationary surveys, something which has previously been found in a comparison of point counts versus line transect surveys (Wilson et al., 2000). This result may be because SailMAV was able to access not only bankside areas but also the open water body, habitats which are likely to contain different species. We also found high site dissimilarity owing to species turnover between data collected using moving and stationary techniques, which is again likely due to the difference in habitats accessed. These differences in α- and β-diversity suggest that stationary and moving approaches to data collection may be complementary. It should be noted that we were not able to use the first few minutes of recordings from moving surveys due to human voices present in the recordings, and it is also likely that our presence on the bankside caused birds not to call; therefore, the differences between survey methods found in both underwater and surface recordings may be exaggerated. For future use of this platform, it is therefore important to cover areas of the waterbody where humans are not present and use the fully functionality of the platform in terms of landing on the body of water from flight.

Invasiveness and operation

Freshwater sampling methodologies can be invasive (Desjonquères et al., 2020; Linke et al., 2018; Radinger et al., 2019), with even PAM requiring bankside access, installation of buoys or use of boats (Desjonquères et al., 2020). SailMAV's ability to land on and take-off from the water surface and autonomously move across the water surface allowed us to overcome many of these challenges, requiring only limited bankside access. SailMAV was able to sail close to and even through flocks of birds with very little effect on their behaviour, improving the welfare standards of sampling and reducing any potential bias in data collection. The only changes in behaviour we observed were individual birds moving out of the path of the robot. Despite observations that SailMAV had little effect on animal behaviour, we are not able to quantify this effect and future studies should focus on how unmanned vehicles are affecting biodiversity.

Conclusions and Future Recommendations

Future work on SailMAV is focused around on increasing the size of SailMAV to reduce the drag effect from the hydrophone and allow it to be placed lower in the water to reduce sounds from water movement and on reducing or eliminating the mechanical sounds produced by the UAAV. Overall autonomy of aquatic research platforms such as SailMAV can be improved with additional visionbased sensing or access to water body limits for mission planning and obstacle avoidance. A multisensing approach can also be achieved with such platforms by integrating open-source acoustic recorders and other environmental sensing platforms with robotics controllers. This would have the additional advantage of all the acquired data being geotagged during mission progress. Finally, aerial-aquatic robotics is still a young topic, so air–water–air transitions have not been fully automated effectively. Work in this area is still necessary to remotely access aquatic ecosystems and autonomously acquire data with minimal impact or study bias.

Our novel approach, integrating PAM and a UAAV, can overcome some of the limitations and inefficiencies of freshwater monitoring, facilitating data collection across wider spatial scales, improving coverage and accessibility and reducing invasiveness. This approach also yielded differences in α- and β-diversity, suggesting that it may be a more suitable approach for certain questions or complementary to other methods. However, with this approach we lose the ability to collect data at fixed points for an extended period and the fine-scale analysis that comes with this. Additionally, unless SailMAV can be operated in a way that allows repeatability of transects over the same time scales, which is currently hindered by weather conditions, we are likely introducing bias and variation into our data. Noise from water movement and mechanical noise from the robot is currently a significant issue and needs to be reduced to optimize analysis of the acoustic data. Due to the differences in stationary versus the moving method with SailMAV, we believe using these methods of collect acoustic data collection in freshwater environments can be complementary and increase understanding of ecosystem dynamics.

Author Contributions

Jenna Lawson contributed to conceptualization, methodology, software, formal analysis, investigation, data curation, writing, visualization, project administration and resources. Raphael Zufferey contributed to software, investigation and data curation. Andre Farinha contributed to methodology, software, investigation, data curation, writing, visualization and resources. Luca Romanello contributed to methodology, software, investigation, data curation and resources. Oscar Pang contributed to investigation and resources. Mirko Kovac contributed to conceptualization, methodology, funding acquisition and supervision.

Acknowledgments

This work was funded by the Engineering and Physical Sciences Research Council (EP/R009953/1, EP/N018494/1, EP/R026173/1, EP/S031464/1), and the EU H2020 AeroTwin project (grant ID 810321), by the National Environmental Research Council (NERC) (grant number NE/R012229/1), and carried out within the framework of the EUROfusion Consortium, funded by the European Union via the Euratom Research and Training Programme (Grant Agreement No 101052200 – EUROfusion). The work of Mirko Kovac is supported by the Royal Society Wolfson fellowship (RSWF/R1/18003). Views and opinions expressed are however those of the author(s) only and do not necessarily reflect those of the European Union or the European Commission. Neither the European Union nor the European Commission can be held responsible for them.

Authors' Declaration

All authors on the manuscript have seen and approved the submitted version of the manuscript, have substantially contributed to the work, and all persons entitled to co-authorship have been included. This manuscript has been submitted solely to *Remote Sensing in Ecology and Conservation* and has not been published elsewhere, either in part or whole, nor is it in press or under consideration for publication in another journal.

Data Availability Statement

Data tables and associated scripts are available in Zenodo—http://doi.org/10.5281/zenodo.7189907.

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. SailMAV metrics.
Table S2. Mission record detailing the date of each mission on Lake Vrana for SailMAV, which season it took place and whether it was in the Ornithological Reserve, 'Reserve', or the remaining area of the lake 'Disturbed'. Rows highlighted in orange are those that were excluded from analysis of surface recording to account for differences in sampling effort.
Table S3. Description of each acoustic index used, together with the reference to the paper where the indices were first developed and presented.
Table S4. Confusion matrix for both stationary and moving datasets showing the true positive (TP), false positive

(FP), true negative (TN) and false negative (FN) results for each set of recordings.

Table S5. Model validation scores for both stationary and moving datasets showing the accuracy, precision, recall and F1 score for each dataset.

Table S6. Eigenvalues, variance explained and cumulative variance for each PC axis for survey type analysis. PC's to be retained are highlighted in grey. PC1 has an eigenvalue over 1 and accounts for over 80% of total variance.

Table S7. Top four loading values for survey type analysis, indicating the strength of contribution of each index to the PC's. Higher values show a stronger contribution. Indices that represent PC1 are highlighted and all have similar strength.

Table S8. Eigenvalues, variance explained and cumulative variance for each PC axis for seasonal and protection analysis.

Table S9. Top four loading values for seasonal and protection analysis, indicating the strength of contribution of each index to the PC's.

Figure S1. Species accumulation curves showing the cumulative number of species across missions between seasonal data and data across different levels of protection.

Figure S2. Boxplots showing the difference in (A) species richness, (B) abundance and (C) Simpson's Index between the ornithological reserve 'protected' and other more disturbed areas of the lake 'disturbed' and seasonal differences.

Figure S3. NMDS plot for site dissimilarity.

Figure S4. Variable correlation plot.

Figure S5. Boxplots showing the difference in PC1 between (A) levels of designated protection and seasons and (B) between survey types.