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Quantitative Analysis of Reproductive Behavior in the Polychaete *Perinereis aibuhitensis* (Grube, 1878) Using a Newly Developed Behavioral Analysis Software

Oshima Shion[1], Satou Kenji[2], Watanabe Narihiro[3], Satoh Takanori[4], Yachiguchi Koji[4], Matsubara Hajime[5], Tabuchi Yoshiaki[6], Ogiso Shouzo[1], Hattori Atsuhiko[7], Hirayama Jun[8], Srivastav Ajai K.[9] and Suzuki Nobuo[1]*

[1]Noto Marine Laboratory, Institute of Nature and Environmental Technology,Kanazawa University, Ogi, Noto-cho, Ishikawa 927-0553, Japan
[2]Institute of Transdisciplinary Sciences for Innovation, Kanazawa University, Kanazawa, Ishikawa 920-1192, Japan
[3]Graduate School of Natural Science and Technology, Kanazawa University, Kanazawa, Ishikawa 920-1192, Japan
[4]Institute of Noto Satoumi Education and Studies, Ogi, Noto-cho, Ishikawa 927-0553, Japan
[5]Noto Center for Fisheries Science and Technology, Kanazawa University, Ossaka, Noto-cho, Ishikawa 927-0552, Japan
[6]Life Science Research Center, University of Toyama, Sugitani, Toyama 930-0194, Japan
[7]Department of Sport and Wellness, College of Sport and Wellness, Rikkyo University, Niiza Saitama 352-8558, Japan
[8]Faculty of Education, Bunkyo University, Koshigaya, Saitama 343-8511, Japan
[9]Department of Zoology, D.D.U. Gorakhpur University, Gorakhpur 273009, India

**Corresponding Author*

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Abstract: Analysis of reproductive behavior provides useful data for understanding the aquaculture of aquatic animals. The Polychaete *Perinereis aibuhitensis* is widely used as bait for recreational fishing. Recently, it has been reported that the use of this species as bait is effective in promoting the growth of kuruma prawns (*Penaeus japonicus*). Consequently, polychaetes, including this species, are increasingly being utilized in aquaculture. In this study, to examine the reproductive behavior of *P. aibuhitensis*, we developed an original behavioral analysis software. Using this software, we analyzed the reproductive behavior of *P. aibuhitensis*. Egg-holding and non-egg holding individuals were placed in an observation rearing apparatus and maintained under a 12 h light–dark cycle, and their behavior was recorded using a video camera. The obtained video data were analyzed with the developed software. As this species is nocturnal, differences in reproductive behavior were observed only during nighttime activity. During the first three hours of the dark period, individuals holding eggs moved more vigorously and exhibited significantly higher movement speeds than individuals not holding eggs. In contrast, during midnight, no significant differences in behavior were observed between egg-holding and non-egg-holding individuals. These results are consistent with previous findings that polychaetes exhibit spawning behavior immediately after sunset. Thus, the software developed in this study is effective for analyzing the reproductive behavior of *P. aibuhitensis* and may be applicable to behavioral analyses of other aquatic animals.

Keywords: Polychaete, *Perinereis aibuhitensis*, Reproductive behavior, Original software, Nocturnal aquatic animals

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Introduction

Analyzing animal behavior can contribute to important objectives in many research fields, including the study of feeding behavior and reproductive physiology, thereby supporting scientists investigating animal physiology, psychology, ecology, and toxicology (Franco-Restrepo *et al.*, 2019). In polychaetes, reproductive swimming behavior has been reported in *Perinereis nuntia* var. *brevicirrus* (Hardege and Bartels-Hardege, 1995), *Nereis japonica* (Bartels-Hardege *et al.*, 1996), *Platynereis dumerilii* (Bentley *et al.*, 2001), and *Neanthes glandicincta* (Azmi *et al.*, 2021). Interestingly, sex pheromones have been identified in polychaetes (Zeeck *et al.*, 1988, 1996). One such pheromone, 5-methyl-3-heptanone, has been identified in *Platynereis dumerilii* (Zeeck *et al.*, 1988). This compound induces spawning behavior, including the nuptial dance (Zeeck *et al.*, 1988). Taken together, behavioral analysis provides important data for understanding reproductive physiology and contributes to the development of aquaculture practices in polychaetes.

On the other hand, recreational fishing is one of the most common leisure activities in coastal regions worldwide and involves large numbers of people (Cole *et al.*, 2018; Font *et al.*, 2018). Polychaetes are important bait organisms in recreational fishing. *Perinereis aibuhitensis* (Grube, 1878) (Fig. 1) is one of the main marine bait species used for recreational fishing in Japan. Because this species is rare and occurs only in the Sumida River basin in Japan, most *P. aibuhitensis* consumed domestically is imported from Korea (Choi and Lee, 1997). Recently, it has been reported that feeding kuruma prawns (*Penaeus japonicus*) with this species promotes their growth (Chen *et al.*, 2024), and that polychaetes, including *P. aibuhitensis*, are increasingly being utilized in aquaculture (Cole *et al.*, 2018; Li *et al.*, 2025).

To accumulate fundamental data on the reproductive physiology of *P. aibuhitensis*, we first developed an original software for analyzing the behavior of aquatic animals. Using this software, we then compared the behaviors of *P. aibuhitensis* individuals according to whether they were holding eggs.

Materials and Methods

Animals

Farmed polychaetes *P. aibuhitensis* from Korea were purchased from a fishing tackle shop in October 2025. For acclimation, the animals were reared in an aquarium at 20°C. A board was placed in the aquarium as a hideout, and the aquarium was illuminated with LED light (LED PLUS Beauty Class 23.6 inches, Zensui Co. Ltd., Osaka, Japan) under a 12 h light–dark cycle for 1 week. The light conditions were controlled using a timer, with the light period from 6:00 a.m. to 6:00 p.m. and the dark period from 6:00 p.m. to 6:00 a.m.

Figure 1 shows immature (A) and mature (B) individuals. As this species matures, the eyes become larger, the body segments shorten, and the overall body length decreases.

Experiments investigating reproductive behavior in P. aibuhitensis

The acclimatized individuals were used for the experiments. *P. aibuhitensis* was placed in the

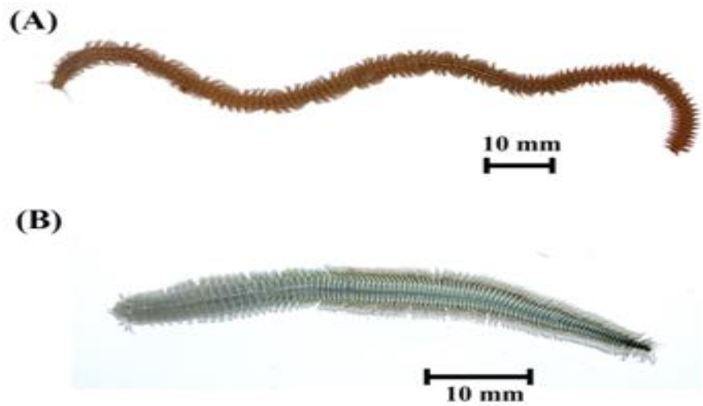

Fig. 1: Photographs of sexually immature (A) and mature (B) polychaetes *Perinereis aibuhitensis*. As this species matures, the eyes become larger, the body segments shorten, and the overall body length decreases.

observation rearing apparatus with LED light (LED Tape Light, Shenzhen Sibi Lighting Co., Ltd., China) under light and dark cycles (12 h:12 h). The light conditions were set by timer as describe above. The behavior of this species was recorded for 24 h using a video camera (HC-VX992M, Panasonic Corporation, Tokyo, Japan). The obtained video was analyzed using the present software we developed.

Statistical analysis

Differences between two groups i.e. egg-holding and non–egg-holding individuals were analyzed using Student's t-test. In all analyses, statistical significance was set at $p < 0.05$.

Results and Discussion

Development of original software for analyzing reproductive behavior in P. aibuhitensis

When analyzing human or animal behavior using video data, various factors must be considered, such as whether the target is a single individual or multiple individuals, whether a single or multiple cameras are used, and whether depth information is incorporated (Perez and Toler-Franklin, 2023). Although several software programs are available for such analyses (Luxem *et al.*, 2023), we developed our own software specifically for analyzing the behavior of *P. aibuhitensis*.

This software analyzes RGB videos acquired from a top-view perspective of a single target recorded by a fixed camera. Based on the recognition of the target's position in each video frame, the software calculates various derivative parameters, including the distance traveled and movement speed. Assuming that the target and the solid-color background can be distinguished by sufficiently high contrast, adaptive thresholding and dynamic background subtraction are applied to extract the contour of the target.

The definition of the target's location depends on the type of animal and the behavior being analyzed. In lugworms such as *P. aibuhitensis*, the elongated body often adopts complex, coiled, or rounded postures, making it difficult to reliably identify specific body parts such as the head or tail. Therefore, in this study, the software first extracts the contour of the target in each video frame and then calculates the centroid by averaging the coordinates of all pixels within the contour. This centroid is defined as the location of the target. This approach is simple and broadly applicable to various animal species.

Using the centroid coordinates obtained for each frame, the software automatically calculates behavioral metrics, including total distance traveled, activity duration, and movement speed.

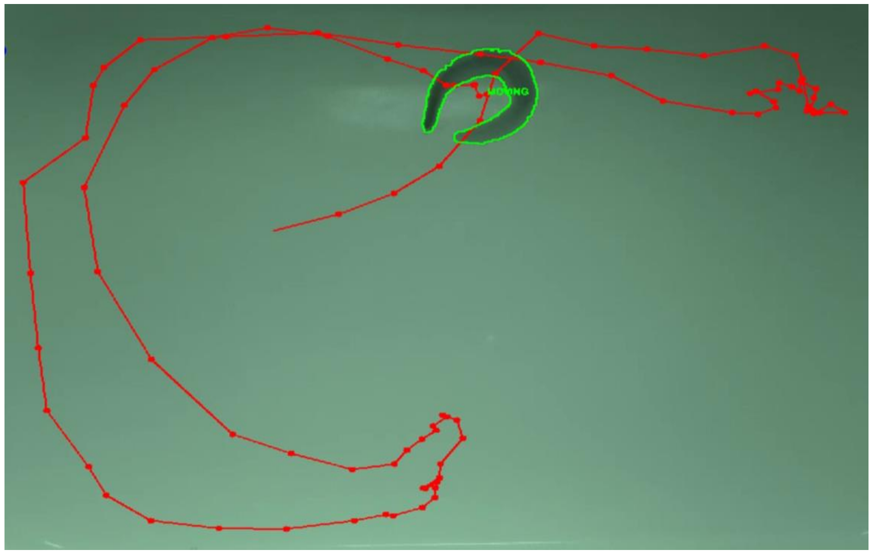

Fig. 2: Investigation of reproductive behavior in *Perinereis aibuhitensis* using Python-based behavioral analysis software. The red line indicates the trajectory of the worm's movement.

The calculated data are exported as CSV files, enabling subsequent statistical analyses using Microsoft Excel or other software. The software is written in Python, with image processing primarily implemented using Open CV and scikit-image. Figure 2 shows representative results of trajectory analyses of *P. aibuhitensis* obtained using this software.

This software does not require machine learning and is adaptable to various imaging devices, lighting conditions, and individual sizes, making it highly versatile and reproducible. Furthermore, the entire process, from detection and tracking to quantification, is fully automated, eliminating the need for manual annotation or threshold adjustment by the analyst and thereby improving usability and efficiency.

In addition to reproductive behavior, this software can be applied to the analysis of other behavioral patterns, and its general design allows for extension to different species and experimental conditions. The software used in this study is available at the following URL: https://github.com/saltrau/PolyTrack

Analysis of reproductive behavior in P. aibuhitensis using behavioral analysis software

Using the software developed in this study, we analyzed the reproductive behavior of *P. aibuhitensis*. Egg-holding and non-egg- holding individuals were placed in an observation rearing apparatus and maintained under a 12 h light–dark cycle, and their behavior was recorded using a video camera. The obtained video data were analyzed using the developed software. Because this species is nocturnal, differences in reproductive behavior were observed only during nighttime activity. During the dark period, distance traveled, activity duration, and movement speed in *P. aibuhitensis* were analyzed.

During the initial three hours of the dark period (6:00 p.m. to 9:00 p.m.), mature individuals holding eggs moved more vigorously (https://ijzi.net/Video-Reproductive-behavior.html). No significant differences were observed in distance traveled (Fig. 3A) or activity duration (Fig. 3B) between mature individuals holding eggs and immature individuals not holding eggs. However, movement speed was significantly higher in mature individuals than in immature individuals ($p = 0.023$) (Fig. 3C).

During the three-hour period around midnight (11:00 p.m. to 1:00 a.m.), no significant differences

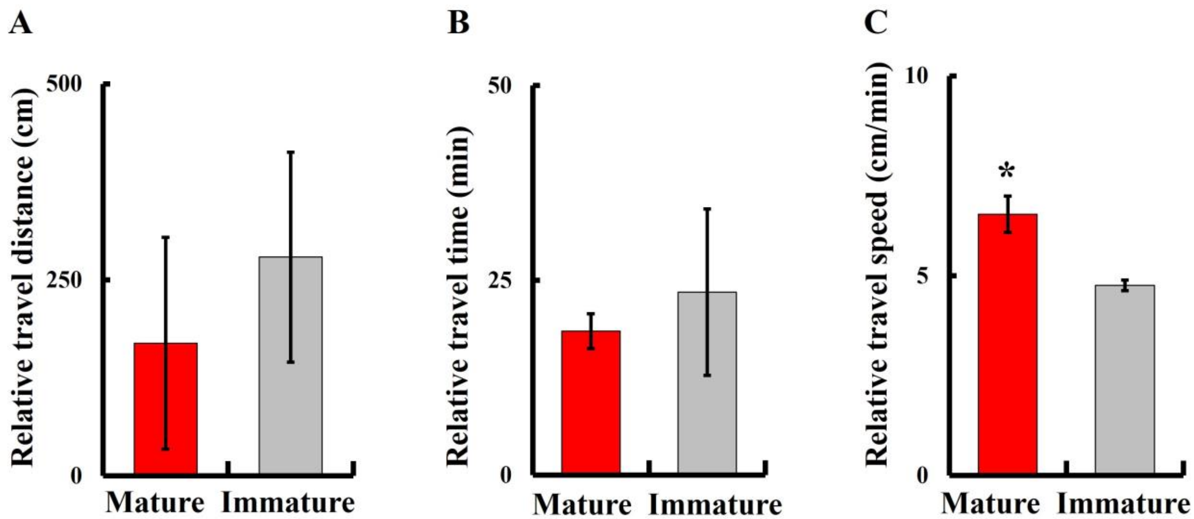

Fig. 3: Travel distance (cm)(A), travel time (min) (B), and travel speed (cm/min)(C) during the initial three hours after dark (6 p.m. to 9 p.m.) in *Perinereis aibuhitensis*. No significant differences were observed in travel distance or travel time between mature (n = 3) and immature individuals (n = 3), whereas travel speed differed significantly between the two groups ($p < 0.05$, Student's t-test). All results are expressed as means ± SEM.

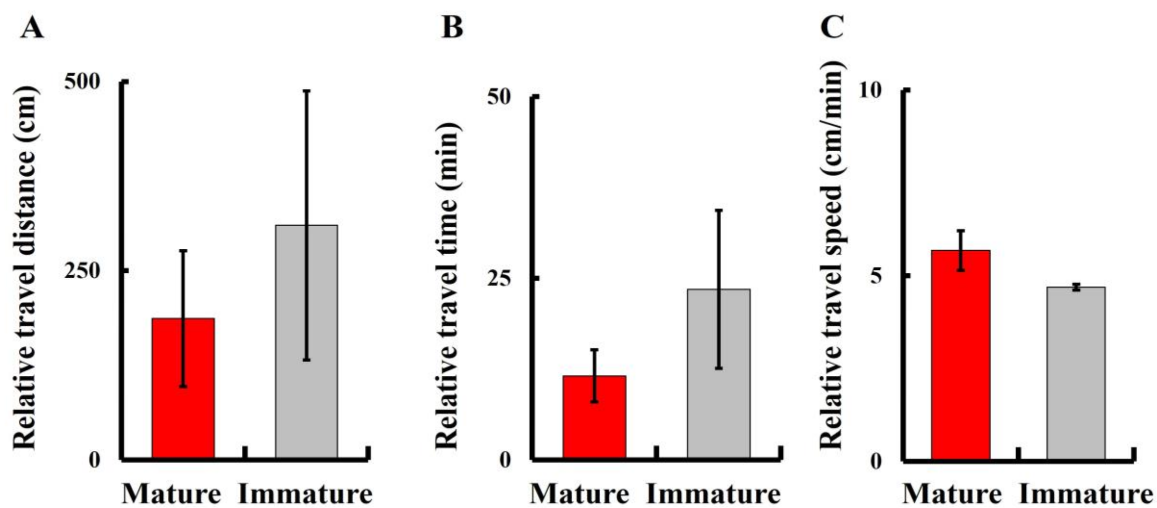

Fig. 4: Travel distance (cm)(A), travel time (min)(B), and travel speed (cm/min)(C) around midnight (11 p.m. to 1 a.m.) in *Perinereis aibuhitensis*. No significant differences were observed in any of the parameters between mature (n = 3) and immature individuals (n = 3). All results are expressed as means ± SEM.

in distance traveled (Fig. 4A) or activity duration (Fig. 4B) were observed between mature and immature individuals. Movement speed in mature individuals tended to be higher than that in immature individuals; however, this difference was not statistically significant ($p = 0.132$) (Fig. 4C). These results are consistent with previous findings showing that polychaetes exhibit spawning behavior immediately after sunset (Bentley *et al.*, 2001). Therefore, the software developed in this study was able to successfully capture and analyze reproductive behavior specific to *P. aibuhitensis*.

In polychaetes, sex pheromones such as 5-methyl-3-heptanone have been identified and are known to induce spawning behavior, including the nuptial dance (Zeeck *et al.*, 1988, 1996). In contrast, sex pheromones in *P. aibuhitensis* have not yet been identified. However, the known pheromone 5-methyl-3-heptanone may also be effective in inducing spawning behavior in *P. aibuhitensis*. In future studies, we plan to add this pheromone to seawater and observe the resulting behavioral responses of *P. aibuhitensis* using the behavioral analysis software developed in this study.

The software developed in this study is effective for analyzing the behavior of this species by tracking centroid movements. However, elongated animals such as polychaetes locomote by wriggling their bodies, and centroid-based tracking alone cannot fully capture these complex body dynamics. Therefore, more advanced software capable of analyzing whole-body movements is required. We are currently developing software that can analyze the full-body movements of polychaetes. Using this software, we aim to investigate the detailed behavioral patterns of polychaetes in the near future.

Conclusion

In the present study we developed an original software to examine the reproductive behavior of *P. aibuhitensis*. Using this software, we observed that egg-holding individuals moved more vigorously and exhibited higher movement speeds than individuals not holding eggs. These results are consistent with previous findings showing that polychaetes exhibit spawning behavior immediately after sunset. Thus, the software developed in this study is effective for analyzing the behavior of this species.

Ethical Statement

This study was conducted in strict accordance with the ethical guidelines of Kanazawa University, Japan.

Author Contributions

Oshima Shion, *Satou Kenji*, *Watanabe Narihiro* and *Suzuki Nobuo*: designed the experiments and wrote the manuscript. *Oshima Shion*, *Satoh Takanori*, *Yachiguchi Koji*, *Matsubara Hajime* and *Suzuki Nobuo*: performed experiments. *Oshima Shion*, *Tabuchi Yoshiaki*, *Hattori Atsuhiko*, *Ogiso Shouzo*, *Hirayama Jun* and *Suzuki Nobuo*: sampling and analyzed the data. *Oshima Shion* and *Suzuki Nobuo*: confirm the authenticity of all the raw data. *Hirayama Jun*, *Srivastav Ajai K*. and *Suzuki Nobuo*: supervision, editing and refining manuscript content. All authors have read and agreed to the published version of the manuscript.

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Conflict of Interest

The authors declare no conflicts of interest.

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