The In situ Plankton Assemblage eXplorer (IPAX): An inexpensive underwater imaging system for zooplankton study

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Abstract
1. Zooplankton play vital ecological roles that maintain aquatic ecosystems. Imaging instruments have enabled in situ observations of these organisms that can be automated and are less invasive than traditional sampling methods. However, these instruments are often costly and require sophisticated engineering expertise to operate.
2. The In situ Plankton Assemblage eXplorer (IPAX) is an open-source low-cost imaging platform for zooplankton studies. The IPAX is a programmable instrument that has powerful LED illumination and a high-resolution camera that can image zooplankton in situ, while material costs are less than USD $450. The optical performance of the instrument was calibrated in the laboratory using a calibration target and preserved zooplankton. The IPAX was then deployed in the field to observe diversity, emergent patterns and phototactic behaviour of demersal zooplankton at night to demonstrate its practicality.
3. Laboratory calibration indicated that the IPAX can resolve 100 µm features with 70% contrast at the focal plane with 5 cm × 3 cm field of view and 5 mm depth of field. The instrument also resolved fine morphological details of preserved zooplankton when in focus. The field deployment demonstrated capability to resolve the myriad of zooplankton present in addition to the different phototactic behaviour that was elicited and observed from the different colour LEDs.
4. The IPAX enables economical and autonomous surveys of zooplankton in various aquatic habitats. Its low cost facilitates construction and deployment of multiple units that can cover large spatial areas, while its versatility also allows adaptations to many experimental needs for aquatic ecology.

KEYWORDS
in situ sensor, open-source hardware, underwater imaging, zooplankton

1 | INTRODUCTION

Zooplankton play many vital ecological roles ranging from supporting aquatic productivity to linking aquatic food webs that maintain both freshwater and saltwater ecosystems (Santhanam, Pachiappan, & Begum, 2018). As a result, investigations of zooplankton are necessary to understand complex aquatic ecosystems. Studies of zooplankton are typically conducted by sampling these animals using...
various methods, such as towed nets, pumps and traps, and then examining them in laboratory environments (Suthers & Rissik, 2009). However, these sampling methods can be labour-intensive and can also exclude certain types of zooplankton from the samples. For examples, fast swimming zooplankton can avoid net tows (Fleminger & Clutter, 1965), and sensitive organisms can be damaged during collection (Ney & Schumacher, 1978).

Advances in electronics, particularly in imaging platforms, have allowed the development of many imaging systems for zooplankton studies (e.g., Briseño-Avena, Roberts, Franks, & Jaffe, 2015; Cowen & Guigand, 2008; Lombard et al., 2019; Olson & Sosik, 2007). These imaging systems enable in situ observations that can be automated and are less invasive, allowing continuous sampling of zooplankton, including those that are rare and sensitive to traditional sampling methods (Brownlee, Olson, & Sosik, 2016; Cowen & Guigand, 2008). Even though these in situ imaging platforms provide valuable insights into the ecology of zooplankton, they are relatively expensive and require engineering expertise to operate. As a result, the development of a less-expensive imaging system that does not need specialized engineering expertise has potential to expand the user base and can lead to increased knowledge in observational ecology.

To take advantage of modern advances in hardware and software at reduced cost, the In situ Plankton Assemblage eXplorer (IPAX) was developed as an open-source and relatively inexpensive underwater imaging system for zooplankton study. The IPAX is capable of imaging zooplankton in their natural habitats with a high-resolution camera and a powerful LED illumination. This article presents the IPAX, a laboratory testing to validate its performance and a deployment to demonstrate its practicality in the field.

2 | MATERIALS AND METHODS

2.1 | Overview

The IPAX consists of six main subsystems: a core computer, a control board, a camera, an LED flash unit, a power regulator board and rechargeable lithium batteries (Figure 1). The core computer is based on a Raspberry Pi Zero W (Raspberry Pi Foundation), and it communicates with the camera and stores images on an on-board SD card. The control board is based on an ATmega328p (Microchip Technology) Arduino-compatible chip, and it performs time keeping with a real-time clock and handles power cycling of the Raspberry Pi to conserve power. The camera is a Raspberry Pi compatible camera based on Sony IMX219 chip with an interchangeable M12 lens (ArduCam); this camera sensor has been characterized in detail for scientific purposes (Ryan et al., 2017), and the M12 mount allows users to switch lenses according to their needs. The focal plane of the camera can also be adjusted manually by moving the lens closer or farther away from the camera sensor. For the following experiments, a 6.0-mm F2.0 lens (PT0620CW, M12 Lenses) was used, and the focal plane was set 50 mm away from the viewpoint of the IPAX in water.

![FIGURE 1 System diagram of the In situ Plankton Assemblage eXplorer showing the communication between subsystems and the voltage used by each subsystem. Optional environmental sensors, not required for zooplankton imaging, are shown in the dashed box](image)

TABLE 1 Costs of each subsystem of the In situ Plankton Assemblage eXplorer for building one unit when parts are ordered as a batch for 10 units. The detailed cost breakdown and the bill of materials are available on the data repository (Lertvilai, 2020)

| Subsystems                          | Cost (USD) |
|-------------------------------------|------------|
| Control board                       | 26         |
| Power regulator board               | 32         |
| Optics and camera                   | 110        |
| Housing                             | 155        |
| Batteries                           | 25         |
| Others (computer and mechanical screws) | 85        |
| Total                               | 433        |

The LED flash unit consists of 10 Cree XPE2 LEDs (Cree Inc.) with focusing lenses (Carclo) and is triggered by signal from the Raspberry Pi to flash during imaging acquisition. The selected LED model has various colours available, and users can install the colours that fit their experimental designs. The 51.8 Wh lithium batteries (IMR Batteries) provide 7.4 V power input to the power regulator board, which then regulates the voltage to appropriate level for each subsystem as shown in Figure 1. The control board, the LED flash unit and the power regulator board are custom-made printed circuit boards, while the Raspberry Pi and the camera are commercially available. All components are packaged inside a cylindrical waterproof housing rated for 100 m (Bluerobotic). The components are securely attached to the interior of the housing by 3D-printed mechanical parts (Figure 2).

Material costs, for building 10 units, are <$450 each (Table 1), and construction of more units, by ordering parts in larger batches, can substantially lower the individual cost. Additional sensors, including depth and temperature sensors from Bluerobotic, can be readily integrated to the waterproof housing and can provide environmental information during each deployment. However,
the incorporation of these sensors will add significant costs to the system.

2.2 | Laboratory calibration

The imaging system of the IPAX was calibrated by arranging the camera and optics attached on a side of a clear acrylic tank filled with saltwater. To facilitate the imaging and reduce stray light, all sides of the tank, except the side with the camera attached, were painted with matte black spray paint. A USAF1951 resolution test chart was then used as a calibration target to evaluate the modulation transfer function (MTF) of the system by calculating the contrast transmittance as a function of the spacing between black bars on white background (Wood et al., 2008). The resolution target was also moved along the camera axis using a translation stage to evaluate the effective depth of field (DOF) of the imaging system.

Additionally, various types of preserved zooplankton were used to evaluate the optical performance of the IPAX. Zooplankton samples of representative taxa preserved in 70% ethanol were mounted on clear glass slides with clear glass cover slips. The slides were immersed in the acrylic tank at the focal plane of the IPAX to optimize the camera setting. Then, the slides were moved along the camera axis to evaluate the effects of DOF on each type of organisms.

2.3 | Field deployment

Two IPAX units were built and deployed in various habitats around Coconut Island in Kaneohe Bay, Hawaii, in August 2019. Both units were identical except for the LEDs used for illumination. One unit had far-red LEDs, with a centre wavelength at 730 nm, and the other unit had 6,500 K white LEDs. The unit with red light was used to record baseline abundance and behaviour of zooplankton as red light above 700 nm reduces observational bias (Cohen & Forward, 2016; Sweatt & Forward, 1985). Comparatively, the unit with white light was used to observe the differences in animal presence that included both natural abundance and phototactic response.

Both IPAX units were deployed simultaneously in protected mangrove lagoons, open lagoons and fringing reefs around the island. The instruments were secured on the seafloor by weighting them with 2 kg lead blocks. The two units were placed approximately 1-m apart and were positioned with the viewports perpendicular to minimize mutual interference. The camera was set to record videos at 30 frames per second and 1,640 × 1,232 pixels resolution with the exposure time of 500 µs and ISO 200. The LED illumination strobed with 50% duty cycle during image acquisition. Each IPAX produced a beam of light in front of the camera during video recording and minimal light from a small green indicator LED on the Raspberry Pi.

Video recordings were repeated over five nights in the same shallow protected lagoon to investigate the emergent pattern and the phototactic behaviour of demersal zooplankton. The IPAX units were programmed to record a 30-s video every 10 min from 6 p.m. to 7 a.m. every night. This deployment time covered the entire night and also an hour before sunset and an hour after sunrise. For each 30-s video, the last 5 s were extracted and manually analysed to obtain cumulative counts of each group of zooplankton that were identified to taxonomic class level. This cumulative count was then used to obtain the frequency of occurrence of zooplankton over different time of the night.

3 | RESULTS

3.1 | Optical performance

As configured, the IPAX had a field of view of 5 cm × 3 cm at the focal plane located 5-cm away from the acrylic viewport in water. At this distance, the exposure time between 500 and 1,500 µs was sufficient to obtain details of zooplankton without saturating parts of the images. An estimate of system resolution using the contrast transmittance as a function of line spacing (Figure 3) indicated that the instrument resolves a 100-µm feature with 70% contrast at the focal plane. At 20% contrast, the IPAX resolves 20 µm. Results also indicated that for a 100-µm feature, the depth of field was 5 mm with 50% contrast, which yields an effective sampling volume of 7.5 cm³.

Observation of the images of the preserved zooplankton indicated that the IPAX was able to resolve fine details that can be used for identification. For a smaller zooplankter, such as a Brachyuran...
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zoea (Figure 4a), the IPAX resolves morphological details well when the object is in focus. The eye spot, the carapace dorsal spine and the abdomen were clearly visible within ±2 mm from the focal plane. The morphological details deteriorated as the zoea moved farther away with the carapace dorsal spine becoming obscure at ±4-mm away. Larger zooplankton, such as a chaetognath (Figure 4b), on the other hand, could be identified within ±10 mm of the focal plane. When within ±5 mm, the fine details, such as the fangs of the chaetognath, were visible.

3.2 | Field results

The IPAX was successfully deployed in the habitats around the island. With fully charged batteries, the instrument lasted for two full nights, equivalent to 26 hr of night-time operation with 80 min of video recording. As observed, although species-level identification was not possible, the video provided sufficient resolution for identification down to major taxonomic groups (Figure 5). For habitats with low currents, such as protected lagoons (Videos S1–S3), zooplankton could stay in the field of view of the camera for longer periods of time, possibly allowing behavioural observations. For example, sinusoidal movements of polychaetes can be easily observed from the videos (Video S1), while this behaviour was reported to be difficult to stimulate in the laboratory (Clark & Hermans, 2009).

In more energetic habitats, such as fringing reefs and open lagoons (Video S4), the flow moved zooplankton out of the field of view very rapidly. Although this precluded behavioural observations of weak swimmers, the relatively short exposure time of the camera provided sharp images of zooplankton that were suitable for identification and enumeration. With the exposure time of 500 µs, a 100-µm feature can still be resolved even with an ambient flow of 20 cm/s. If shorter exposure times are needed when deployed in energetic habitats, the camera can be set to lower exposure time and higher gain to handle higher flow velocity.

Since the IPAX captured images of open space using backscatter illumination, each image also contained many zooplankton that were out of focus. When plankton density is high, such as in a swarming event, out-of-focus organisms reduced the contrast of the in-focus plankton. Fortunately, the typical abundance was not high enough to cause problems in this field deployment.

The frequency of occurrence of each type of zooplankton over the extracted last 5 s of videos were averaged over five nights, and the 6-point moving average of the counts is shown in Figure 6. This enumeration reveals emergent patterns of zooplankton from the in situ observation. Overall zooplankton were present throughout the deployment time, though the counts were much higher from 8 p.m. to 4 a.m. (Figure 6a). Copepods were present with relatively constant density. Brachyuran zoea were more abundant towards dusk, while chaetognaths were more abundant towards dawn, and polychaetes showed two distinct peaks at dusk and dawn (Figure 6b–e). These emergent patterns were similar to previous reports (Alldredge & King, 1980; Yahel, Yahel, Berman, Jaffe, & Genin, 2005). The comparison between the IPAX units with white light and with red light shows...
**FIGURE 5** Examples of zooplankton images taken from the video recordings. (a) The full field of view from a video recorded by the In situ Plankton Assemblage eXplorer with white light during the deployment in Hawaii. (b) a Stomatopod pseudozoea. (c) a Brachyuran zoea, (d) a Stomatopod antizoea, (e) a chaetognath, (f) a polychaete carrying egg mass and (g) a copepod. The scale bar indicates 5 mm in (a) and 1 mm in (b–g).

**FIGURE 6** The 6-point moving average of frequency of occurrence of zooplankton over five nights from the same shallow lagoon habitat from two In situ Plankton Assemblage eXplorer units with different colour LEDs. The sampling period was 10 min, and each point is the cumulative organism count over extracted 5 s of recordings. The solid lines indicate the average over five nights, and the shaded error bars indicate the standard errors. (a) The total count of all zooplankton. (b–e) The count of each major taxonomic group.
an overall strong positive phototactic behaviour of zooplankton in this shallow habitat similar to the report by Tranter et al. (1981). The phototaxis is particularly strong in chaetognath and polychaetes compared to copepods and brachyuran zoea.

4 | DISCUSSION

The laboratory calibrations and the field deployment demonstrate that the IPAX is an imaging platform suitable for in situ observation of zooplankton. It is designed to be autonomous and fully self-contained within one compact waterproof housing with internal batteries and data storage. The simplicity and versatility of the IPAX allow it to be incorporated into many platforms in shallow waters, such as moorings and autonomous vehicles or simply secured on the seafloor. However, for a long-term deployment, bio-fouling might reduce the image quality overtime, so additional anti-fouling capability would then be needed.

Even though this study only used the IPAX to observe photographs of zooplankton, the instrument can potentially be adapted to accommodate various in situ experiments. For instance, deploying two units to observe the same sampling volume will allow three-dimensional tracking within the sampling volume to observe swimming behaviours. With some modifications to the illumination, the IPAX can also be used for studies in benthic habitats, such as examining larval settlement and investigating macroalgal growth. However, caution should be exercised in environments with extremely high flow rates, such as streams and rivers, because the minimum exposure time might not be sufficient to obtain high quality images. Additionally, the system may not work well in very turbid water because high concentration of ambient particles can significantly reduce the contrast of zooplankton, causing unusable images and videos.

The IPAX is also low cost and is designed to be accessible to research laboratories with minimal engineering expertise. Most components, except the main circuit boards, are commercially available, which simplifies fabrication. The main computer is a Raspberry Pi, which can be easily programmed with Python code to set the routine and set camera parameters. The control board can be programmed with an Arduino platform, which bypasses the complexity of low-level programming on the microcontroller. Each subsystem in the IPAX is also designed to be modular, so that users can easily adapt one subsystem to fit their experimental designs without the need to redesign the whole instrument. This modularity is particularly advantageous because technology is rapidly advancing, and better components can become available at a time.

The affordability of the IPAX also allows multiple units to be built, and the bulk construction will also further reduce manufacturing cost of each unit. Multiple IPAX units will allow an experiment to cover larger spatial area in a single deployment, which can expand our knowledge of gradients and patchiness in zooplankton distribution across multiple habitats. IPAX units can potentially be deployed on mobile platforms, such as the autonomous explorers by Jaffe et al. (2017), to create a swarm of instruments that can track zooplankton distribution in a semi-Lagrangian fashion. Even though multiple units of IPAX can produce large amount of data that can overwhelm manual analyses, there are multiple image processing techniques and machine learning tools for zooplankton classification, such as Ecotaxa (Picheral, Colin, & Irisson, 2017), that can facilitate analyses of the data from IPAX to produce scientifically quantitative data (Benfield et al., 2007).

5 | CONCLUSIONS

In summary, the IPAX is a low-cost underwater imaging system that is capable of observing zooplankton in their natural habitat. The laboratory calibration and the field testing have proven that the IPAX is a suitable tool for in situ zooplankton studies. This autonomous instrument will enable researchers to investigate zooplankton in various aquatic habitats without the need for intensive field labour and specialized engineering expertise. These benefits will facilitate advances in knowledge of zooplankton and ultimately, their role in aquatic ecosystems.

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PEER REVIEW

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DATA AVAILABILITY STATEMENT

The design of the IPAX is publicly available on https://github.com/pichertvilai/IPAX (http://doi.org/10.5281/zenodo.3735054).

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**SUPPORTING INFORMATION**

Additional supporting information may be found online in the Supporting Information section.

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