water

MDPI

Article

Mass Mortality of Asari Clams (*Ruditapes philippinarum*) Triggered by Wind-Induced Upwelling of Hypoxic Water Masses

Ryota Sone [1,*], Mitsuyasu Waku [1], Satoshi Yamada [1,†], Dai Miyawaki [1], Toshiro Ishida [1], Satoru Kamohara [1,‡], Tetsunori Inoue [2,3,4] and Teruaki Suzuki [5]

1 Aichi Fisheries Research Institute, 97 Wakamiya, Miya, Gamagori 443-0021, Aichi, Japan
2 Marine Pollution Management Group, Port and Airport Research Institute, 3-1-1 Nagase, Yokosuka 239-0826, Kanagawa, Japan
3 Estuary Research Center, Shimane University, 1060 Nishikawatsu-cho, Matsue-shi 690-8504, Shimane, Japan
4 Department of Transdisciplinary Science and Engineering, Tokyo Institute of Technology, 4259 Nagatsuta-cho, Midori-ku, Yokohama 226-8503, Kanagawa, Japan
5 Graduate School of Environmental and Human Science, Meijo University, 1-501 Shiogamaguchi, Tempaku, Nagoya 468-8502, Aichi, Japan
* Correspondence: riyouta_sone@pref.aichi.lg.jp; Tel.: +81-533-68-5196
† Current address: Japan Fisheries Science and Technology Association Tokai/Hokuriku Branch, 3-4-10 Marunouchi, Naka-ku, Nagoya 460-0002, Aichi, Japan.
‡ Current address: IDEA Consultants, Inc., 1-7-15 Irifune, Minato-ku, Nagoya 455-0032, Aichi, Japan.

Abstract: To investigate the mass mortality of the macrobenthos community, primarily asari clams, triggered by upwelling-driven hypoxia, we conducted continuous observations of temperature, salinity, and DO, and monthly macrobenthos monitoring on the Rokujo tidal flat in Mikawa Bay, central Japan, from 2014 to 2016. Additionally, laboratory experiments were conducted using sediments on a tidal flat containing macrobenthos to examine the possibility of hydrogen sulfide formation in tidal flats. The bottom layer at the offshore station was intermittently hypoxic, and the station of the tidal flat was occasionally hypoxic in August and September for three years. Hypoxia was mostly observed on the tidal flat when constant easterly winds were recorded offshore. The biomass of asari clams decreased considerably from September to October 2016 when hypoxia was intermittent. Hypoxia persisted for approximately one week from 20 September, which was associated with the calm weather and stagnation of tidal currents owing to the neap tide. Conversely, the hydrogen sulfide concentration in the water directly above the sediment exceeded 30 mg L^{-1} after 3 days of incubation in the laboratory experiment. Therefore, the possibility of oxygen consumption on tidal flats due to hydrogen sulfide formed by biological die-offs was considered in the long-term persistence of hypoxia.

Keywords: asari clams; upwelling of hypoxic water masses; mass mortality; hydrogen sulfide; biogenic organic load

check for updates

Citation: Sone, R.; Waku, M.; Yamada, S.; Miyawaki, D.; Ishida, T.; Kamohara, S.; Inoue, T.; Suzuki, T. Mass Mortality of Asari Clams (*Ruditapes philippinarum*) Triggered by Wind-Induced Upwelling of Hypoxic Water Masses. *Water* **2023**, *15*, 3997. https://doi.org/10.3390/w15223997

Academic Editor: Achim A. Beylich

Received: 11 October 2023
Revised: 11 November 2023
Accepted: 15 November 2023
Published: 17 November 2023

1. Introduction

The upwelling of hypoxic water masses that reach shallow water areas (called 'Nigashio' in Mikawa Bay and 'Aoshio' in Tokyo Bay, Japan) severely damages fisheries and significantly influences the nutrient cycle in the inner bay by the collapse of the tidal flat ecosystem (e.g., [1,2]). The Rokujo tidal flat, located in the inner part of Mikawa Bay, central Japan (Figure 1), is a sea area with large numbers of asari clams, *Ruditapes philippinarum* (Adams and Reeve, 1850) [3]. Fishermen transplant juvenile clams from this area to fishing grounds in order to encourage population re-stocking. This transplantation contributes to the fishery in the bay. In addition, high-density juvenile asari clams can efficiently remove suspended matter by filter feeding and play important roles in the nutrient cycle in the bay, including maintaining water quality [4]. During summer, hypoxic water masses with a dissolved oxygen concentration (DO) below 2 mg L^{-1} [5] accumulate in offshore areas [1,6]. The upwelling of these masses has caused mass mortality of thousands of tons of asari clams (e.g., [6,7]), which is extremely concerning for the fishery and nutrient cycle. The mass mortality of asari clams is thought to be a direct result of exposure to hypoxic water [8,9]. However, there have not been many observations of hypoxic upwelling and its effects on tidal flat ecosystems. In addition, because asari clams are highly tolerant of hypoxia or anoxia [10,11], the upwelling of a hypoxic water mass does not necessarily result in the mass mortality of clams. Therefore, meteorological, oceanographical, or biological conditions influencing the mass mortality of asari clams triggered by wind-induced upwelling of hypoxic water masses are not clear. Kajiyama [12] shows cases of mass mortality of asari clams triggered by wind-induced upwelling of hypoxic water masses in Tokyo Bay. In these cases, hypoxia was observed to persist for more than 3 days. There is a need to verify that long-term hypoxia causes mass mortality of asari clams and to examine the factors that contribute to long-term hypoxia. On the other hand, hydrogen sulfide is produced using organic matter under anoxic conditions through sulfate reduction by sulfate-reducing bacteria, which are ubiquitously present in lakes, river sediments, and estuaries [13–15]. Hydrogen sulfide is known to consume a large amount of oxygen in the water [16–18] and is highly toxic to aquatic organisms [19–21]. Biogenic organic loads associated with biological die-off might produce reducing substances, such as hydrogen sulfide, prolong hypoxia on tidal flats, and accelerate biological mortality.

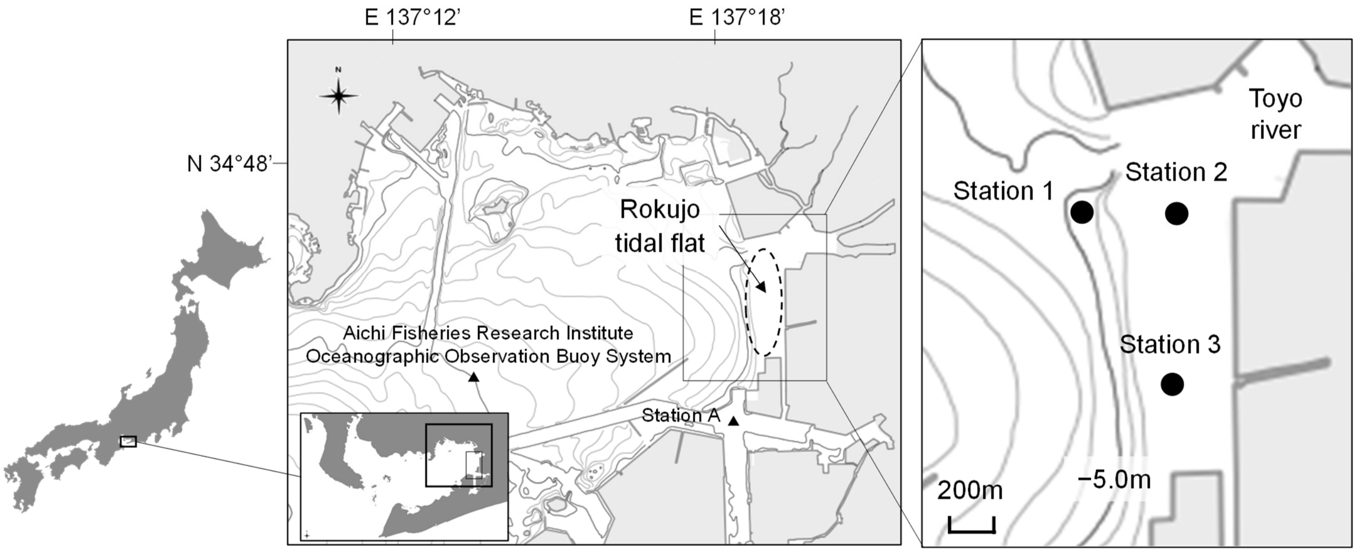

Figure 1. Location of biological and oceanographic observation stations in Mikawa Bay, central Japan. Periodic and continuous observations of water quality were conducted at Station 1. Continuous observations of water quality and macrobenthos monitoring were conducted at Stations 2 and 3. Station A shows a facility for the navigation channel.

To elucidate the effects of the upwelling of hypoxic water masses on the tidal flat ecosystem, mainly the asari clam population, we conducted biological observations in the Rokujo tidal flat and water quality observations in the surrounding sea area, including the tidal flat in three years. Additionally, we conducted a simple laboratory experiment using sediments from a tidal flat containing benthic organisms to examine the possibility of hydrogen sulfide formation in tidal flats.

2. Materials and Methods

2.1. Study Area

The Rokujo tidal flat is a north–south extended estuarine tidal flat on the left bank of the Toyo River mouth, covering an area of 3.06 km^2 ([22]; Figure 1). The tidal flat terrace extends approximately 1.0 km offshore westward, and the tidal flat edge is −0.7 m below the chart datum level (CDL), with the offshore side dropping to a depth of approximately 6–7 m. The surrounding water area, including the Rokujo tidal flat, is located at the port of Mikawa. The Rokujo tidal flat is a conservation area with high social value for fisheries and the environment; however, natural beaches, such as tidal flats, are scarce owing to coastal development [23].

2.2. Oceanographic Observation and Macrobenthos Monitoring

Periodic and continuous observations of water quality were conducted to measure the development of hypoxic water masses and upwelling onto the tidal flat. To monitor water quality outside the tidal flat, water temperature, salinity, and DO in the water column were measured once a month from June to September 2014, from June to October 2015, and from June to October 2016 at Station 1 (34°46.20′ N, 137°18.41′ E, CDL: −4.6 m), a station offshore the tidal flat, using a water quality sensor (AAQ1182, JFE Advantech Co., Ltd., Hyogo, Japan). When DO was below 0.2 mg L^{-1} in 2016, water was collected using a Kitahara water sampler (Rigosha Co., Ltd., Tokyo, Japan), treated with zinc acetate, and taken to the laboratory to determine hydrogen sulfide concentration using the methylene blue method [24]. In addition, water temperature and salinity (Infinity-CTW, JFE Advantech Co., Ltd., Hyogo, Japan), and DO (RINKO-W, JFE Advantech Co., Ltd., Hyogo, Japan) were observed 0.3 m above the sea floor at 10 min intervals from June to October for 3 years offshore the tidal flat (Station 1 in 2014–2016) and on the tidal flat (Station 2 (34°46.21′ N, 137°18.86′ E, CDL: −0.4 m) in 2014 and 2016 and Station 3 (34°45.37′ N, 137°18.88′ E, CDL: −0.6 m) in 2015). In addition, the current velocity (Infinity-EM, JFE Advantech Co., Ltd., Hyogo, Japan) was observed 0.3 m above the sea floor at 10 min intervals from June to October 2016 on the tidal flat (Station 2). The wind direction and velocity during the observation period were obtained from the oceanographic observation buoy system operated by the Aichi Fisheries Research Institute [25], and the actual tidal level was obtained from the Mikawa Port Office as a factor affecting the flow environment in the study area.

To elucidate the effects of the upwelling of hypoxic water masses on the tidal flat ecosystem, macrobenthos communities were observed at two stations on the tidal flat (Stations 2 and 3). Macrobenthos in the bottom sediments were collected monthly within a quadrat (25 × 25 × 15 cm, n = 1) in July and September 2014, from June to October 2015, and from June to October 2016 at each station. Thus, there were 2 samples collected at 2 stations in 2014 (n = 4), 5 samples collected at 2 stations in 2015 (n = 10), and 5 samples collected at 2 stations in 2016 (n = 10). Individuals collected from sieved samples through a 1 mm mesh were counted and identified to the lowest possible taxon (usually to species levels), and their wet weight was recorded to the nearest 0.1 g. They were aggregated at the class level.

2.3. Tidal Flat Sediment Incubation Experiment

Tidal flats with numerous organisms, such as the Rokujo tidal flats, may exhibit high concentrations of hydrogen sulfide with a chain of biological die-offs triggered by the upwelling of hypoxic water masses. To investigate this possibility, we conducted a static incubation experiment in tidal flat sediments containing macrobenthos. Intact sediment cores of approximately 20 cm thickness were collected on 18 August 2016 at Station 2 using acrylic tubes (4.4 cm inner diameter, 25 cm length). Five sediment cores were sampled from the same site and sealed at the bottom end with a rubber stopper. Upon return to the laboratory, sediment cores were carefully filled with filtered seawater and sealed at the top end with a rubber stopper. The cores were incubated statically at 28 °C, which is the water temperature in tidal flats during summer, in the shaded constant-temperature room. The cores were provided for measurements after 0, 3, 4, 6, and 20 days of incubation, one at a time. The DO of the water directly above the sediment was measured using an optical DO meter (ProODO, YSI Inc., Yellow Springs, OH, USA), and the hydrogen sulfide concentration was determined using the methylene blue method [24].

3. Results

3.1. Oceanographic Observation

3.1.1. Monthly Observation of Water Quality Outside the Tidal Flat

Figures 2–4 show the vertical profiles of water temperature, salinity, and DO at Station 1, a station offshore the tidal flat, in 2014–2016, respectively. The water temperature of the surface and bottom layer were 23.8 °C and 22.4 °C in June 2014, 23.2 °C and 19.8 °C in June 2015, and 24.0 °C and 23.0 °C in June 2016, respectively. The salinity of the surface and bottom layer were 30.1 and 31.4 in June 2014, 28.1 and 32.1 in June 2015, and 29.4 and 30.8 in June 2016, respectively. Thus, in June 2014–2016, water temperature and salinity were vertically homogeneous, and no obvious stratification was observed. After July 2014–2016, stratification tended toward high water temperature and low salinity in the upper layer and low water temperature and high salinity in the lower layer. The water temperature of the surface and bottom layer were 27.5 °C and 24.0 °C in August 2014, 32.5 °C and 22.6 °C in August 2015, and 26.9 °C and 26.5 °C in September 2016, respectively. The salinity of the surface and bottom layer were 20.2 and 32.2 in August 2014, 24.3 and 31.2 in August 2015, and 25.9 and 31.6 in September 2016, respectively. In those times, the bottom layer DO decreased to nearly 0 mg L^{-1}. In September 2016, the bottom layer DO was 0.12 mg L^{-1} and no hydrogen sulfides were detected. In October 2015 and 2016, the oxygen environment recovered.

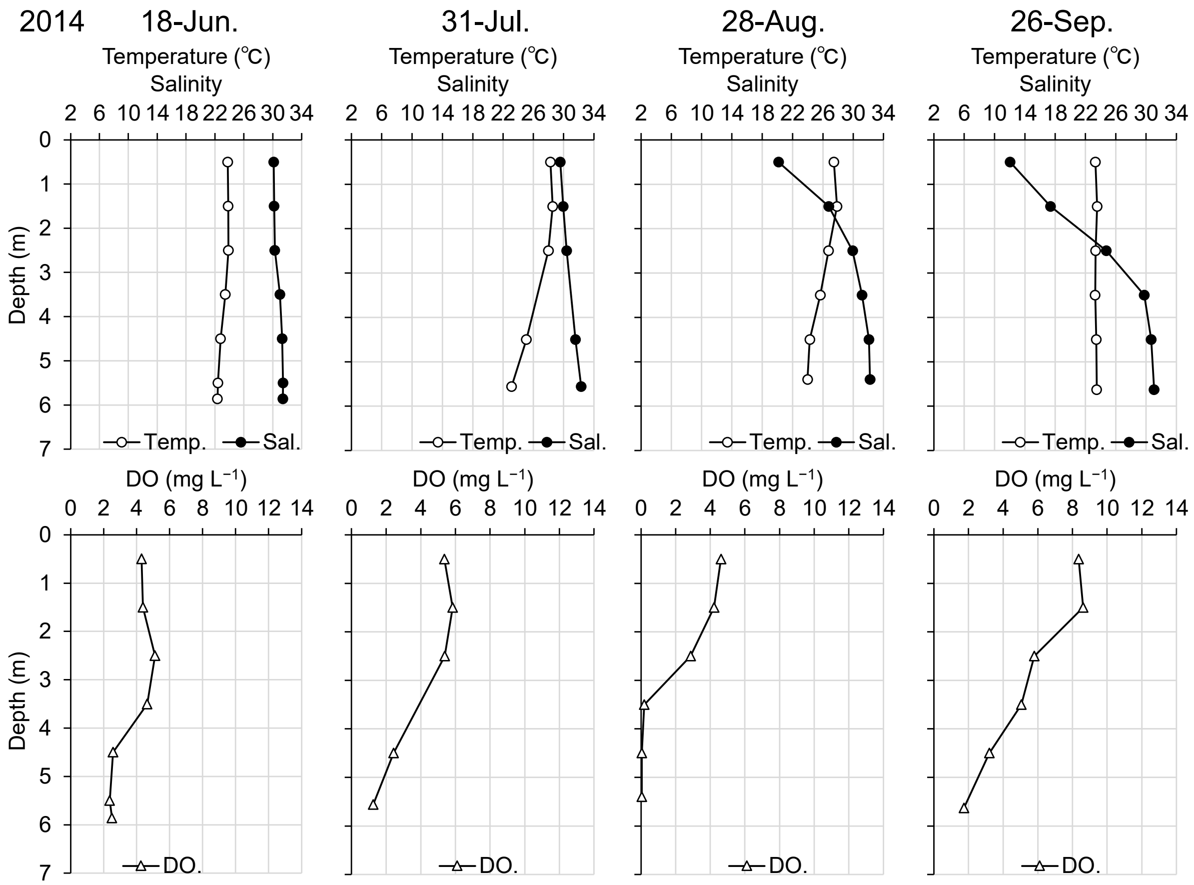

Figure 2. Monthly changes in the vertical profile of temperature, salinity, and dissolved oxygen (DO) for the water column at Station 1 from June to September 2014.

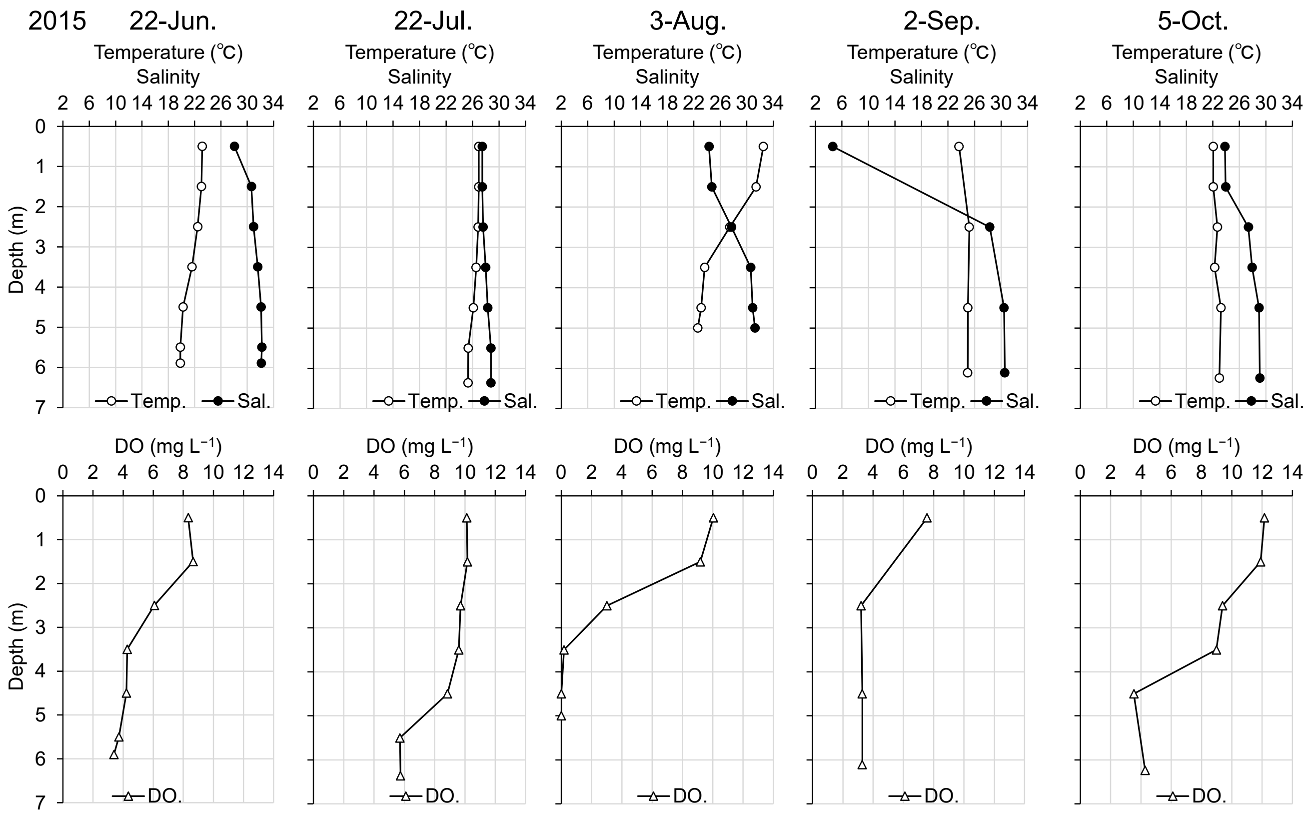

Figure 3. Monthly changes of vertical profile in the temperature, salinity, and dissolved oxygen (DO) for the water column at Station 1 from June to October 2015.

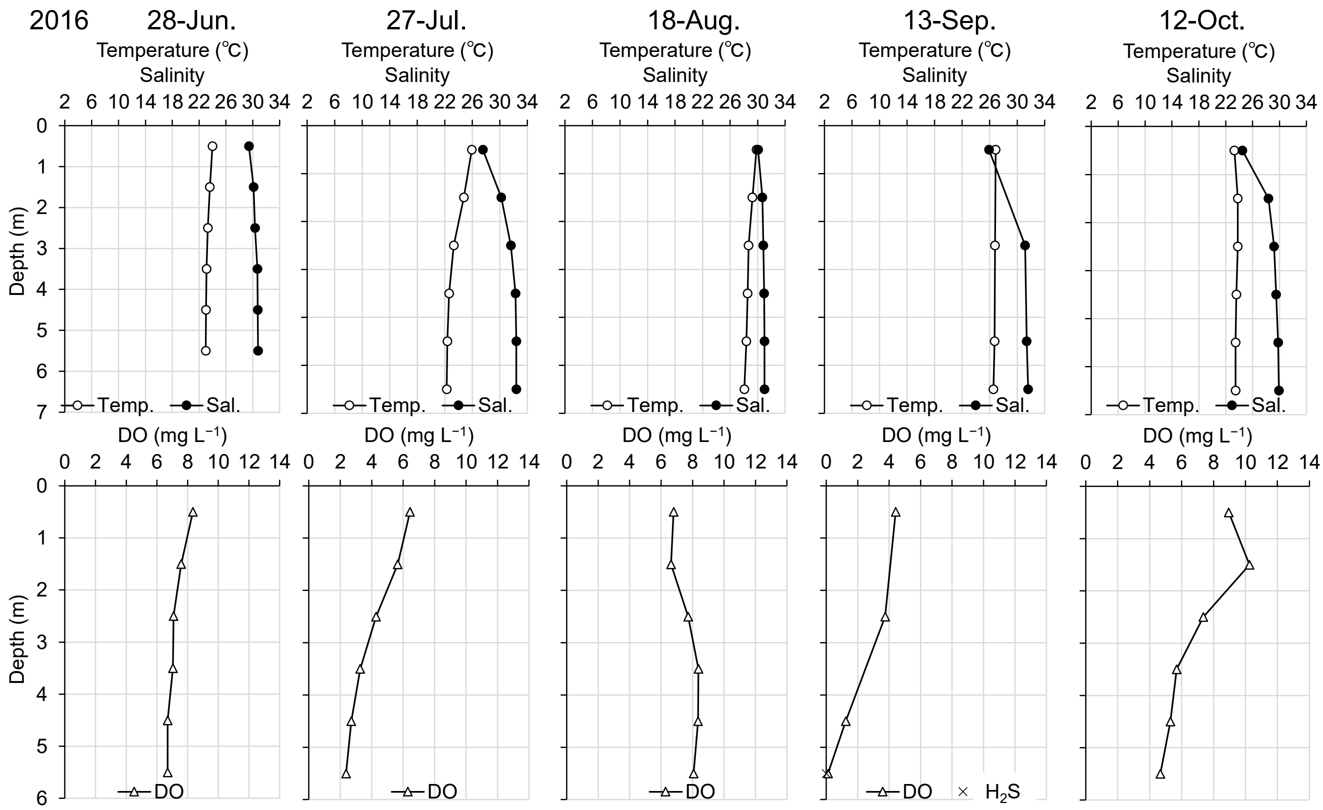

Figure 4. Monthly changes in the vertical profile of temperature, salinity, dissolved oxygen (DO), and hydrogen sulfide (H_2S) for the water column at Station 1 from June to October 2016.

3.1.2. Continuous Observation of Water Quality Outside and on the Tidal Flat

Figures 5–7 show the results of continuous observations of the DO on the tidal flat (Station 2 or 3) and offshore (Station 1), with temperature and salinity on the tidal flat (Station 2 or 3), the wind direction and velocity at the oceanographic observation buoy system and the measured tide level in the Port of Mikawa in August and September 2014–2016, respectively. DO frequently dropped to approximately 0 mg L^{-1} at Station 1 in 2014–2016. On the tidal flat (Station 2 or 3), DO tended to be higher than at Station 1; however, hypoxia, DO below approximately 2 mg L^{-1}, was observed approximately 17 times in 2014–2016 (Arrows in Figures 5–7). Easterly winds of approximately 5 m s^{-1} or more were blowing in the events of hypoxia on the tidal flat. They were observed 13 times during the observation period (e.g., 1 September, 5 September, and 24 September in 2014, 5 August, 6 August, 17 August, 25 August, 16 September, 24 September, and 27 September in 2015, 15 August, 13 September, and 20 September in 2016). And in those cases, low water temperature below 25 °C, high salinity over 30, or both were observed on the tidal flat.

No hypoxic period lasted more than 3 days in 2014 and 2015. In 2016, instances of severe DO reduction were observed at Station 2 on 9, 13, and 20 September. In particular, the hypoxic period from September 20 lasted approximately one week. Figure 8 shows the east-west and north-south components of the current recorded at 10 min intervals and their 25-h moving averages at Station 2 in August and September 2016. In the subsequent sections, we describe certain observation cases at Station 2 in 2016, focusing on the flow environment before and after the reduction in the DO. For example, on 13 September, when DO decreased, easterly winds of more than 10 m s^{-1} blew from the previous day (Figure 7), and an increase was observed in the eastward flow velocity of the bottom (Figure 8). From 20 to 27 September, the hypoxic period continued (Figure 7). Strong winds exceeding 15 m s^{-1} were observed (Figure 7), and the eastward flow velocity in the bottom layer increased temporarily on September 20 (Figure 8), when the DO declined rapidly. Thereafter, a period of low wind velocity continued for approximately a week, and at the beginning of the neap tide (Figure 7), flow velocities remained relatively low until approximately 28 September (Figure 8).

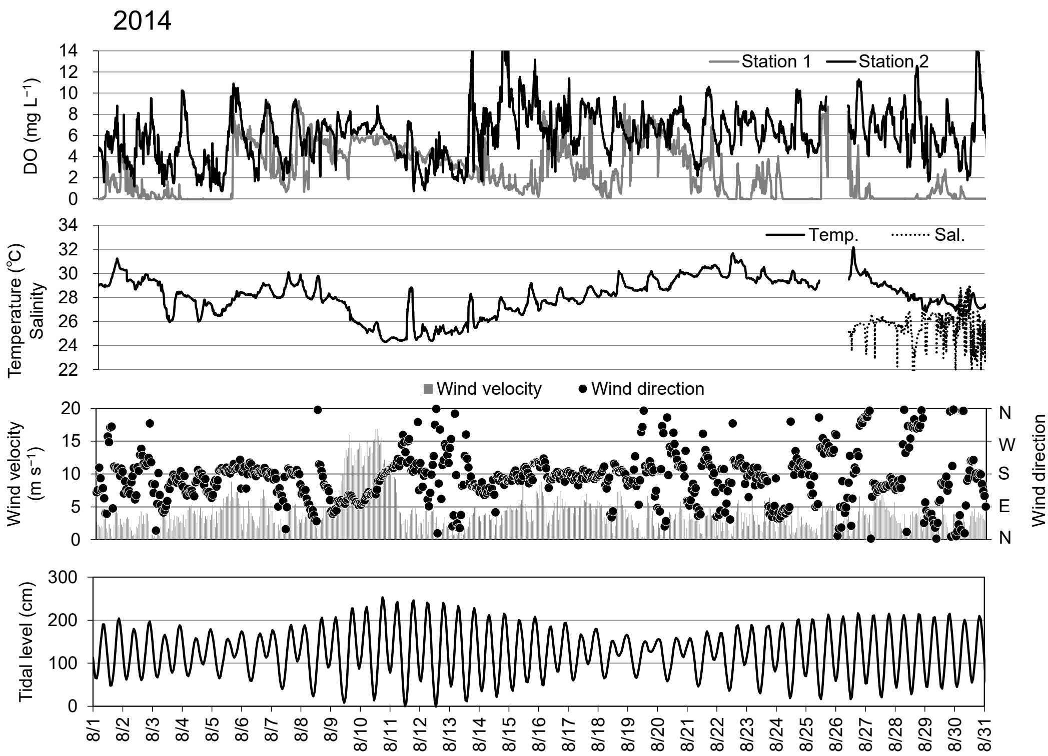

Figure 5. *Cont.*

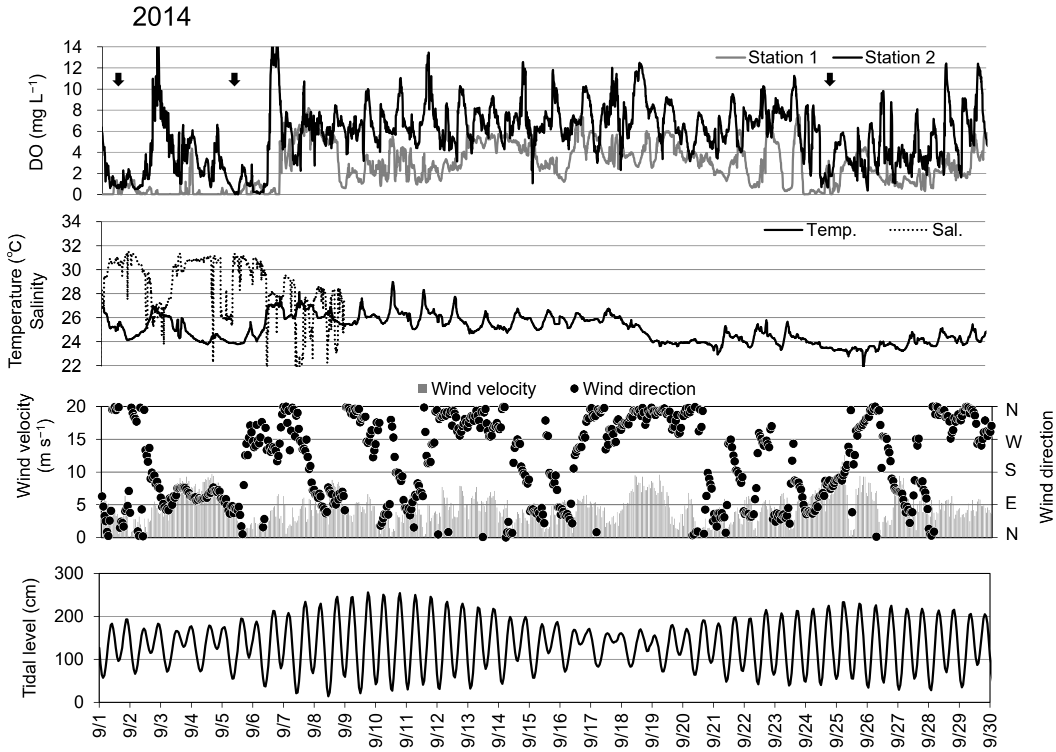

Figure 5. Time–series of dissolved oxygen (DO) at Stations 1 and 2, temperature and salinity at Station 2, wind velocity and direction from the oceanographic observation buoy system operated by Aichi Fisheries Research Institute, and tidal level at the Mikawa Port from August to October 2014 (upper graphs: in August, lower graphs: in September). Arrows indicate a large decline in the DO below approximately 2 mg L^{-1}.

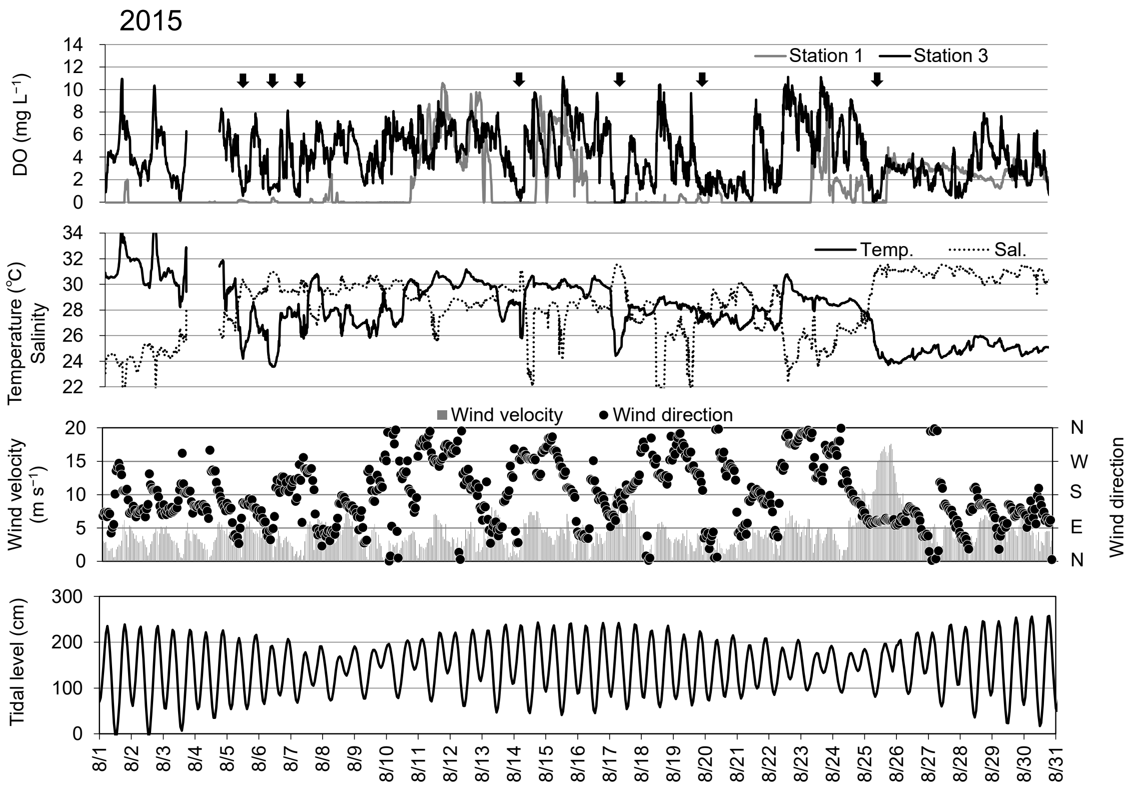

Figure 6. *Cont.*

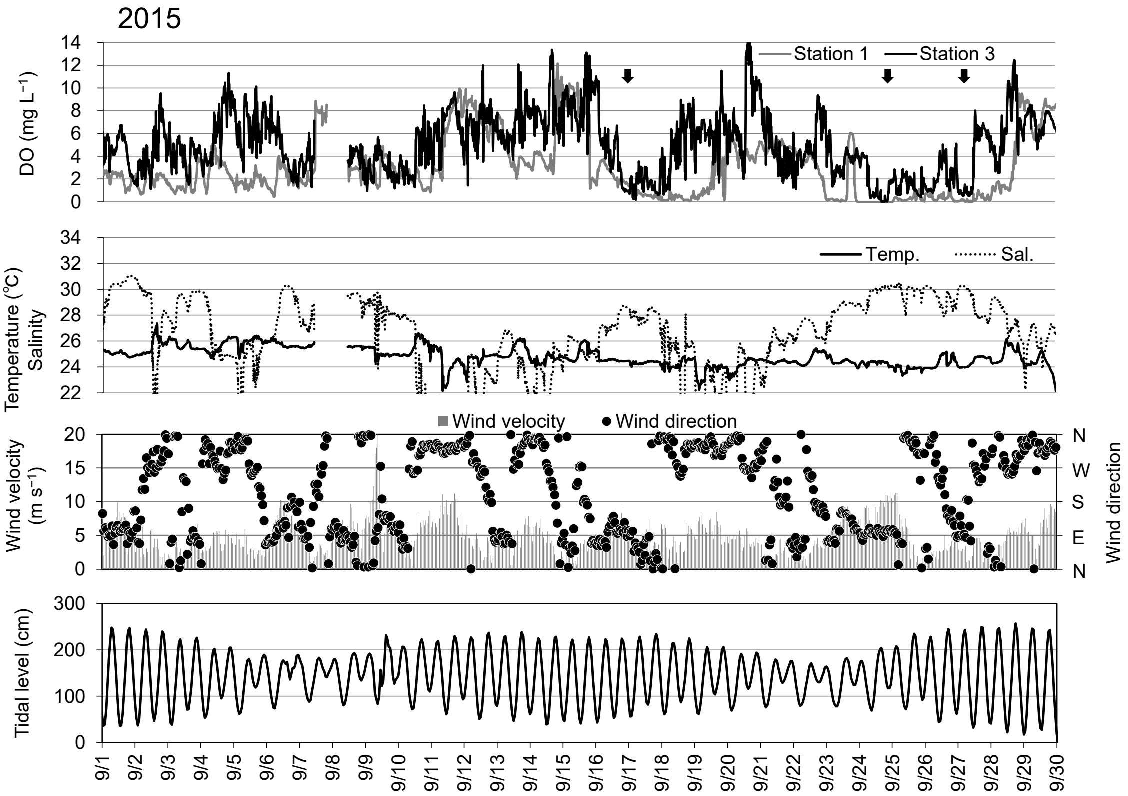

Figure 6. Time–series of dissolved oxygen (DO) at Stations 1 and 3, temperature and salinity at Station 3, wind velocity and direction from the oceanographic observation buoy system operated by Aichi Fisheries Research Institute, and tidal level at the Mikawa Port from August to October 2015 (upper graphs: in August, lower graphs: in September). Arrows indicate a large decline in the DO below approximately 2 mg L^{-1}.

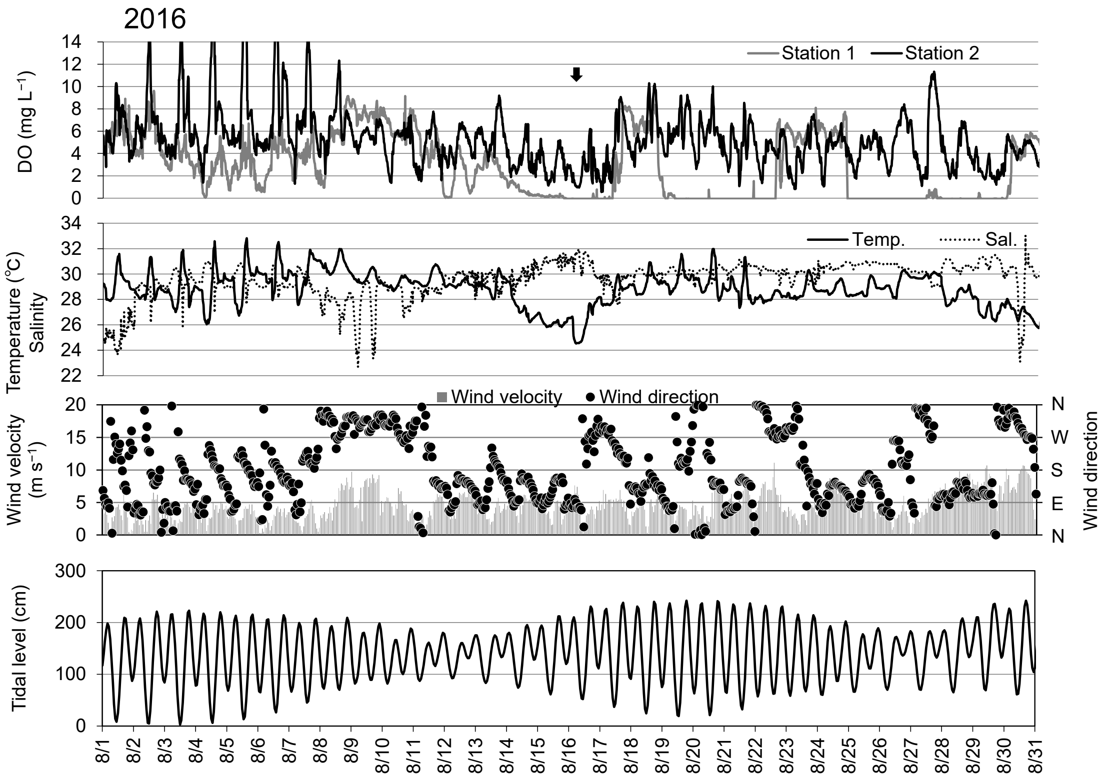

Figure 7. *Cont.*

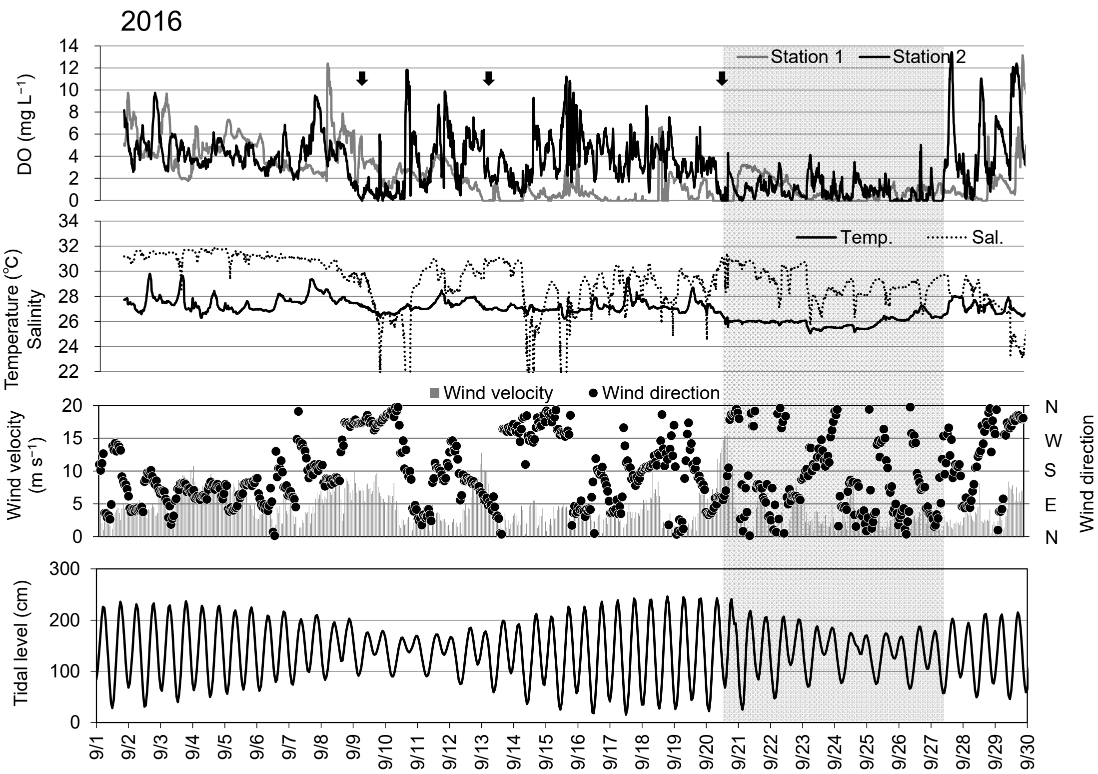

Figure 7. Time–series of dissolved oxygen (DO) at Stations 1 and 2, temperature and salinity at Station 2, wind velocity and direction from the oceanographic observation buoy system operated by Aichi Fisheries Research Institute, and tidal level at the Mikawa Port from August to October 2016 (upper graphs: in August, lower graphs: in September). Arrows indicate a large decline in the DO below approximately 2 mg L^{-1}. Gray hatch indicates a period of severe hypoxia.

3.1.3. Macrobenthos Monitoring on the Tidal Flat

Figure 9 shows the monthly changes in the biomass of macrobenthos at Stations 2 and 3 on the tidal flats in 2014, 2015, and 2016. Total biomass throughout the three years was dominated by bivalves in both stations, followed by polychaeta and then gastropods. The biomass of asari clams was predominant at both stations each year. The biomass of macrobenthos at Station 2 ranged from approximately 320–5370 g m^{-2} and at Station 3 from approximately 210–4290 g m^{-2} throughout the three years. The biomass of macrobenthos, especially asari clams, at Station 2 tended to be higher than Station 3. In July 2014, macrobenthos biomass was 1508 g m^{-2} and 432 g m^{-2} at Stations 2 and 3, respectively. In September 2014, the biomass increased to 5371 g m^{-2} and 615 g m^{-2} at Stations 2 and 3, respectively. In 2015, the biomass of macrobenthos remained generally flat and ranged from 1318 g m^{-2} to 2714 g m^{-2} and from 855 g m^{-2} to 1643 g m^{-2} at Stations 2 and 3, respectively.

In June 2016, macrobenthos biomass was 321 g m^{-2} and 210 g m^{-2} at Stations 2 and 3, respectively. In August, the biomass increased to 4757 g m^{-2} and 4289 g m^{-2} at Stations 2 and 3, respectively. The biomass leveled off in September but declined sharply in October at Station 2. From September to October, the biomass, mainly asari clams, decreased to approximately 10% at Station 2. At Station 3, macrobenthos biomass declined from August to October, and in particular, the biomass of asari clams decreased to approximately 50% from September to October. In October 2016, macrobenthos biomass was 493 g m^{-2} and 1033 g m^{-2} at Stations 2 and 3, respectively. Supplementary Materials shows the biomass and densities of macrobenthos at both stations in 2014, 2015, and 2016.

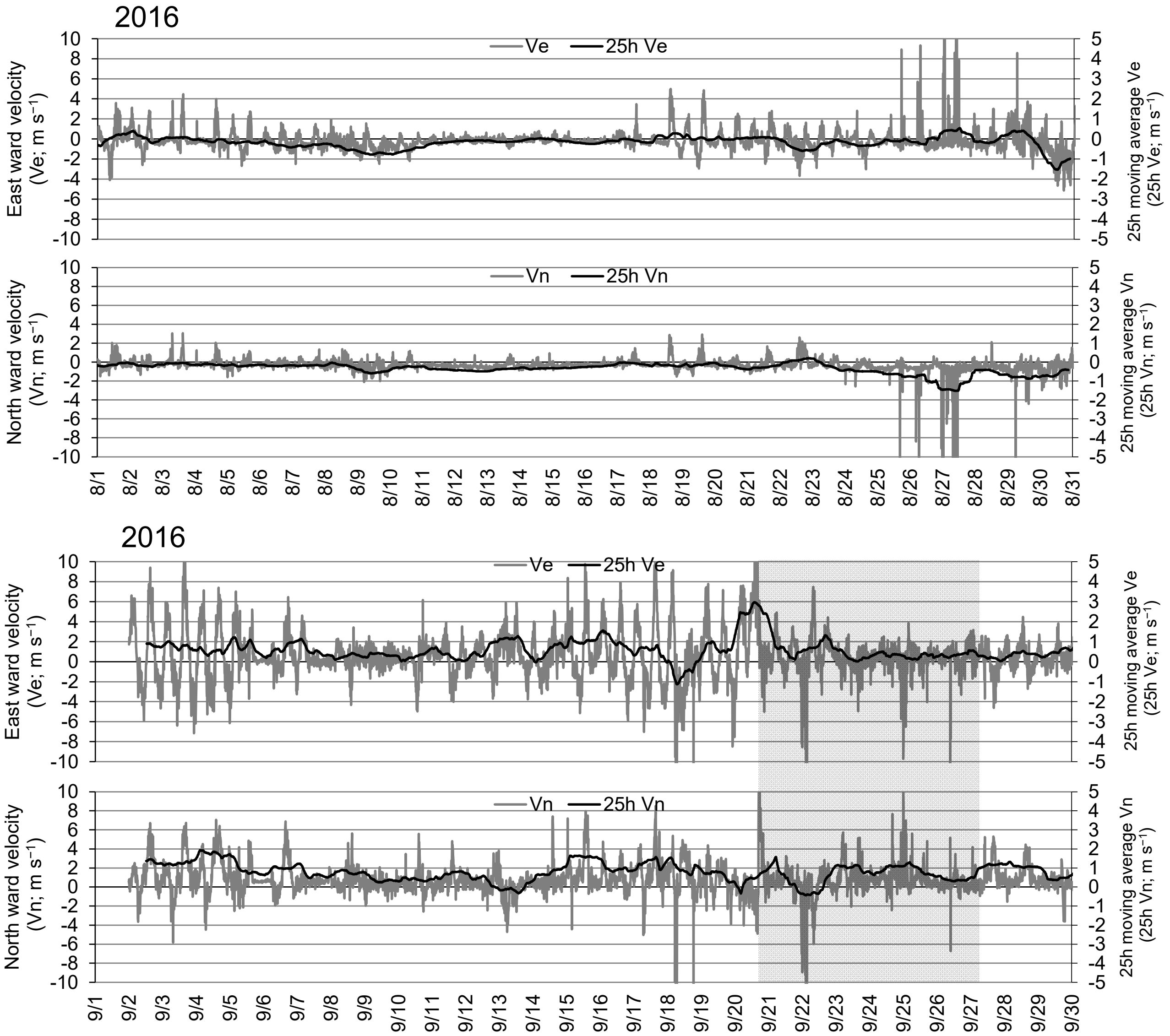

Figure 8. Time-series of east-west and north-south component velocity of bottom current at Station 2 from August to October 2016. Gray hatch indicates the period of severe hypoxia at Station 2.

3.2. Tidal Flat Sediment Incubation Experiment

Figure 10 shows the DO and hydrogen sulfide concentrations in the water directly above the sediment during the incubation experiment. On the third day of incubation, the water directly above the sediment became anoxic, and the hydrogen sulfide concentration reached approximately 30 mg L^{-1}, after which it remained almost unchanged.

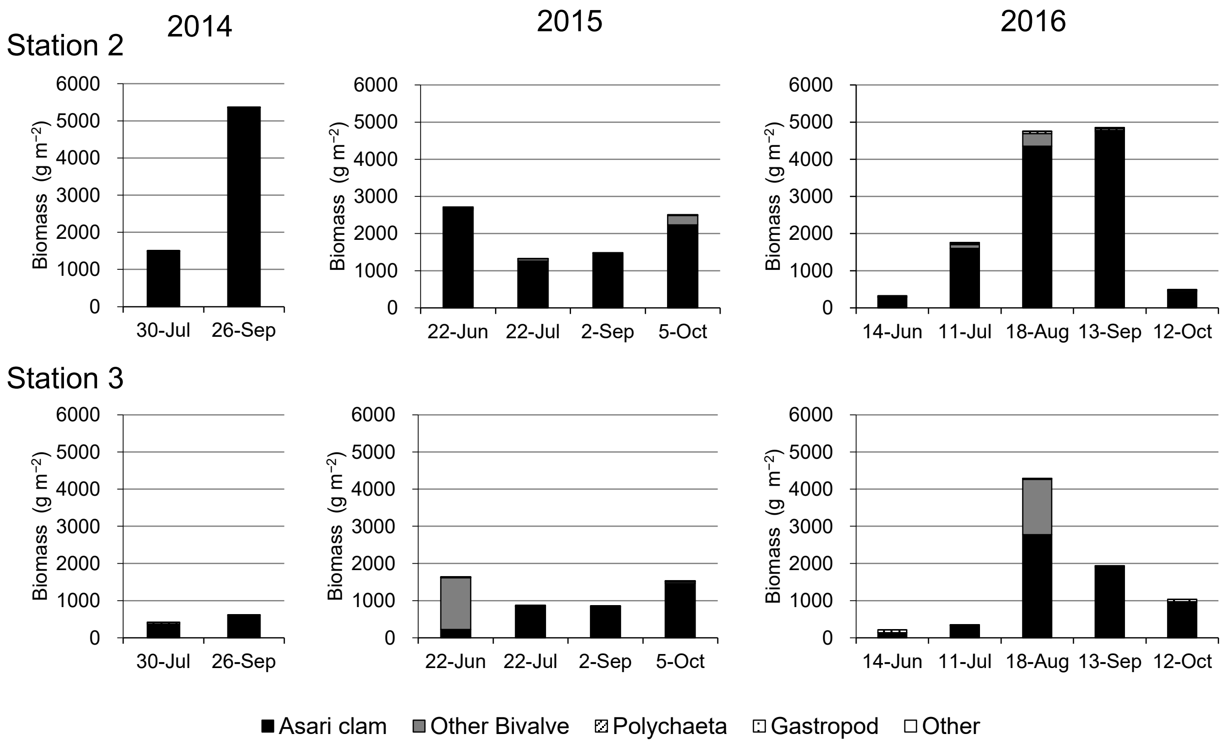

Figure 9. Monthly changes in the biomass of macrobenthos at Stations 2 and 3 from June to October 2014–2016.

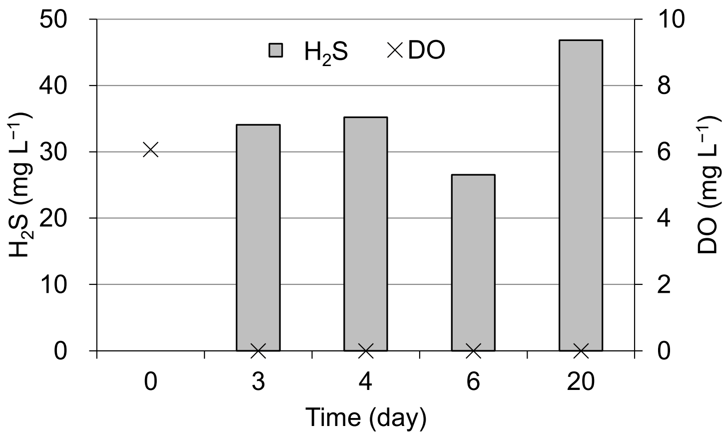

Figure 10. Changes in the dissolved oxygen (DO) and hydrogen sulfide (H_2S) concentration of the water directly above the sediment incubated at 28 °C.

4. Discussion

4.1. Upwelling of Hypoxic Water Masses on the Tidal Flat

In the Mikawa Bay, summer stratification progresses offshore hypoxia [1]. In 2014, 2015, and 2016, stratification developed from July at Station 1, a station offshore of the tidal flat area, and resulted in hypoxia in the bottom layer (Figures 2–4). In August and September, the bottom layer at Station 1 was intermittently hypoxic (Figures 5–7). Compared to the offshore station, DO tended to be higher on the tidal flats, but hypoxia was occasionally observed (Figures 5–7). When constant easterly winds of approximately 5 m s^{-1} or more were observed offshore, hypoxic water masses with low water temperature below 25 °C, high salinity over 30, or both were mostly observed on the tidal flat, which was similar to offshore bottom waters of the same period. Focusing on 2016, wind and tidal current observations indicate that the bottom water flowed eastward when easterly winds blew hard on 13 and 20 September (Figures 7 and 8). These results suggest that the current direction in the bottom layer of the tidal flat was opposite that in the surface layer, and that hypoxic water masses with low temperature and high salinity in the offshore bottom layer were upwelling onto the tidal flat. Wind is a major factor in causing the intrusion of hypoxic water masses into the shallow area [26–28]. Aoki et al. [29] reported that based on various field data in the Rokujo tidal flat, onshore translation of offshore bottom waters induced by eastly wind forcing of about 5 m s^{-1} for several days and vertical mixing near the edge of the tidal flat may be main causes of the upwelling phenomena. This supports our results of the field observation.

4.2. Effects of Hypoxia on the Macrobenthos Biomass

In general, river mouth tidal flats have high biological productivity [30,31], and the Rokujo tidal flat is no exception. No significant depletions of macrobenthos biomass were observed in 2014 and 2015. The dominant species of macrobenthos on the tidal flat were asari clams. Because asari clams were found to be tolerant to 48 h of anoxia [11], they likely did not experience a major depletion of macrobenthos in 2014 and 2015. However, clam biomass decreased by approximately 90% at Station 2 and 50% at Station 3 from September to October 2016, even though no such large depletion was observed in other periods. High water temperature, low salinity (flood), and strong winds (waves) may be factors influencing clam depletion (e.g., [32–36]), but we found nothing special about these factors when comparing 2016 to 2014 and 2015 in terms of water temperature, salinity, and wind conditions. Conversely, hypoxia continued for about a week, starting 20 September 2016. This was the only time in three years that such a prolonged period of hypoxia occurred. Asari clams are highly tolerant of anoxia [11], but it has been reported that the effects of anoxia begin to occur from 48 h to 96 h [8]. Therefore, prolonged hypoxia was thought to have caused the depletion of macrobenthos, including asari clams, in September 2016. Wind and tidal conditions may have had an influence on the duration of anoxia for more than 3 days in September 2016. The period of anoxia was considered to have been caused by a combination of calm wind conditions (generally within 5 m s^{-1}) and the beginning of the neap tide. Kakino [9] also suggested that the inhibition of seawater exchange associated with calm conditions and neap tide after the hypoxic upwelling had caused severe mortality of asari clams in Tokyo Bay.

4.3. Possibility of Hydrogen Sulfide Formation on the Tidal Flat

In addition, the possibility of oxygen consumption on tidal flats due to biological die-offs will be considered in the long-term persistence of hypoxia. The incubation experiments of tidal flat sediments containing macrobenthos resulted in hydrogen sulfides exceeding 30 mg L^{-1} after 3 days of incubation (Figure 10). The hydrogen sulfide release from the sediment was estimated to be at least 570 mg m^{-2} day^{-1} in this laboratory experiment, assuming that the sulfide concentration in the water was saturated on the third day. Table 1 shows the results of sediment incubation in the present study compared with the previous study, the incubation experiment of sediment severely polluted with organic matter in the navigation channel, Station A (Figure 1). The release rate at Station A was estimated to be approximately 390 mg m^{-2} day^{-1} [37], which was lower than the result of the present experiment in tidal flat sediment. Because sediment organic matter (total organic matter + Biological organic matter) at Station 2 on the tidal flat was higher than that at Station A in the navigation channel (Table 1), tidal flat sediments are considered to have a potential release of highly concentrated hydrogen sulfide depending on hypoxic condition. Sulfate-reducing bacteria use organic matter and sulfate as a respiratory substrate, and hydrogen sulfide, carbon dioxide, and water are produced through anaerobic respiration [14], according to the following equation:

$$SO_4^{2-} + 2CH_2O + 2H^+ \rightarrow H_2S + 2CO_2 + 2H_2O$$

The biological organic matter (macrobenthos biomass) was 93.2 g-C m^{-2}, which was approximately 3 times larger than TOC at Station 2 (Table 1). The theoretical hydrogen sulfide production potential estimated from biological organic matter was 132 g m^{-2}. The hydrogen sulfide release estimated by the laboratory experiment was 570 mg m^{-2} day^{-1} and hydrogen sulfide production over 3 days was calculated to be 1.7 g m^{-2}. The theoretical values estimated from biological organic matter were enough to explain the experimental values. Therefore, biogenic organic loads were considered to contribute to the production of hydrogen sulfide on the tidal flat under anoxic conditions.

Sugahara et al. [38] reported that a relatively high concentration of hydrogen sulfide was detected after the death of an individual, despite hypoxic conditions with no hydrogen sulfide, in an experiment using the brackish water clams *Corbicula japonica* (Prime, 1864). In the study, sulfate-reducing bacteria produce hydrogen sulfide from organic acids in the bodies of *C. japonica* and are released from the inside of the shell to the environment upon their death. This indicates that dead bivalves become the source of hydrogen sulfide produced by sulfate reduction, thus supporting our results of the incubation experiment.

Hydrogen sulfide is known to be a potent oxygen consumer as well as a strong biotoxin. Asari clams are tolerant to hydrogen sulfide, but high concentrations of hydrogen sulfide ($\geq$13.5 mg L^{-1}) at 24 °C resulted in reduced survival of clams after 24 h [11]. In this study, although hydrogen sulfide was not detected offshore in 2016 (Figure 4), hydrogen sulfide produced on the tidal flat seems to have contributed to the persistence of hypoxia. In addition, the exhibited result (approximately 30 mg L^{-1}) of the incubation experiment exceeded the reported value of lethal level and this seems to have accelerated biological mortality.

However, to test this hypothesis, several issues have to be addressed. The sediment incubation experiment in this study did not reveal the detailed processes of hypoxia and hydrogen sulfide formation. Additionally, the quantitative balance of sediment and water volume is different between the experiment and in the field which could not be directly compared for the discussion. Another important issue is the in situ measurement of hydrogen sulfide concentrations in tidal flats during the upwelling of hypoxic water masses. The dynamics of hydrogen sulfide in the field during upwelling hypoxia need to be elucidated by observing the related enhancement. In addition, it is important to build a numerical model to simulate the chain of biological die-off, hydrogen sulfide release, and massive oxygen consumption in tidal flats triggered by upwelling hypoxia.

Table 1. The flux of hydrogen sulfide, biological organic matter, and sedimentary organic matter in the sediments on the tidal flat and the navigation channel.

Sediment Collection Site	Hydrogen Sulfide Release (mg m^{-2} day^{-1})	Total Organic Matter (TOC; g-C m^{-2})	Biological Organic Matter (Macrobenthos Biomass; g-C m^{-2})
Tidal flat (Station 2)	567.7	30.6 *2	93.2 *3
Navigation channel (Station A)	387.5 *1	76.8 *2	-

*1: Average value of reference core in Inoue and Hagino [37]; *2: Sone et al., unpublished data; *3: Converted from wet weight to carbon weight in reference to Sone et al. [4] and Nakata and Hata [39].

5. Conclusions

We conducted continuous observations of water quality, temperature, salinity, and DO, with flow conditions and biological observations, monthly macrobenthos monitoring, on the Rokujo tidal for three years. Additionally, we conducted a simple laboratory experiment using sediments from a tidal flat containing benthic organisms and examined hydrogen sulfide production caused by biogenic organic loads. The field observation results showed that constant easterly winds caused upwelling hypoxia on the Rokujo tidal flat from August to September and long-term hypoxia, approximately one week in 2016, caused large depletion of asari clams on the tidal flat, which was associated with stagnation of tidal currents. Conversely, the incubation experiment results showed that a high concentration of hydrogen sulfide was formed from the sediments on the tidal flat. These findings may indicate the following scenario for the mass mortality of asari clams in the Rokujo tidal flats triggered by upwelling hypoxia. First, hypoxic water masses with or without hydrogen sulfide in offshore areas upwell on tidal flats when strong easterly winds blow. Subsequently, hypoxia on tidal flats is prolonged by mild meteorological and hydrographic conditions, such as slight winds and neap tides. This results in the death of asari clams with relatively high tolerance to hypoxia. Moreover, accumulated hydrogen sulfide on the tidal flat seems to have prolonged the period of hypoxic conditions and led to mass mortality of asari clams and other macrobenthos.

The shallow area, including tidal flats, plays the role of purification around coastal areas via their ecosystem function (e.g., [40]), but immediately turns from a sink to a source of nutrient load following the occurrence of hypoxia [1,41]. Moreover, the results of the present study suggest that the tidal flat ecosystem is a potential source of biogenic organic loads and hydrogen sulfide released by the chain of biological die-off triggered by upwelling hypoxia.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/w15223997/s1, Table S1: The biomass and densities of macrobenthos at Stations 2 and 3 in 2014, 2015, and 2016.

Author Contributions: R.S. designed the study, measured environmental factors, sampled macrobenthos, conducted experiments, and drafted the article. M.W. designed the study, measured environmental factors, sampled macrobenthos, and drafted the manuscript. S.Y. measured environmental factors and sampled macrobenthos. D.M. measured environmental factors and sampled macrobenthos. T.I. (Toshiro Ishida) measured environmental factors and sampled macrobenthos. S.K. designed the Study and gave final approval of the article. T.I. (Tetsunori Inoue) drafted the manuscript and provided final approval of the same. T.S. designed the study and provided final approval of the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: This study was supported by the Environment Research and Technology Development Fund of the Ministry of Environment, Japan (5-1404, principal investigator Prof. Yoshiyuki Nakamura).

Data Availability Statement: Data cannot be made publicly available; readers should contact the corresponding author for details.

Acknowledgments: We are thankful to Shogo Sugahara of Shimane University for his help and expertise with the analytical methods for hydrogen sulfide. We also appreciate the members of the Fisheries Environment Research Department at the Aichi Fisheries Research Institute.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Suzuki, T. Oxygen-deficient waters along the Japanese Coast and their effects upon the estuarine ecosystem. *J. Environ. Qual* **2001**, *30*, 291–302. [CrossRef] [PubMed]
2. Diaz, R.J.; Rosenberg, R. Spreading dead zones and consequences for marine ecosystems. *Science* **2008**, *321*, 926–929. [CrossRef] [PubMed]
3. Ministry of Agriculture, Forestry and Fisheries of Japan. White Paper on Fisheries. 2014. Available online: https://www.jfa.maff.go.jp/j/kikaku/wpaper/h24/attach/pdf/09_3shou3setu.pdf (accessed on 4 February 2023).
4. Sone, R.; Kamohara, S.; Yamada, S.; Suzuki, T.; Takabe, T. Water purification in the river mouth tidal flat with an abundant supply of the Manila clam *Ruditapes philippinarum*: A model study in June in Rokujo tidal flat, at the mouth of the Toyo River in Mikawa Bay, Japan. *Bull. Jpn. Soc. Fish. Oceanogr.* **2015**, *79*, 117–129. (In Japanese with English abstract)
5. Diaz, R.J. Overview of hypoxia around the world. *J. Environ. Qual.* **2001**, *30*, 275–281. [CrossRef]
6. Takeda, K.; Ishida, M. Mass mortality of the short-necked clam, *Ruditapes philippinarum*, caused by a niga-shio (bitter tide) in Mikawa Bay and dissolved oxygen deficiency in a borrow pit. *J. Adv. Mar. Sci. Tech. Soc.* **2006**, *12*, 51–58.
7. Iimura, A.; Kobayashi, H.; Ogura, H. Occurrence of Blue Tide in Tokyo Bay in 2010. Available online: https://www.pref.chiba.lg.jp/wit/suishitsu/report/documents/ar2010suishitsu008.pdf (accessed on 15 November 2023).
8. Kakino, J. Mortality of Manila clam caused by blue tide; effects of hypoxic water and sulfides. *Bull. Chiba Pref. Fish. Exp.* **1982**, *40*, 1–6.
9. Kakino, J. Mass mortality of shellfish by hypoxic water at the head of Tokyo Bay. *Fish. Eng.* **1986**, *23*, 41–47.
10. Higano, J. Influence of environmental factors as oxygen deficiency, hydrogen sulfide and suspended mud on the survival and growth of Manila clam. *Bull. Fish. Res. Agency Suppl.* **2005**, *3*, 27–33.

11. Kodama, K.; Waku, M.; Sone, R.; Miyawaki, D.; Ishida, T.; Akatsuka, T.; Horiguchi, T. Ontogenetic and temperature-dependent changes in tolerance to hypoxia and hydrogen sulfide during the early life stages of the Manila clam *Ruditapes philippinarum*. *Mar. Environ. Res.* **2018**, *137*, 177–187. [CrossRef]
12. Kajiyama, M. Occurrence of blue tide and fishery damage in Tokyo Bay. *Bull. Chiba Pref. Fish. Res. Ctr.* **2019**, *13*, 61–76.
13. Capone, D.G.; Kiene, R.P. Comparison of microbial dynamics in marine and freshwater sediments: Contrasts in anaerobic carbon catabolism 1. *Limnol. Oceanogr.* **1988**, *33*, 725–749. [CrossRef]
14. Fossing, H. *A Model Set-Up for an Oxygen and Nutrient Flux Model for Aarhus Bay (Denmark)*; NERI Technical Report No. 483; National Environmental Research Institute, Ministry of the Environment: Roskilde, Denmark, 2004.
15. Middelburg, J.J.; Levin, L.A. Coastal hypoxia and sediment biogeochemistry. *Biogeosci. Discuss.* **2009**, *6*, 1273–1293. [CrossRef]
16. Brüchert, V.; Jorgensen, B.B.; Neumann, K.; Riechmann, D.; Schlosser, M.; Schulz, H. Regulation of bacterial sulphate reduction and hydrogen sulphide fluxes in the central Namibian coastal upwelling zone. *Geochim. Cosmochim. Acta* **2003**, *67*, 4505–4518. [CrossRef]
17. Lavik, G.; Stuhrmann, T.; Brüchert, V.; Van der Plas, A.; Mohrholz, V.; Lam, P.; Mussmann, M.; Fuchs, B.M.; Amann, R.; Lass, U.; et al. Detoxification of sulphidic African shelf waters by blooming chemolithotrophs. *Nature* **2009**, *457*, 581–586. [CrossRef]
18. Rolff, C.; Walve, J.; Larsson, U.; Elmgren, R. How oxygen deficiency in the Baltic Sea proper has spread and worsened: The role of ammonium and hydrogen sulphide. *Ambio* **2022**, *51*, 2308–2324. [CrossRef]
19. Smith, L.; Kruszyna, H.; Smith, R.P. The effect of methemoglobin on the inhibition of cytochrome c oxidase by cyanide, sulfide or azide. *Biochem. Pharmacol.* **1977**, *26*, 2247–2250. [CrossRef]
20. Vismann, B. Sulfide species and total sulfide toxicity in the shrimp *Crangon crangon*. *J. Exp. Mar. Biol. Ecol.* **1996**, *204*, 141–154. [CrossRef]
21. Marumo, K.; Yokota, M. Review on Aoshio and biological effects of hydrogen sulfide. *Rep. Mar. Ecol. Res. Inst.* **2012**, *15*, 23–40.
22. Nature Conservation Bureau, Environment Agency. *The Report of the Coastline Survey in the 4th National Survey on the Natural Environment*; Nature Conservation Bureau, Environment Agency: Tokyo, Japan, 1994.
23. Mikawa Port Office, Chubu Regional Development Bureau, Ministry of Land, Infrastructure, Transport and Tourism. Sea Blue Project. Available online: https://www.mikawa.pa.cbr.mlit.go.jp/gaiyou/jigyou/seablue/ (accessed on 4 February 2023).
24. Cline, J.D. Spectrophotometric determination of hydrogen sulfide in natural waters. *Limnol. Oceanogr.* **1969**, *14*, 454–458. [CrossRef]
25. Aichi Fisheries Research Institute Oceanographic Observation Buoy Systems. Available online: http://suisanshiken-buoy.jp/top/index.html (accessed on 4 February 2023).
26. Sanford, L.P.; Sellner, K.G.; Breitburg, D.L. Covariability of dissolved oxygen with physical processes in the summertime Chesapeake Bay. *J. Mar. Res.* **1990**, *48*, 567–590. [CrossRef]
27. Breitburg, D.L. Near-shore hypoxia in the Chesapeake Bay: Patterns and relationships among physical factors. *Estuar. Coast. Shelf Sci.* **1990**, *30*, 593–609. [CrossRef]
28. Chan, F.; Barth, J.A.; Kroeker, K.J.; Lubchenco, J.; Menge, B.A. The dynamics and impact of ocean acidification and hypoxia. *Oceanography* **2019**, *32*, 62–71. [CrossRef]
29. Aoki, S.; Mase, T.; Kamohara, S. Upwelling of bottom oxygen-depleted waters in shallow coastal seas. *Ann. J. Coastal Eng.* **2014**, *70*, 1141–1145.
30. Nixon, S.W. Physical energy inputs and the comparative ecology of lake and marine systems. *Limnol. Oceanogr.* **1988**, *33*, 1005–1025. [CrossRef]
31. Sasaki, K. Bio-productivity and bio-diversity in tidal flat. *Aquabiology* **2003**, *25*, 180–186.
32. Isono, R. Upper temperature effect on rates of growth and oxygen consumption of the Japanese Litteleneck Clam. *Ruditapes philippinarum. Nippon Suisan Gakkaishi* **1998**, *64*, 373–376. [CrossRef]
33. Kakino, J. Study on the effects of physical and chemical environmental factors on the Japanese little neck clam *Ruditapes philippinarum*. *Fish Eng.* **2006**, *43*, 117–130.
34. Matsukawa, Y.; Cho, N.; Katayama, S.; Kamio, K. Factors responsible for the drastic catch decline of the Manila clam *Ruditapes philippinarum* in Japan. *Nippon Suisan Gakkaishi* **2008**, *74*, 137–143. [CrossRef]
35. Toba, M. Revisiting recent decades of conflicting discussions on the decrease of Asari clam *Ruditapes philippinarum* in Japan: A review. *Nippon Suisan Gakkaishi* **2017**, *83*, 914–941. [CrossRef]
36. Matsuda, M.; Hirano, K. Effects of low-salinity seawater exposure to Manila clam *Ruditapes philippinarum*. *Bull. Nagasaki Pref. Inst. Fish* **2019**, *44*, 7–13.
37. Inoue, T.; Hagino, Y. Effects of three iron material treatments on hydrogen sulfide release from anoxic sediments. *Water Sci. Technol.* **2022**, *85*, 305–318. [CrossRef] [PubMed]
38. Sugahara, S.; Suzuki, M.; Yamamuro, M.; Semura, H.; Kamiya, H.; Senga, Y.; Noda, K.; Egawa, M.; Seike, Y. Sulfide tolerance of *Corbicula japonica* by the difference in shell size. *Aquacult. Sci.* **2017**, *65*, 83–87.
39. Nakata, K.; Hata, K. Evaluation of the cleansing function of coastal tidal flats. *J. Soc. Water Environ.* **1994**, *17*, 158–166. (In Japanese)

40. Matsukawa, Y.; Sasaki, K. Budgets of nitrogen, phosphorus and suspended solid in an intertidal flat. *Bull. Jpn. Soc. Sci. Fish* **1986**, *52*, 1791–1797. [CrossRef]
41. Waku, M.; Hata, K.; Kaneko, K.; Suzuki, T.; Takabe, T. Adverse effects of dead zones in the coastal waters on the bay-wide material cycle and its remedial measures—An analyze with the ecosystem model in Mikawa Bay, Japan. *J. Adv. Mar. Sci. Tech. Soc.* **2013**, *19*, 15–27.