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Physiological Effects of Ship Noise on Yellowfin Sea Breams (*Sparus latus*)

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Abstract

Objective: This research is to explore the effects of ship noise on the physiological status of yellowfin sea breams after short-time stimulations. Methods: The noise of the Pearl River Estuary was collected by the sound acquisition system, then the yellowfin sea breams were stimulated by the sound replay system, and finally the blood samples were tested every 24 hours. Results: The concentrations of red blood cells, white blood cells, lymphocytes and the hemoglobin in the experimental group are all higher than those in the control group after noise exposure. Conclusion: A short-time noise exposure can induce the stress response and promote the immune response to the adverse environment, and at the same time, excessive immune response will increase the death rate of yellowfin sea breams.

Keywords: Ship noise; Noise exposure; Yellowfin sea bream; Stress response; Blood index.

1. Introduction

The Pearl River Estuary has gradually become one of the busiest shipping waters in China. The average shipping volume in the Pearl River Estuary has been more than 4000 vessels daily, and the noise generated by large vessels can reach more than 170dB. At the same time, the Pearl River Estuary is also one of the main living water areas of yellowfin sea breams (*Sparus latus*), a euryhaline, omnivorous, benthal-inhibited species, and warm-water species of fish [1]. The living environment of yellowfin sea breams has been severely affected by continuous ship noise.

The harm of noise to the animal body can be divided into two types: the hearing system (specific) and the non-hearing system (non-specific) [2]. The latter mainly includes the effects on the nervous system, the cardiovascular system, the respiratory system, the digestion system, the endocrine system, the immune function, the metabolic function, and the reproductive function and behaviors, etc. The severity of the harm of noise to the animal body is related to noise intensity, exposure duration and exposure mode. High-intensity low-frequency noise will affect the Caspase-3-mediated apoptosis process of the inner ear cells, resulting in the significant hearing impairment in rats [3]. A large number of experiments show that noise can disorder the endocrine system and affect the immune functions by altering the secretion of neurotransmitters and hormones. Wu, *et al.* [4] find that the conditioned reflex activity of the mice will become difficult under strong noise, indicating that noise can cause damage to the brain and nerve functions of mice. According to Li, *et al.* [5], strong noise can damage the follicle cells and hence affect the reproduction of mice. It can be seen from the above studies that noise has various effects on the physiological functions of animals.

Studies of the effects of noise on fish [6-13] show that ship noise may hinder the development of fish larvae, reduce the growth rate, weaken the ability of marine mammals to accumulate waste gases, and even plug blood vessels to cause tissue disruption. The study of fish in the nature protection zones in Italy conducted by Codarin, *et al.* [6] shows that ship noise can seriously affect the hearing of local fish and interfere in the communication of fish, and the number of fish long exposed to ship noise is decreasing gradually. They are more likely to be frightened and more difficult to feed on Voellmy, *et al.* [14]. The frequency of feeding also decreases with the increase of ship noise [8]. Celi, *et al.* [13] find that the ship noise stimulation will lead to obvious increase of tens of blood indexes, such as corticotropin and cortisol, of the experimental fish.

With the ship noise pollution becoming increasingly serious in the Pearl River Estuary, the growth of yellowfin sea breams, a major economic fish in the Pearl River Estuary, will surely be affected more and more severely. Therefore, in this research, we will expose the yellowfin sea breams to different types of simulated ship noises of different time durations and then conduct blood tests to explore the physiological effects of ship noise on yellowfin sea breams, to provide reference data for the assessment of the harm of ship noise to yellowfin sea breams, and to provide suggestions for the improvement of the yellowfin sea bream breeding. This study will be of practical significance for the protection of yellowfin sea breams in the Pearl River Estuary.

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2. Methodology

2.1. Reagents and Equipment

The reagents used in this research include heparin sodium, eugenol and sea crystal salt. The equipment includes the sound acquisition system and the sound replay system. The former consists of a hydrophone (See Figure 1a), a band-pass filter amplifier (See Figure 1b), a A/D conversion acquisition device (See Figures 1c and 1d) and a control storage device.

Figure-1. Main components of the sound acquisition system

A single hydrophone (TC 4013, Reson, Slangerup, Denmark) is applied in this experiment. This hydrophone has very wide frequency response range, being 1Hz-170KHz (±3 dB), the sensitivity being -211±3dBre:1V/μPa^{-1}. The hydrophone is connected to the EC6081 pre-positive filter amplifier (VP2000; Reson, Slangerup, Denmark), which has a 6-gear 1MHz bandwidth, with 0-50 dB each. The A/D conversion acquisition device is composed of a chassis (NI cDAQ-9178 TX, USA) and a A/D conversion acquisition card (NI c9223，±10V, TX, USA). The control storage device is a portable computer (Thinkpad-E40). The signal received from the hydrophone is filtered and amplified by the filter amplifier, and then the analog signal is converted into digital signal by the A/D conversion acquisition device and stored in the computer hard disk.

The sound replay system is consists of an underwater microphone (LL916, LubellLabs), a power amplifier (Pvi4B) and an isolation voltage regulator (ACE203). The working frequency response bandwidth of the underwater microphone is 200Hz–23kHz(500-21000Hz+/-10dB) and the maximum output sound pressure is 180dB/uPa/m@1kHz.

2.2. Experimental Fish

36 strong-build 1.5-year-old yellowfin sea breams are used in this experiment (See Figure 2). They are temporarily kept in the experimental tank for four days. The specifications of the yellowfin sea breams are shown in Table 1.

Figure-2. Experimental yellowfin sea bream

Table-1. The physical signs of the experimental yellowfin sea breams

Physical sign	length (cm) M±SD	weight (g) M±SD
Indicator	21.50±2.70	190.83±38.18

2.3. Methods

2.3.1. Temporary Breeding of Yellowfin Sea Breams

The selected healthy yellowfin sea breams are transferred to the experimental tank. Yellowfin sea breams are a kind of aggressive fish. To prevent body surface damage or death from struggling during the transfer process, they are anaesthetized with eugenol before transferred to the tank.

The size of the water tank is 1.5m x 1.2m x 1.0m (See Figure 3), in which about half tank of water (0.9 m^3) is filled. Some sea crystal salt is added into the water to simulate the seawater environment, so that the water density is maintained at 1.018-1.028g/cm^3. The temperature of the water was kept at that of natural water (26-31℃). The air was pumped into the water by an oxygen pump. The yellowfin sea breams were not fed during the temporary breeding period. A half of the water was changed each day to avoid disturbance.

Figure-3. Experimental tank

2.3.2. Noise Stimulation Experiment

The ship noise was collected from the 4000 ton bulk carriers in the Pearl River Estuary. The water depth was 9.8m when the noise was collected. The parameters of the instrument are as follows: The high-pass and the low-pass of the filter amplifier were set to 10Hz and 250kHz respectively, and the gain was set to 30dB. The whole acquisition process was controlled by the program designed with the Labview 2012 (National Instruments, Austin, TX, USA). The sampling rate is 500kHz.

Before the experiment, the selected yellowfin sea breams were randomly divided into two groups, 16 of which were in the experimental group and the other 20 in the control group (still other 4 in reserve). The two groups of yellowfin sea breams were placed in two experimental tanks of the same size. In the experiment, the ship noise was played to the experimental group using the sound replay system. At the same time, the ambient noises of both the experimental group and the control group were recorded and analyzed using the sound acquisition system.

The ambient noise wave and the power spectral density of the experimental group and the control group of yellowfin sea breams are shown in Figures 4 and 5.

Figure-4. Underwater noise waveform diagram

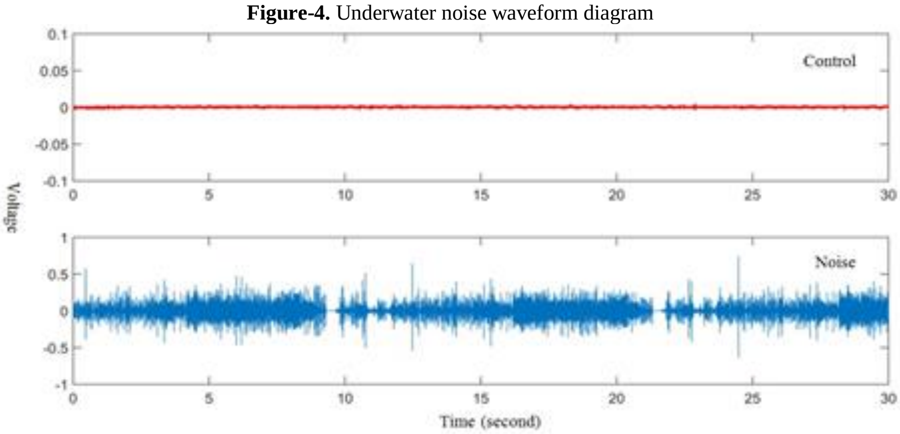
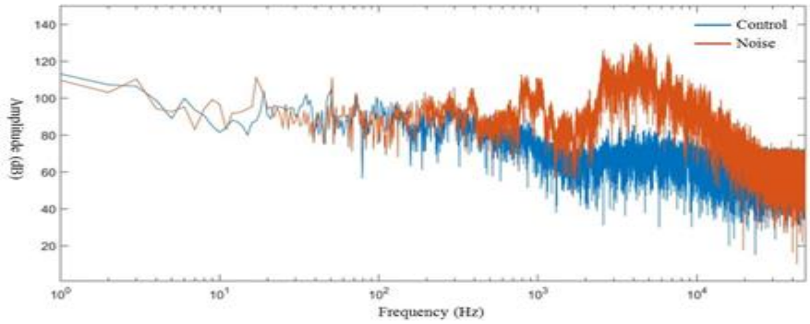


Figure-5. Underwater noise spectrum diagram



The ambient noise mean-square root pressure of the experimental group and that of the control group are 80dB and 150dB respectively. The maximum power of the control group is 130dB within the 100-21000Hz bandwidth range, and the corresponding frequency is 5075Hz. The maximum power of the experimental group is 97dB within the 100-21000Hz bandwidth range, and the corresponding frequency is 100Hz.

2.3.3. Blood Sample Collection and Test

The 1% heparin sodium anticoagulants was prepared before blood samples were collected, with 0.5g heparin sodium, 0.45g NaCl and 50ml distilled water. Then, 0.1ml of the mixture was added to the 1.5ml centrifuge tube to reserve.

After noise exposure, the blood samples were collected at intervals of 24 hours from three yellowfin sea breams of the experimental group and three others of the control group. The blood samples were taken from the tail vein of the fish. The needle was injected into the tail vein at at the place of 2 to 3 scales of the lateral line of the yellowfin sea bream hip position with the 1ml syringe. The 1-2cm^3 blood was collected and transferred to the centrifuge tube. Note that the mucus and tissue fluid of the fish skin should be avoided during blood collection. The collected blood samples were stored in the -70℃ cryogenic freezer. The weight (g) and the length (cm) of each fish were at the same time recorded.

The collected blood samples were immediately sent to Zhuhai maternal and child health care hospital for routine blood test on the day of blood collection. The test indexes include red blood cell (RBC), hemoglobin concentration (HGB), white blood cell (WBC) and lymphocyte absolute value (LY#).

3. Results

3.1. Yellowfin Sea Bream Blood Index Levels in the Stress Period of Environment Change

After transferred to the experimental water tank, the yellowfin sea breams experienced the stress and adaptation period of the environmental change. In this period, the yellowfin sea bream blood samples were extracted for the hemoglobin concentration (HGB) test, the lymphocyte absolute value (LY#) test, the red blood cell (RBC) test and the white blood cell (WBC) test. The results are shown in Figure 6

Figure-6. Blood index change in environmental adaptation period: A: Hemoglobin concentration change; B: Lymphocyte absolute value change; C: Red blood cell count change; D: White blood cell count change

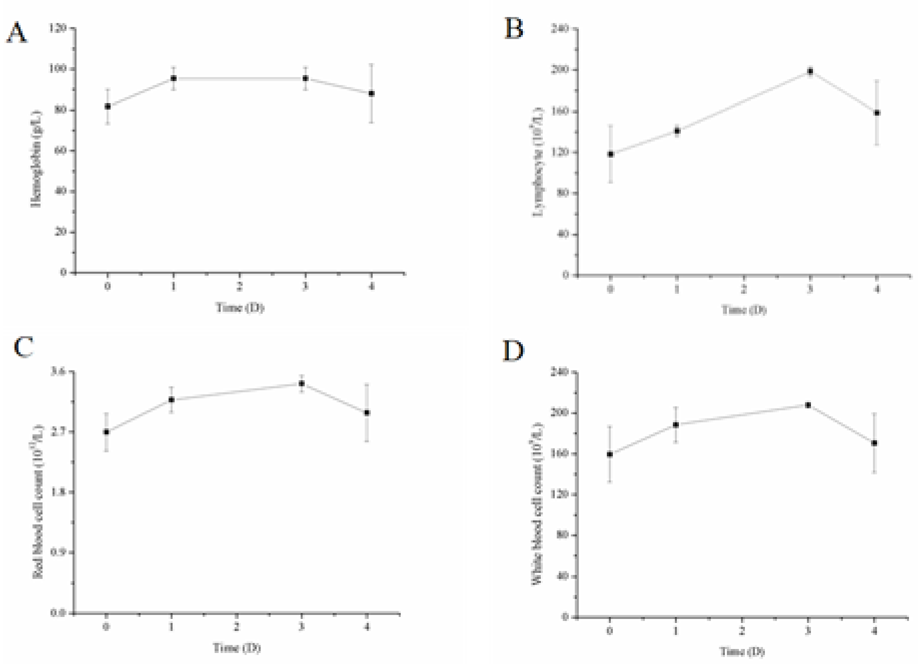


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Figure 6 shows that all the blood indexes of the yellowfin sea breams experienced a trend of rise-and-fall change at different degrees in this period. All reached their peak in the third day after the environmental change and ended slightly higher than the original level.

3.2. Survival Rates of Yellowfin Sea Breams after Noise Stimulation

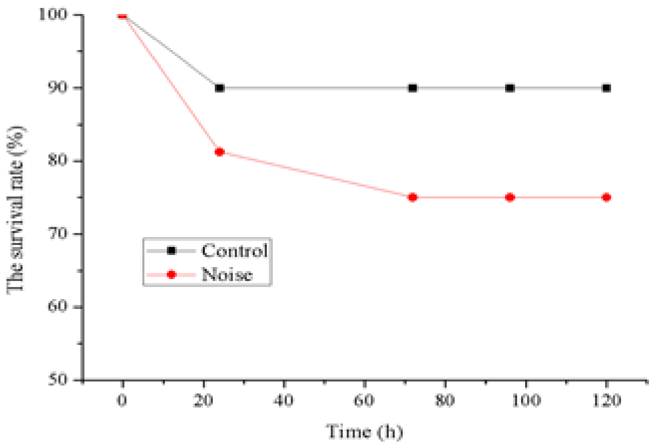
The numbers of the yellowfin sea breams survived the noise stimulation are shown in Table 2.

Table-2. Noise stimulation survivals

	0h	24h	72h	96h	120h
Experimental group	16	13	12	12	12
Control group	20	18	18	18	18

During the five days' exposure to ship noise, the number of survivals in the experimental group and that in the control group were significantly different. Both the survival rates began to decrease from the first day of noise exposure. However, the survival rate of the experimental group became stable after 72 hours' noise stipulation, while the control group came to be stable after only 24 hours. Comparatively, with the increase of the time span of noise exposure, the survival rate of the experimental group decreased faster than that of the control group. The survival rate of the experimental group was 75% and that of the control group was 90% after 120 hours' noise stipulation. See Figure 7.

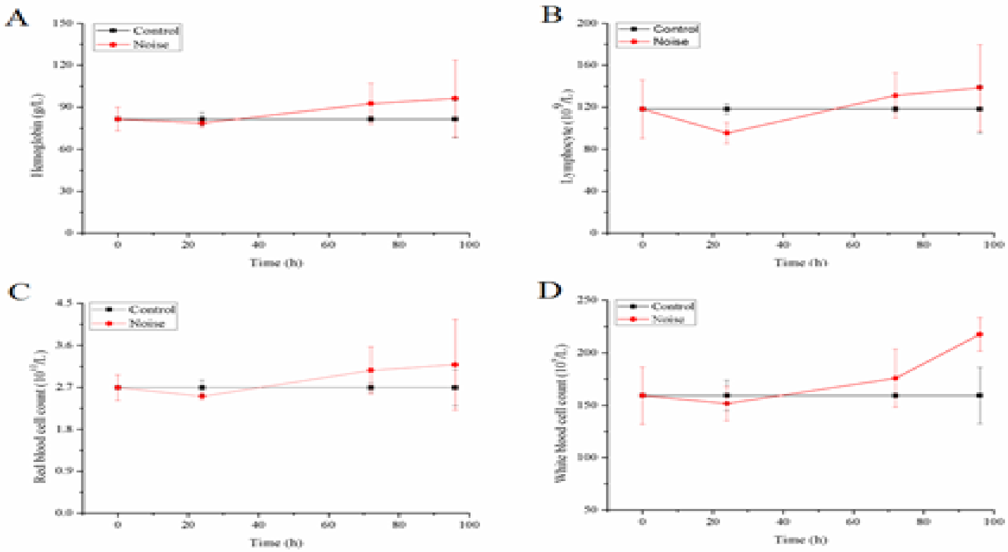
Figure-7. Survival rate of yellowfin sea breams after noise stimulation



3.3. Yellowfin Sea Bream Blood Index Change after Noise Stimulation

Within the 0h-96h exposure to ship noise, the levels of concentration of the hemoglobin, the lymphocyte absolute value, the red blood cells and the white blood cells in the control group did not change significantly, while those in the experimental group changed over time. Specifically, during the first 24 hours, all the four blood indexes decreased to some extent in the control group. After 24 hours, the index levels increased gradually, and began to run over those of the control group after about 40 hours' exposure to noise. The four indexes in the experimental group were significantly higher than those in the control group at the end of the experiment. See Figure 8.

Figure-8. Changes in yellowfin sea bream blood indexes after noise stimulation: A: Change of hemoglobin concentration; B: Change of lymphocyte; C: Change of red blood cell counting; D: Change of white blood cell counting D: Change of white blood cell counting



4. Discussion

4.1. Environmental Change and Stress Response

Stress is an individual non-specific response induced by stressors, including behavior response and physiological response. Both are regulated by the central nervous system and the endocrine system. As to fish, the hypothalamus pituitary kidney medulla is the most important regulatory region of stress response [15]. When the fish body is stimulated from the outside world, its metabolism, growth and immunity will be affected, which can be manifested by the change of blood components.

In this study, the period of time following the transfer of the yellowfin sea breams from the aquaculture pool to the experimental water tank is the stress period after environmental change. The changes in a variety of environmental conditions (such as water quality, water salinity, water temperature and dissolved oxygen) are external stimuli and stress sources as well for the yellowfin sea breams. These stimuli induced the stress response of the yellowfin sea breams. Therefore, we can detect the increase of the white blood cells and the lymphocyte absolute value during the stress period of environmental change. The reason may be related to the increase of catecholamine secretion in the stress. Catecholamine is one of the most popularly used stress hormones in present studies. It can increase the number of white blood cells by promoting white blood cells in the marginal pool to enter into the circulatory pool [16]. This is the reason for the change of the peripheral blood lymphocyte group of many animals after stress. The functions of the hemoglobin concentration and the red blood cells are to carry and transport oxygen. The increase of these two indexes may have been resulted from the accelerated metabolism rate, the increase of oxygen consumption and the increase of oxygen demand of the yellowfin sea breams under stress.

After three days, the indexes of yellowfin sea breams began to recover gradually and then to the normal level on the fourth day. This is because the biological adaptability changed the characteristics of the living body with the change of the external environment of the living body [17]. This is closely related to the stress. After the stress response occurs, the living body will regulate to maintain a steady state, such as negative feedback regulation, and eventually achieve a new dynamic balance.

4.2. Effects of Ship Noise on the Survival Rate of Yellowfin Sea Breams

Research on animals [18, 19] already shows that stress hormones can alter the functions of the immune system. Acute stress can increase the total number of white blood cells and natural killer cells in the blood [20], and enhance the immune response in a short time. It can be speculated that the immune response will be intensified under the condition that the environmental change causes stress of the living body. In addition, the immune function is easily disturbed by the noise stimulation.

In this experiment, the survival rate of yellowfin sea breams in the experimental group is significantly lower than that in the control group. The reason may be that the stress response promoted the enhancement of the immune function of yellowfin sea breams. At the same time, it was stimulated by noise, which increased the immune response. Excessive immune response destroyed the body’s own cells and tissues, resulting in the death of yellowfin sea breams.

4.3. The Reason for the Noise Effects on the Blood Indexes of Yellowfin Sea Breams

High level noise is of intensive stimulation, which will cause strong stress response in the fish. Under such intensive stimulation, the blood indexes will change more significantly. The stress response of carps can stimulate the secretory function of the head-kidney and promote a large number of new red blood cells into the blood circulation in a short time [21]. It is speculated that the increases of concentration of the red blood cells and the hemoglobin of yellowfin sea breams are also related to the regulation of the head-kidney. The stimulation of noise can also promote the secretion of epinephrine and cortisol in carps [22]. The increase of the cortisol level will inhibit the transfer of white blood cells from blood to tissue, prevent cells from programmed death, and hence prolong the life of white blood cells [23]. Therefore, the increase of the red blood cells and the lymphocyte absolute value of yellowfin sea breams is also associated with the increased level of epinephrine and cortisol secretion. In addition, the main function of white blood cells is to protect the living body and to resist the invasion of disease. The main function of lymphocyte absolute value is to produce antibodies, which play an important role in specific immunity. The increase of these two levels in this experiment suggests an enhanced immune response of yellowfin sea breams.

Noise may enter into the living body as an antigenic substance to stimulate a certain protein to form a kind of antigen which is known as the noise antigen out of the conformation change. This antigen can combine with the receptors of the body’s immune cells, triggering the immune response. Therefore, under a certain intensity and a short time of noise stimulation, the body stress system of yellowfin sea breams can respond rapidly to produce a large number of lymphocytes to resist the adverse environment [24]. It is presumed that in this process the living body has an immune response similar to that of inflammation. Zheng and Ariizumi [19] find that the three days of acute noise exposure could enhance the humoral and cellular immune responses in BALB/c mice, and that four weeks of chronic noise stress could inhibit the humoral immune function and the cellular immune function. According to Van Raaij, *et al.* [18], the 85dB noise stimulation of rats will increase the activity of the non-specific immunity related to spleen NK cells in 7 days, but this activity begins to decrease after 3 weeks of noise exposure.

The above mentioned studies indicate that noise can promote the specific and non-specific immune responses of the animal body within a short time (3-7 days), but the immune functions of the body will be inhibited after a relatively longer time (3-4 weeks). Therefore, we can propose that the enhancement of the immune response of yellowfin sea breams occurring in this experiment is a regulatory response to acute noise stimulation, but a longer noise stimulation will inhibit or damage the immune function, resulting in the decrease of the indexes of white blood cells and the lymphocyte absolute value.

5. Conclusion

This research concludes that environmental changes and noise stimulation can lead to the stress of the body of yellowfin sea breams, promote its immune response to cope with the adverse environment. With the increase of noise intensity, the stress response and the effect on the immune function may also increase. It is also found that excessive immune response or the disorder of immune response will damage the structure and function of yellowfin sea breams themselves and even cause death of them. In addition, longer chronic noise stimulations will inhibit the immune function of yellowfin sea breams.

Relavant research shows that noise has a cumulative effect on the physiological response in rainbow trout [25] and in cannabis harnishes [26]. Shi, *et al.* [22] also find that repetitive noise stimulations have cumulative effects in large yellow croakers and weavers. It hence can be speculated that long-term and repeated noise stresses have a more serious effect on the body functions of yellowfin sea breams.

Both red blood cells and white blood cells of yellowfin sea breams change with noise stimulation. They both show an upward trend after 96 hours of stress, indicating that the yellowfin sea breams are sensitive to noise stimulation. In addition, the changes in red blood cells and white blood cells can also be taken as an indicator of the stress response and immune response of yellowfin sea breams, which can be used to determine the health of yellowfin sea breams. As an important safeguard for the body, the yellowfin sea bream immune system plays an important role in fighting against external adverse factors. The stress response caused by environmental change and noise stimulation will affect the structure and function of the immune system of yellowfin sea breams, threaten their health and growth, and cause economic loss to the aquaculture industry. What concerns the aquaculture industry is how to reduce ship noise exposure and create a suitable environment for yellowfin sea breams.

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