# 多毛類Perinereis aibuhitensisの頭部で合成されるインドール化合物の日内リズム


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# Diurnal rhythm of indole compounds synthesized in polychaete *Perinereis aibuhitensis* heads

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**Abstract:** Polychaete *Perinereis aibuhitensis* is in great demand as fishing bait in Japan. However, *P. aibuhitensis* for fishing bait is imported from Korea and China. In preparation for coming restrictions such as resource depletion, it is indispensable to cultivate *P. aibuhitensis* domestically. Understanding the life history of *P. aibuhitensis* is necessary for domestic aquaculture. In the present study, we analyzed serotonin, which is involved in diurnal rhythm regulation in vertebrates, as well as tryptophan metabolites (kynurenine and indole-3-acetic acid). Mass spectrometric analyses were used in this study to detect serotonin, kynurenine, and indole-3-acetic acid in the head of the *P. aibuhitensis*. Serotonin production, as well as kynurenine production, was found to have no significant diurnal variation in *P. aibuhitensis*. Notably, there was a diurnal rhythm in indole-3-acetic acid synthesis in *P. aibuhitensis*. These results indicate that indole-3-acetic acid, rather than serotonin, plays an important function in the diurnal rhythm of *P. aibuhitensis*. These findings suggest that *P. aibuhitensis* could be efficiently reared and produced in Japan, if we can artificially support its optimal living environment that can maintain the diurnal rhythm of indole-3-acetic acid even in high-density farming.

**Key words:** *P. aibuhitensis*; Indole-3-acetic acid; Diurnal rhythm

Polychaetes belonging to Polychaeta are important as bait for marine anglers. The main marine fishing bait polychaetes in Japan are the following (Saito et al. 2011)—the family Nereididae: *Perinereis aibuhitensis* Grube, 1878, *P. wilsoni* Glasby and Hsieh, 2006, *Nectoneanthes oxypoda sensu* Imajima, 1972, and *Alitta virens* (M. Sars, 1835); the family Glyceridae: *Glycera nicobarica* Grube, 1968; the family Onuphidae: *Diopatra sugokai* Izuka, 1907; the family Eunicidae: *Marphysa sanguinea* (Montagu, 1815); the family Lumbrineridae: *Scoletoma heteropoda* (Marenzeller, 1879); the family Oenonidae: *Halla okudai* Imajima, 1967. Among these, *P. aibuhitensis* and *P. wilsoni* are the most common and traded throughout Japan. The former is widely distributed from the Korean Peninsula to India (Uchida 1992). However, the only description of this species in Japan is from the estuary of the Sumida River (Imajima 1972), making it a rare species in Japan (Saito et al. 2011). *P. aibuhitensis* ("Aogokai"), known as "Aoisome", "Aomushi", and "Akaisome", costs about 6,000 yen per 1 kg and is imported from Korea and China. In contrast, the latter is distributed from the

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Korean Peninsula to the Yellow Sea, Taiwan, and Japan. *P. wilsoni* ("Ishiisogokai"), known as Ishigokai and Jyarime, costs about 8,000 yen per 1 kg and is the mainly sold Japanese product. However, annual imports of these marine fishing bait polychaetes have been decreasing year by year due to declining stock resources caused by the development of their living areas and other factors in exporting countries (Saito et al. 2011). So far, the aquaculture of *P. wilsoni* has been established in the 1980s (Yoshida 1984), and stable supplies of the species have been cultivated in Shikoku and Kyushu, Japan. However, *P. aibuhitensis* aquaculture has not been established in Japan.

The family Nereididae is considered to die after one spawning (Olive 1983). In many species of the family Nereididae, mature individuals swim together and release eggs and sperm, known as swarming (Imajima 1972; Wu et al. 1985; Hanafiah et al. 2006). The season in which Nereididae swarming occurs varies from species to species; however, the synchrony of the date and time is related to lunar and tidal cycles (Izuka 1903, 1908; Sato and Tsuchiya 1987; Fukao 1996). In addition, it has been reported that the synchrony of Nereididae swarming can be altered by providing cycles of nocturnal illumination (artificial moonlight condition) through indoor breeding experiments (Hauenschild 1960; Hardege et al. 1990; Fischer and Dorresteijn 2004). In short, Nereididae are thought to synchronize their reproduction with the lunar cycle (Yoshida 1984). Although no lunar cycle is observed in the *P. wilsoni* reared indoors, Yoshida (1984) reported that reversing the day/night sunlight may promote *P. wilsoni* spawning. In fact, farmers reverse the day and night sunlight to spawn *P. wilsoni.* However, the relationship between light and spawning of *P. aibuhitensis* is unknown. It has been observed that *P. aibuhitensi* feed on seaweed at night (N. Suzuki, personal communication). Therefore, studying diurnal rhythms in *P. aibuhitensi* is likely to contribute to the study of their aquaculture.

The correct regulation and maintenance of physiological processes are required for the efficiency and survival of organisms (Hirayama et al. 2019). In addition, the activities associated with these physiological processes are organized in a daily manner. In particular, diurnal rhythms of physiological functions in animals have important roles for their feeding and growth (Mogi et al. 2015; Panda 2016; Yúfera et al. 2017; Galal-Khallaf et al. 2020).

Tryptophan, an amino acid, is a precursor of various bioactive substances and plays an important role in the diurnal rhythm of living organisms. Tryptophan is important as a hormone precursor, for example, to serotonin; to niacin *via* kynurenine, and to indole-3-acetic acid, an important plant-growth hormone (Mano and Nemoto 2012; Kałużna-Czaplińska et al. 2019; Castro-Portuguez and Sutphin 2020; Zhang et al. 2020) (Fig. 1). Serotonin, a tryptophan metabolite, is a hormone associated with diurnal rhythms in vertebrates. In

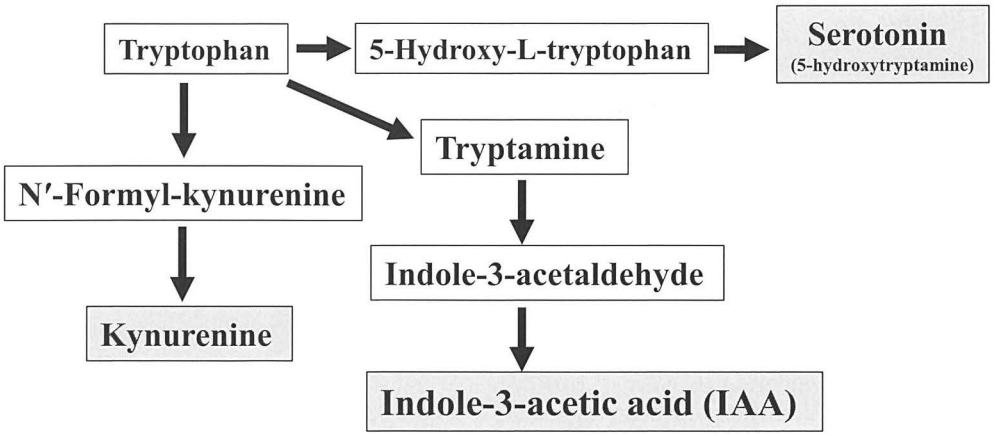



**Fig. 1.** Tryptophan metabolic pathway
Tryptophan is metabolized in the animal body to serotonin, kynurenine, and indole-3-acetic acid.

humans, serotonin links rhythmic activity in the basal forebrain and pre-optic area to the circadian rhythm signaled *via* the suprachiasmatic nucleus (Hornung 2003; Daut and Fonken 2019). This regulation is mediated by the autonomic nervous system in the hypothalamus, into which the dorsal raphe nucleus serotonergic neurons are projected (Hornung 2003; Daut and Fonken 2019; Bacqué-Cazenave et al. 2020). In invertebrates, serotonin seems to be an ancient neurotransmitter that exists throughout the animal kingdom, suggesting the origin of the nervous system from this neurotransmitter (Hay-Schmidt 2000). Serotonin production in the nervous systems of invertebrates, such as cnidarians, planarians, crustaceans, insects, and mollusks, has been reported (Muszyńska-Pytel and Cymborowski 1978; Itoh and Igarashi 2000; Bacqué-Cazenave et al. 2020). Furthermore, sea hares (Corrent et al. 1978; Yeung and Eskin 1987; Colwell 1990; Hattar et al. 2002), crayfish (Calderón-Rosete et al. 2006), and *Drosophila* (Yuan et al. 2005) have been found to possess rhythmic serotonin production in their nervous systems, similar to vertebrates. On the other hand, serotonin production in the nervous system of annelids has been reported (Forest and Lindsay 2008; Starunov et al. 2017). However, rhythmic serotonin production in annelids' nervous systems has not been investigated thus far.

Understanding serotonin synthesis, which is related to the correct regulation and maintenance of physiological processes, in polychaetes will not only clarify the effects of serotonin on its nervous system, which still remains largely unknown, but might also contribute to the mass rearing and breeding of polychaetes. Therefore, we studied the relationship between serotonin synthesis and diurnal rhythms in the *P. aibuhitensis* head, which might be considered rich in nervous system. In addition, the diurnal rhythms of indole-3-acetic acid and kynurenine, which are tryptophan metabolites, were also investigated in the head of *P. aibuhitensis*.

## Materials and methods

*Animals*

Polychaete *P. aibuhitensis* (n = 300) farmed in China were purchased from a fishing tackle shop in July 2016. For acclimation, these were reared in an aquarium (900 mm wide, 600 mm deep, 500 mm high, Density 2.7–individuals/L, Flow velocity 2.8 mm/sec) at 20°C. We put a board as a hideout and exposed an aquarium to light with an LED light (GEX Corporation, CLEAR LED POWER III 900, Osaka, Japan) under light and dark cycles (12 h:12 h) for 1 week.

*Experiments to investigate diurnal rhythms in P. aibuhitensis*

After acclimation, these were used for experiments. Sampling was conducted nine times every 4 h from noon on July 28, 2016, to midnight on July 30, 2016. These were quickly frozen using liquid nitrogen to avoid causing them pain, and the heads were cut off with scissors. The prepared heads were stored at −80°C until use.

*Indole compound extraction and measurements*

Five heads of *P. aibuhitensis* were placed in one sample tube, to which 200 µl of Milli-Q water was added. The collected heads were homogenized with a pestle. Five heads of *P. aibuhitensis* were used to prepare one analytical sample. Five analytical samples were prepared at each time point. A volume of acetone (800 µl) was then added to the tubes and stirred with a vortex mixer for 5 min. The tubes were centrifuged at 4°C and 18,000 g for 10 min. The separated upper layer was stored at −80°C until analysis. The sample was dried and solidified just before analysis by liquid chromatography–tandem mass spectrometry (LC–MS/MS) (LCMS-8050; Shimadzu Co., Kyoto, Japan) by spraying $N_2$ gas. A volume of 100 µl of Milli-Q water was added to the dried sample and stirred for 5 min to redissolve the sample. After centrifugation, the sample was passed through a 0.22 µm filter, and the solution was used as a sample for LC–MS/MS analysis.

The serotonin, kynurenine, and indole-3-acetic acid contents in the *P. aibuhitensis* heads were simultaneously analyzed by using a previously described LC-MS/MS protocol (Iwashita et al. 2021).

*Statistical analyses*

All results are expressed as the means ± standard errors (SEs). A COSINOR program (http://www.circadian.org/softwar.html) was used to calculate the diurnal rhythm periodicity. In all cases, the selected significance level was $p < 0.05$.

## Results

*Serotonin, kynurenine, and indole-3-acetic acid detection in P. aibuhitensis heads by LC-MS/MS*

Serotonin (Fig. 2a), kynurenine (Fig. 2b), and indole-3-acetic acid (Fig. 2c) were detected in the *P. aibuhitensis* heads. The transitions were *m/z* 160.0-132.0, *m/z* 146.0-192.0, and *m/z* 130.0-77.0 for serotonin, kynurenine, and indole-3-acetic acid, respectively. Each prepared sample peak was in complete agreement with the standard peak.

*Changes in serotonin, kynurenine, and indole-3-acetic acid contents in P. aibuhitensis heads*

The diurnal rhythm results for serotonin, kynurenine, and indole-3-acetic acid are shown in Figure 3. The diurnal rhythm periodicity was calculated with a COSINOR program. A significant ($p$ = 0.008) diurnal rhythm (period: 26.7 hr) in indole-3-acetic acid levels was observed in the *P. aibuhitensis* heads (Fig. 3c). The mesor, acrophase, and amplitude were 234.0, 10, and 103.7, respectively. However, no significant diurnal rhythm was observed in serotonin levels (period: > 34.1 hr) (Fig. 3a) and kynurenine levels (period: 26.5 hr) (Fig. 3b) in *P. aibuhitensis* heads.

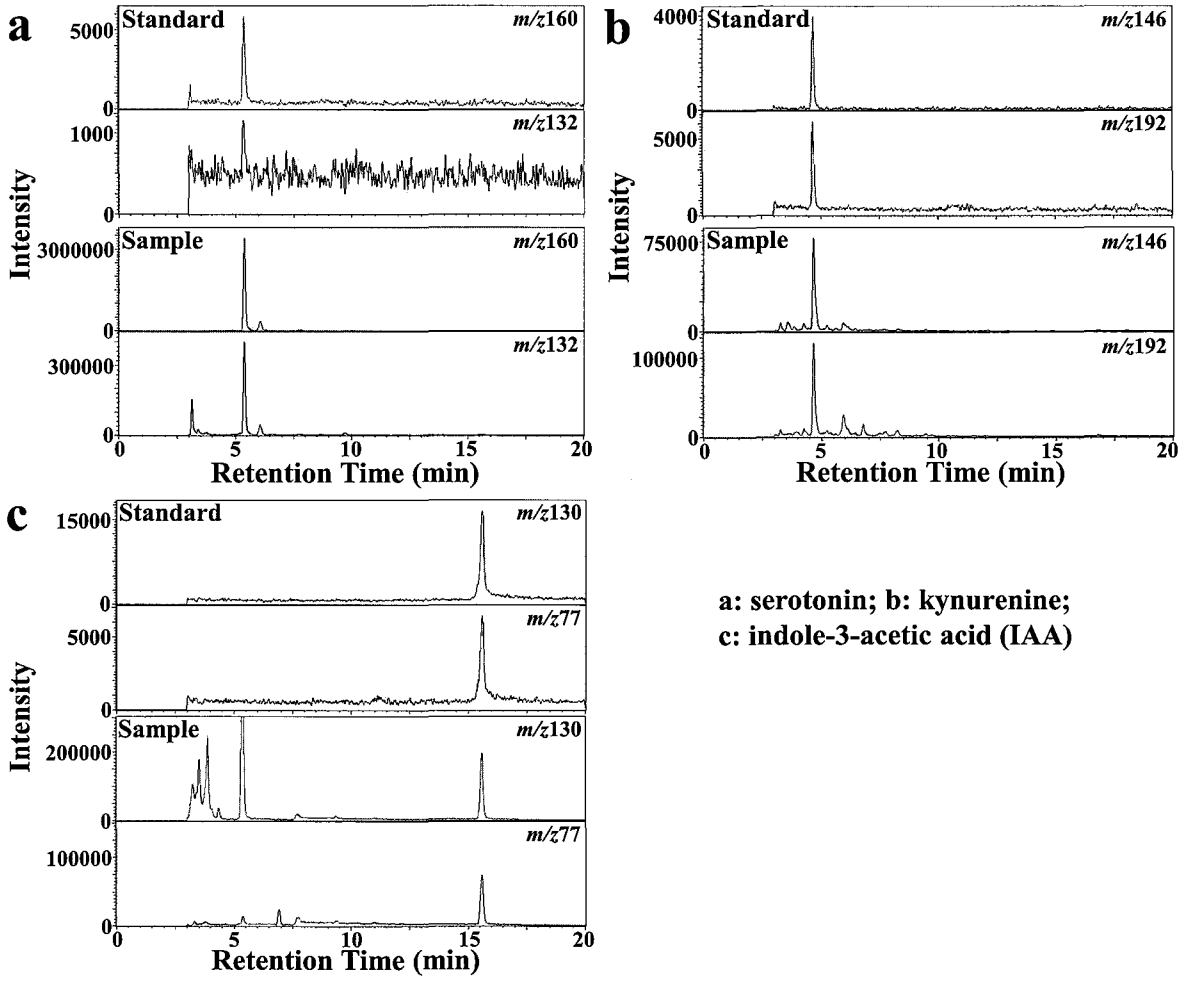



**Fig. 2.** Serotonin (a), kynurenine (b), and indole-3-acetic acid (c) detection in the polychaetes heads by LC-MS/MS analysis.

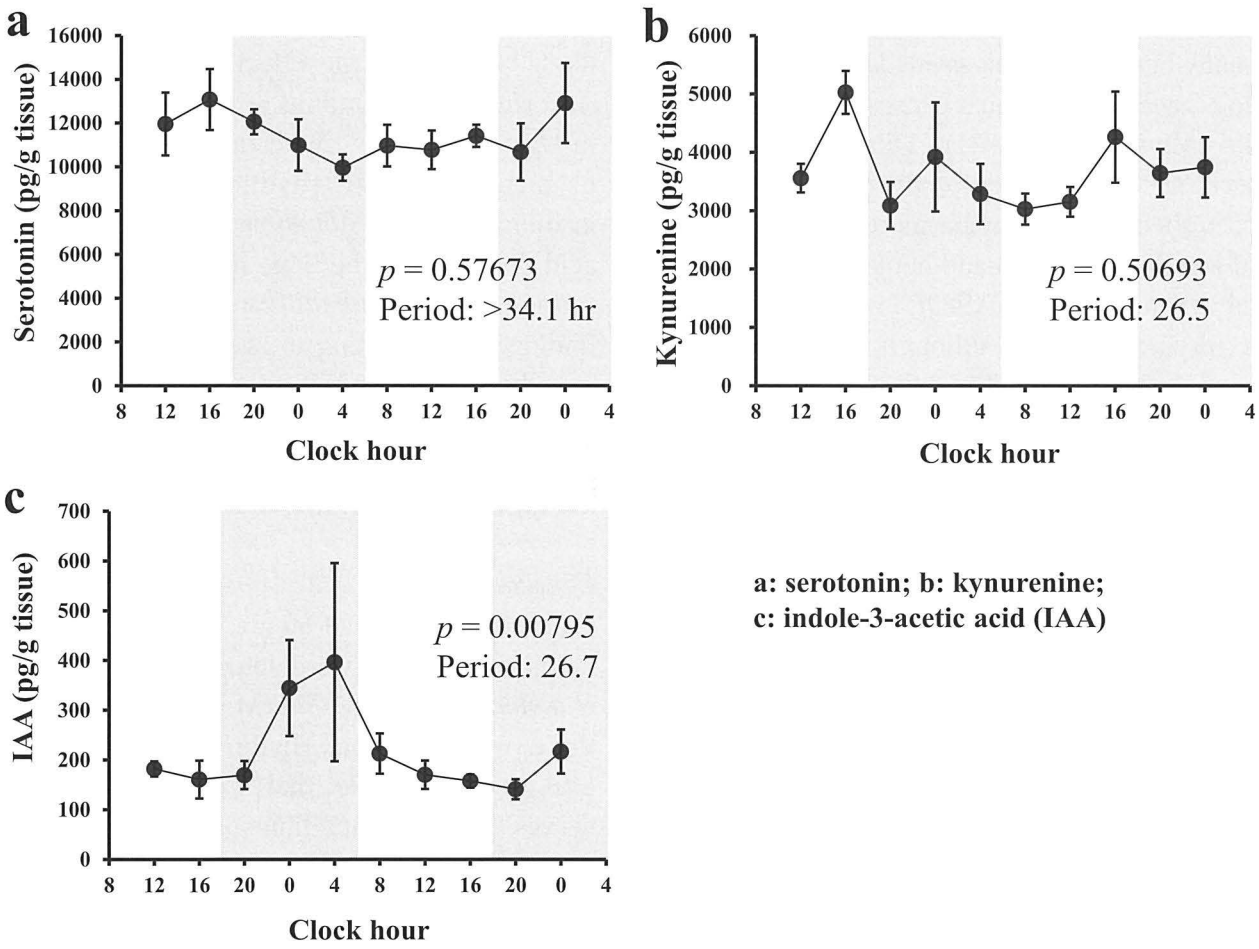



**Fig. 3.** Serotonin (a), kynurenine (b), and indole-3-acetic acid (c) changes in the polychaetes heads over 36 h
Data indicate means ± standard errors. Five samples were analyzed at each time point. To prepare each of the analytical samples, five heads were used.

## Discussion

In the present study, changes in the serotonin, kynurenine, and indole-3-acetic acid in the heads of *P. aibuhitensis* over 36 h were analyzed to examine the diurnal changes of these indole compounds. Interestingly, indole-3-acetic acid in addition to serotonin and kynurenine was detected in *P. aibuhitensis*. Moreover, the indole-3-acetic acid showed the significant diurnal rhythm of *P. aibuhitensis* according to analysis by a COSINOR program; therefore, it may have important physiological functions in *P. aibuhitensis*.

On the other hand, the serotonin, kynurenine and indole-3-acetic acid levels in the head of *P. aibuhitensis* were around 10–14 ng/g tissue, 3–5 ng/g tissue, and 150–600 pg/g tissue, respectively. However, the profile of serotonin and kynurenine showed no significant diurnal variation. In *P. aibuhitensis*, serotonin and kynurenine may have some significant roles in the neural activity of the brain. In the central nervous system, serotonin and kynurenine are known to function as neuromodulators and neurotransmitters in mammals (O'Mahony et al. 2015; Ramírez et al. 2018). Furthermore, kynurenine metabolism is significantly involved in the synthesis of serotonin in mammals. Namely, the indole-2, 3-dioxygenase enzyme of the kynurenine metabolic pathway was activated with an associated reduction of serotonin synthesis (Ramírez et al. 2018). In Annelida, notably, serotonin neurons directly activated the feeding behavior of leeches *Hirudo medicinalis* (Lent 1985; Tierney 2020). In *P. aibuhitensis*, therefore, serotonin and kynurenine may act as neuromodulators and neurotransmitters, and have some roles in feeding behavior.

In gall-inducing insects such as sawflies (*Pontania* sp.), indole-3-acetic acid production

has been reported (Miyata et al. 2021). It has recently been found that sawfly larvae synthesize indole-3-acetic acid, which causes plants to make galls (Miyata et al. 2021). This indicates that insects such as sawflies produce indole-3-acetic acid in their bodies. Furthermore, indole-3-acetic acid was detected in rat and human cerebrospinal fluid (Young et al. 1980), which supports our present study. Although the existence of indole-3-acetic acid synthetic enzymes in *P. aibuhitensis* will need to be confirmed in the future, these findings and the present study supported the possibility of synthesizing indole-3-acetic acid in the head of *P. aibuhitensis*.

As the profiles of indole-3-acetic acid over 36 h are summarized in Figure 4, this study's results obviously indicate that *P. aibuhitensis* has a unique tryptophan metabolite–mediated mechanism generating the diurnal rhythm, in which the indole-3-acetic acid, not serotonin, serves an important function. In Japanese flounder (*Paralichthys olivaceus*), we have recently found that indole-3-acetic acid alleviates high-density stress (Japanese Patent 7093961, Suzuki et al. 2022). Namely, when flounder were reared under 6.25-fold density stress, plasma cortisol (stress marker) levels in flounder supplemented with indoleacetic acid ($10^{-6}$ M) were reduced compared to controls. In *P. aibuhitensi* as well as in flounder, indole-3-acetic acid is likely to alleviate the effects of density stress. Accordingly, our finding of the diurnal rhythm in *P. aibuhitensis* indicates the importance of maintaining the rhythm for the efficient farming of *P. aibuhitensis*. Indole-3-acteic acid levels may be low under high density rearing of *P. aibuhitensis*. Based on our finding, it is tempting to speculate that feeding polychaetes with a bait on seaweeds containing indole-3-acetic acid (Ertani et al. 2018) may help stabilize their diurnal rhythm and contribute to the high-density farming of this species (Fig. 4).

*Conclusion*

In this study, serotonin, kynurenine, and indole-3-acetic acid were detected in the heads of *P. aibuhitensis* by LC–MS/MS analyses. Notably, there was a diurnal rhythm in indole-3-acetic acid. It is possible that the indole pathway serves an important function in the Annelida diurnal rhythm (polychaete species). Serotonin and kynurenine may be involved in feeding behavior, and indole-3-acetic acid may modulate the rhythm in *P. aibuhitensis*. The activities associated with the physiological processes are organized in a daily manner, which increases the organism's physiological efficiency. Accordingly, identifying the diurnal rhythm of polychaete species may contribute to their efficient farming.

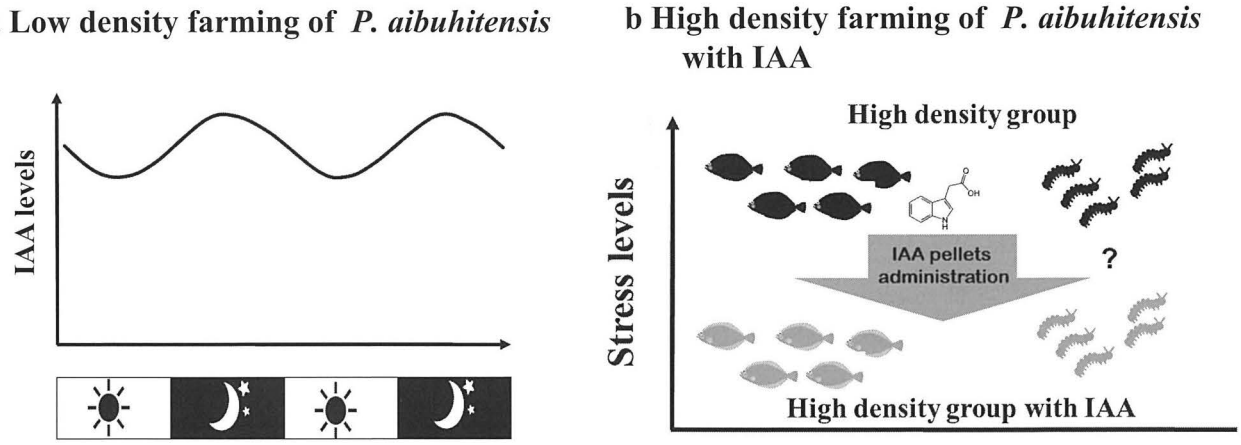



**Fig. 4.** Efficient farming considering the diurnal rhythm of polychaete species
a: Indole-3-actic acid (IAA) remains high and diurnal rhythms remain normal conditions when reared *P. aibuhitensis* at low density, as was done in the present study.
b: Stress hormone (plasma cortisol) levels increased when reared Japanese flounder (*Paralichthys olivaceus*) at high density. By addition of IAA, cortisol levels decrease in Japanese founder. Similar to Japanese flounder, stress level may increase when *P. aibuhitensis* reared at high density. We hypothesize that IAA functions to decrease stress levels and restores the diurnal rhythm to normal in *P. aibuhitensis*. The gray parts are our assumed phenomena.

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# 多毛類 *Perinereis aibuhitensis* の頭部で合成されるインドール化合物の日内リズム

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多毛類 *Perinereis aibuhitensis* は，国内では釣り餌として大きな需要がある。しかし，*P. aibuhitensis* は全て輸入品で，資源枯渇など来る輸入規制に備え，*P. aibuhitensis* の国内養殖が不可欠である。*P. aibuhitensis* の国内養殖を行うためには，その生活史を理解することが必要である。そこで *P. aibuhitensis* の日内リズム，特にそれを調整するとされるセロトニン，キヌレニンそしてインドール-3-酢酸に着目し，*P. aibuhitensis* 頭部におけるそれらの日内リズムを調べた。セロトニンとキヌレニンは，インドール-3-酢酸より多く合成されていたが，有意な日内変動がなかった。一方，セロトニンとキヌレニンに比べ合成量は少なかったが，インドール-3-酢酸合成には日内リズムがあった。これらのことは，高密度養殖においてもインドール-3-酢酸の日内リズムを維持できる最適な生活環境を人工的に維持できれば，*P. aibuhitensis* を日本国内で効率的に飼育・生産できる可能性を示している。