Journal of
*Marine Science
and Engineering*

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Article

Epizoans on Floating Golden Tide Macroalgae in the Southern Yellow Sea

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Abstract: Golden tide macroalgae have been accumulating in the Southern Yellow Sea (SYS) for several years, causing serious damage to local coastal economy and ecosystems. However, little is known about the environmental and ecological significance of the epizoans drifting on the floating macroalgae. In this study, floating macroalgae collected from four stations were identified as *Sargassum horneri*. Furthermore, morphological and molecular (based on the Cytochrome C Oxidase Subunit I, COI) identification revealed that the 28 epizoans on the macroalgae consisted of four crustacean (*Ampithoe lacertosa*, *Idotea metallica*, *Apohyale* sp., and *Peramphithoe tea*). *Apohyale* sp. and *P. tea* were found at all stations, while *A. lacertosa* is found at only one station. The weight range of *Apohyale* sp., *P. tea*, *I. metallica*, and *A. lacertosa* is 0.0037~0.0420 g, 0.0057~0.0304 g, 0.0222~0.6592 g, and 0.0047 g, respectively. The specific roles of these epizoans in the golden tide in SYS deserve further study. Our results provide a reference for future studies of invasive macroalgae and epizoans.

Keywords: macroalgae; harmful algal bloom; golden tide; *Sargassum horneri*; epizoan

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Citation: Xia, J.; Li, C.; Tang, Y.; Li, J.; Wu, T.; Liu, J.; Zhang, J. Epizoans on Floating Golden Tide Macroalgae in the Southern Yellow Sea. *J. Mar. Sci. Eng.* **2023**, *11*, 479. https://doi.org/10.3390/jmse11030479

Academic Editor: Juan J. Dorantes Aranda

Received: 24 January 2023
Revised: 13 February 2023
Accepted: 18 February 2023
Published: 23 February 2023

1. Introduction

In recent years, fast-growing macroalgal blooms have occurred frequently all over the world, particularly in developed countries and developing countries undergoing rapid industrialization [1–5]. Frequent macroalgal blooms may cause the degradation of the coastal ecosystems and are detrimental to local aquaculture development, tourism, and other economic activities [6–10]. In China, some largest macroalgal blooms have been reported [11,12], mainly due to the proliferation of macroalgae or irregular offshore macroalgal aquaculture activities [13–15]. The causes of macroalgal blooms are likely related to global warming and eutrophication [16–19].

Blooms of macroalgae *Ulva* and *Sargassum*, often called the green and golden tides, have been the most reported in the world [20–22]. In China, green tides have been occurring for many years [23–27] while golden tides have bloomed more frequently in the Southern Yellow Sea (SYS) in recent years [18,28–30]. Massive golden tides have accumulated in offshore aquaculture areas (e.g., *Neopyropia* and *Saccharina* raft aquaculture areas and fish farms), causing substantial economic losses [18,31,32]. Additionally, the decay of massive macroalgae biomass accumulated in the intertidal zones of the Shandong and Jiangsu provinces resulted in the emission of toxic gases such as H_2S. Consequently, pollution negatively impacts the coastal environment and human health [33].

To date, few studies on the golden tide have mainly focused on the benthic and floating macroalgae [9,31,32,34,35] or the diversity of their symbiotic microorganisms [36]. Epizoans (specifically epizoan communities constituted of small invertebrates), such as amphipods, isopods, polychaetes, and echinoderms, transfer the primary production to higher-level consumers and are critical links in the marine food chain, and play a vital role in ecosystems' material cycling and energy flow [37–39]. Macroalgae serve as epizoans' feeding grounds, habitats, and sanctuaries [40,41]. The epizoans feed on the surface debris and microorganisms from the thalli [38,42,43], promoting the growth of macroalgae. Therefore, the epizoan community is an essential part of macroalgae-based ecosystems. However, in general terms, there are few information on the composition of the epizoan community inhabiting the golden tides.

The combination of morphological and molecular biological species identification technology has been a powerful methodology for species identification [39]. Among the molecular techniques available, DNA barcoding identification technology is a commonly-used reliable method [44–46]. For the *Phaeophyta*, the Cytochrome C Oxidase Subunit I (COI), COIII, Internally Transcribed Spacer gene (ITS), 23S rDNA (23S), and Large Subunit of Ribulose 1,5 Bisphosphate Carboxylase (*rbc*L) genes are typically used for DNA barcoding [32,47–49]. For epizoans, researchers use the COI, 16S rDNA (16S), 18S rDNA (18S), and 28S rDNA (28S) genes for species identification [39,50,51].

In this study, we used morphological and molecular (COI gene) identification techniques to identify floating macroalgae [47,52] and epizoans [50] collected from the SYS. The frequency of occurrences of epizoans from each station was counted, and the fresh weight of the collected sample was also measured. We also assessed the diversity of epizoans living on the floating golden tide macroalgae and evaluated their roles in the floating macroalgal ecosystem.

2. Materials and Methods

2.1. Sample Collection of Macroalgae and Epizoans

Samples were collected on a research cruise from 22–25 March 2021, offshore in Jiangsu Province. Macroalgae and epizoan samples were taken at four stations (2021SYSA; 2021SYSB; 2021SYSC; 2021SYSD) in the SYS on March 25 (Figure 1); the macroalgae were identified as *Sargassum* on-site based on morphological observation [53] (Figure 1).

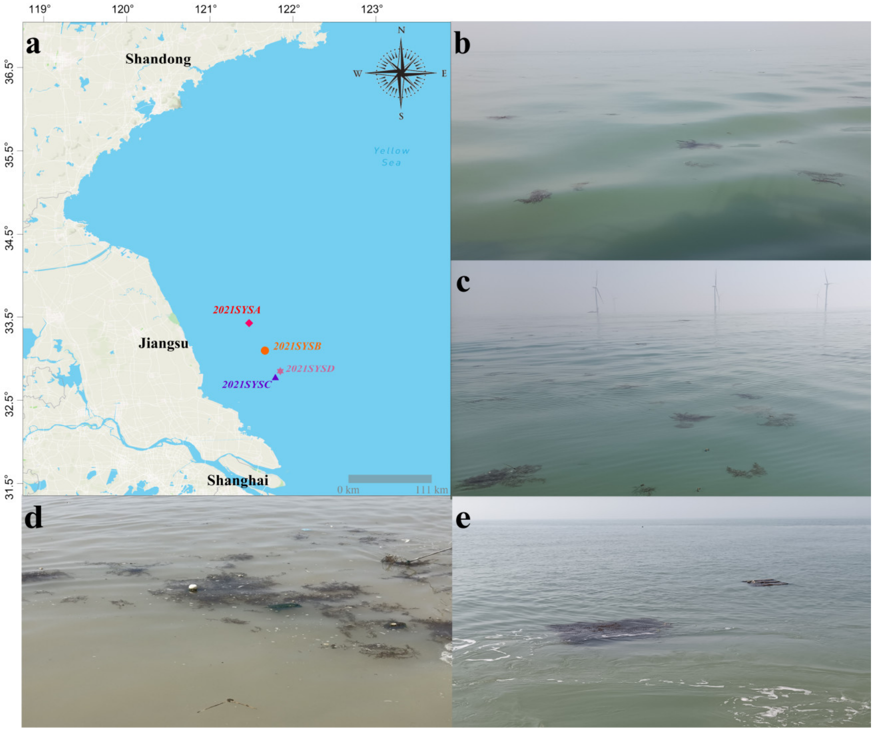

Figure 1. (**a**) Sampling sites of golden tide macroalgae in the Southern Yellow Sea; (**b**–**e**) Marine habitats of stations 2021SYSA, 2021SYSB, 2021SYSC, and 2021SYSD, respectively.

Thalli of macroalgae were collected onto the deck with grappling hooks and cleaned with filtered seawater by using the Vacuum Pump filter (GM-0.33A, Jinteng, Tianjin, China) and filter membrane (0.45 μm, Xinya, Shanghai, China). Meanwhile, 0.5 kg of macroalgae material was stored at 4 °C (BD-138W, Haier Group, Qingdao, China). All epizoans were separated from the macroalgae by sterilized tweezers and numbered, and the fresh weight was weighed using an electronic balance (AL-104, Mettler Toledo, Switzerland), and preserved in a prepared 75% alcohol solution (A500737-0005, Sangon Biotechnology Co., Ltd., Shanghai, China). For subsequent morphological and molecular biology identification, samples were transported back to the laboratory at 4 °C (HYUNDAI-6L, Hyundai Group, Seoul, South Korea).

2.2. Morphological and Molecular Identification of Macroalgae

The macroalgae sample were further identified, and pictures of morphological characteristics were recorded using a Nikon camera (D5600, Nikon Corporation, Tokyo, Japan). Per sample, 0.3 g of fresh algae biomass was separated from three single thalli from each station. A total of 12 samples were processed for molecular identification by DNA barcoding. After grinding the samples in liquid nitrogen (Sangon Biotechnology) using a mortar and pestle, the DNA from the samples were extracted using the Ezup Spin Column Super Plant Genomic DNA Extraction Kit (Sangon Biotechnology) following manufacturer's instructions. The COI sequences were amplified through polymerase chain reaction (PCR) with forwarding primer GazF1 (5′-TCAACAAATCATAAAGATATTGG-3′) [54] and reverse primer R686 (5′-CCACCWGMAGGATCAA-3′) [55]. The 50 μL PCR reaction volume contained 25 μL of 2 × PCR-mix, 19 μL of dd-H_2O, 2 μL of each primer (10 mM), and 2 μL of DNA. The PCR amplification protocol featured an initial denaturation at 94 °C for 1.5 min, followed by 40 cycles at 94 °C for 30 s, 47 °C for 40 s, and 72 °C for 40 s, and a final extension at 72 °C for 5 min [56] using an Applied Biosystems device (Thermo Fisher Scientific, Singapore). Amplicon size was confirmed by 1% agarose gel electrophoresis (PowerPac Basic & Gel Doc^{TM} XR+ with Image Lab^{TM} Software, Bio-Rad Laboratories, Hercules, CA, USA); all qualified PCR products were sent to Sangon Biotechnology for sequencing.

The obtained sequences were checked using BioEdit sequence software [57]. The sequences were compared by the Basic Local Alignment Search Tool (BLAST) of the National Center for Biotechnology Information (NCBI, https://www.ncbi.nlm.nih.gov/, accessed on 4 February 2022) database to identify the species composition of the golden tide macroalgae. To further identify species based on DNA barcoding, the COI sequences of *Sargassum* species (*S. horneri*, *Sargassum fusiforme*, *Sargassum confusum*, *Sargassum fallax*, *Sargassum muticum*, and *Sargassum thunbergii*) were downloaded from the NCBI database for comparisons of COI sequences present identity similarity based on BLAST results (Table 1). *Neopyropia yezoensis*, also known as *Pyropia yezoensis* [58], was chosen as the outgroup for subsequent analysis (Table 1). All sequences were aligned using the MEGA 11 program [59] based on the Maximum Composite Likelihood (MCL) method with the Tamura–Nei model [60], and the phylogenetic tree was optimized using the iTOL database (https://itol.embl.de/, accessed on 4 February 2022) [61]. After establishing the phylogenetic tree, the COI sequences of the 12 samples were uploaded to NCBI, and GenBank accession numbers were obtained subsequently (Table 1).

Table 1. Detailed information about the macroalgae used to build the phylogenetic tree.

Species Number	Authority	Collection Locality	Latitude, Longitude	GenBank Information	Source	Specimen Location	Reference
2021SYSA1, 2021SYSA2, 2021SYSA3	This study	Southern Yellow Sea, China	33.4283° N, 121.4723° E	OM444204, OM444205, OM444206	This study	Shanghai Ocean University	This study
2021SYSB1, 2021SYSB2, 2021SYSB3	This study	Southern Yellow Sea, China	33.0997° N, 121.6630° E	OM444207, OM444208, OM444209	This study	Shanghai Ocean University	This study

Table 1. *Cont.*

Species Number	Authority	Collection Locality	Latitude, Longitude	GenBank Information	Source	Specimen Location	Reference
2021SYSC1, 2021SYSC2, 2021SYSC3	This study	Southern Yellow Sea, China	32.7661° N, 121.7887° E	OM444210, OM444211, OM444212	This study	Shanghai Ocean University	This study
2021SYSD1, 2021SYSD2, 2021SYSD3	This study	Southern Yellow Sea, China	32.8477° N, 121.8477° E	OM444213, OM444214, OM444215	This study	Shanghai Ocean University	This study
dl-3	*Sargassum horneri*	Qingdao, China	N.A.	KC782896	NCBI	Chinese Academy of Sciences	[62]
HY20130629.201	*Sargassum horneri*	Yantai, China	36.7900° N, 120.9700° E	KY047195	NCBI	Ocean University of China	[56]
FJ20130427.193	*Sargassum fusiforme*	Fujian, China	25.1800° N, 119.2700° E	KY047223	NCBI	Ocean University of China	[56]
RZ20121016.86	*Sargassum confusum*	Rizhao, China	35.3900° N, 119.5700° E	KY047204	NCBI	Ocean University of China	[56]
TJS0168	*Sargassum fallax*	Tasmania, Australia	N.A.	GQ368266	NCBI	Muséum National d’Histoire Naturelle of Paris	[63]
SAM539	*Sargassum muticum*	Celestia, Italy	45.4386° N, 12.3496° E	KY682972	NCBI	University of Messina	[64]
QD20120918.77	*Sargassum thunbergii*	Qingdao, China	36.09° N, 120.49° E	KY047198	NCBI	Ocean University of China	[56]
mbccc20	*Pyropia yezoensis*	Qingdao, China	N.A.	JQ619144	NCBI	Chinese Academy of Sciences	[62]

Note: N.A. means no information.

2.3. Morphological and Molecular Identification of Epizoans

The preliminary on-site morphological identifications based on historical literature indicated that the collected epizoans were mainly species of Ampithoidae, Hyalidae, and Idoteidae [65–68]. In the lab, the 28 epizoans collected from the four stations (8 at station 2021SYSA; 3 at station 2021SYSB; 7 at station 2021SYSC, and 10 at station 2021SYSD) were identified based on morphology. Subsequently, all epizoans were identified by DNA barcoding. The DNA from the samples were extracted by the Ezup Column Animal Genomic DNA Purification Kit (Sangon Biotechnology) and PCR amplified. The COI sequences of Amphipoda were amplified with forward primer COI-F (5′-GGTCAACAAATCATAAAGATATTGG-3′) and reverse primer COI-R (5′-TAAACTTCAGGGTGACCAAAAAATCA-3′) [39]. The PCR cycle was: predenaturation at 94 °C for 4 min, followed by 30 cycles at 94 °C for 30 s, 45 °C for 30 s, and 72 °C for 30 s, and a final extension at 72 °C for 5 min [39]. Meanwhile, the COI sequences of Isopoda were amplified with forward primer LCO1718 (5′-TWGGDGCNCCDGAYATGGCHTTYCCDCG-3′) and reverse primer HCO2386 (5′-AAAATTTTAATTCCAGTAGGAACTGCAATAATTAT-3′) [69]. The PCR cycle was: predenaturation process at 95 °C for 2 min, following by 41 cycles at 95 °C for 50 s, 45 °C for 1 min 30, 72 °C for 1 min 30 s, and a final extension at 72 °C for 10 min [69]. The PCR reaction volume was 40 μL and contained 20 μL of $2\times$ PCR-mix, 15.5 μL of dd-H_2O, 1.5 μL of each primer (10 mM), and 1.5 μL of DNA. Then all PCR amplification products were produced using the Applied Biosystems device. The agarose gel electrophoresis, sequencing, sequence checking, and BLAST processes were the same as those described above in Section 2.2.

To further identify species based on DNA barcoding, the most similar COI sequences of epizoan species (*Ampithoe lacertosa*, *Ampithoe helleri*, *Apohyale* sp., *Apohyale* cf. *pugettensis*, *Idotea metallica*, *Idotea emarginata*, and *Peramphithoe tea*) were downloaded from the NCBI database based on BLAST results. Table 2 provides detailed information about these samples. *Oratosquilla oratoria* [70] was chosen as the outgroup for subsequent analysis (Table 2). The alignment of sequences and construction of the phylogenetic tree were conducted following the processes described in Section 2.2. After establishing the phylogenetic tree, COI sequences of the 28 samples were uploaded to NCBI, and GenBank accession numbers were subsequently obtained (Table 2).

Table 2. Detailed information about the epizoans used to build the phylogenetic tree.

Species Number	Authority	Collection Locality	Latitude, Longitude	GenBank Information	Source	Specimen Location	Reference
2021SYSAe1, 2021SYSAe2, 2021SYSAe3, 2021SYSAe4, 2021SYSAe5, 2021SYSAe6, 2021SYSAe7, 2021SYSAe8	This study	Southern Yellow Sea, China	33.4283° N, 121.4723° E	OM714522, OM714523, OM723195, OM714524, OM714525, OM723196, OM723197, OM723198	This study	Shanghai Ocean University	This study
2021SYSBe1, 2021SYSBe2, 2021SYSBe3	This study	Southern Yellow Sea, China	33.0997° N, 121.6630° E	OM665403, OM723205, OM714526	This study	Shanghai Ocean University	This study
2021SYSCe1, 2021SYSCe2, 2021SYSCe3, 2021SYSCe4, 2021SYSCe5, 2021SYSCe6, 2021SYSCe7	This study	Southern Yellow Sea, China	32.7661° N, 121.7887° E	OM723199, OM723200, OM723201, OM714527, OM701800, OM701801, OM701802	This study	Shanghai Ocean University	This study
2021SYSDe1, 2021SYSDe2, 2021SYSDe3, 2021SYSDe4, 2021SYSDe5, 2021SYSDe6, 2021SYSDe7, 2021SYSDe8, 2021SYSDe9, 2021SYSDe10	This study	Southern Yellow Sea, China	32.8477° N, 121.8477° E	OM723206, OM723207, OM714528, OM723202, OM723203, OM714529, OM723204, OM701797, OM701798, OM701799	This study	Shanghai Ocean University	This study
17	*Ampithoe lacertosa*	Zhoushan, China	30.7400° N, 122.8300° E	OK480914	NCBI	Shanghai Ocean University	[39]
SFAM13-003	*Ampithoe helleri*	Minho, Portugal	41.6940° N, 8.8510° W	KX223985	NCBI	University of Minho	[71]
YSGT2021	*Apohyale* sp.	Southern Yellow Sea, China	36.1369° N, 121.4222° E	OK180438	NCBI	Ministry of Natural Resources	[68]
BCAMP0132	*Apohyale* cf. *pugettensis*	British Columbia, Canada	48.8580° N, 125.1600° W	MG315613	NCBI	University of Guelph	[72]
NRM-CGI-000108	*Idotea metallica*	Helgoland Island, Germany	54.1726° N, 7.8791° E	KU530515	NCBI	Swedish Museum of Natural History	[73]
NRM-CGI-000072	*Idotea emarginata*	Helgoland Island, Germany	54.1726° N, 7.8791° E	KU530492	NCBI	Swedish Museum of Natural History	[73]
AP312	*Peramphithoe tea*	Pado-ri, South Korea	36.705° N, 126.13° E	JN575608	NCBI	Inha University	[74]
H38	*Oratosquilla oratoria*	Lianyungang, China	N.A.	JX522527	NCBI	Yancheng Teachers University	[70]

Note: N.A. means no information.

3. Results

3.1. Identification of Macroalgae

The floating macroalgae collected from the four stations were morphologically identified as *Sargassum* sp., which had the main branch and branched structure (Figure 2a,b), and secondary branches that grew out of the extended leaf axils of the main branch. The top of the thalli had split leaves, and the thalli had a distinct gas bladder structure (Figure 2b,c). The gas bladders grew from the leaf axils and were cylindrical, with spikes at both ends (Figure 2c), and they were often arranged in racemose form. Very few thalli had receptacles, but when present, they were cylindrical and long; both ends were thin (Figure 2d) and grew from the terminal or inner area of the leaf axils [53]. Based on these morphological observations, the samples were preliminarily identified as *S. horneri* (Section Spongocarpus) [53].

Figure 2. (**a**) Morphology of *Sargassum* thalli collected in the Southern Yellow Sea; (**b**) Blades; (**c**) Gas bladders; (**d**) Receptacles.

The length of the COI gene sequence obtained from Sangon Biotechnology was about 630 base pairs (bp). After checking and proofreading these sequences, the COI gene sequences of the 12 samples were obtained (each sequence length was 596 bp), available at GenBank, accession numbers are OM444204–OM444215 (Table 1). A comparison of the sequences with those in the NCBI database using BLAST showed that these samples had 100% similarity with *S. horneri*. The MCL phylogenetic tree further supported the molecular biology identification results. All 12 sequences clustered with *S. horneri* (KC782896 and KY047195) as the main clade (marked with orange), which was different from the clade of *S. fusiforme*, *S. confusum*, *S. fallax*, *S. muticum*, and *S. thunbergii*, with high genetic distance (Figure 3).

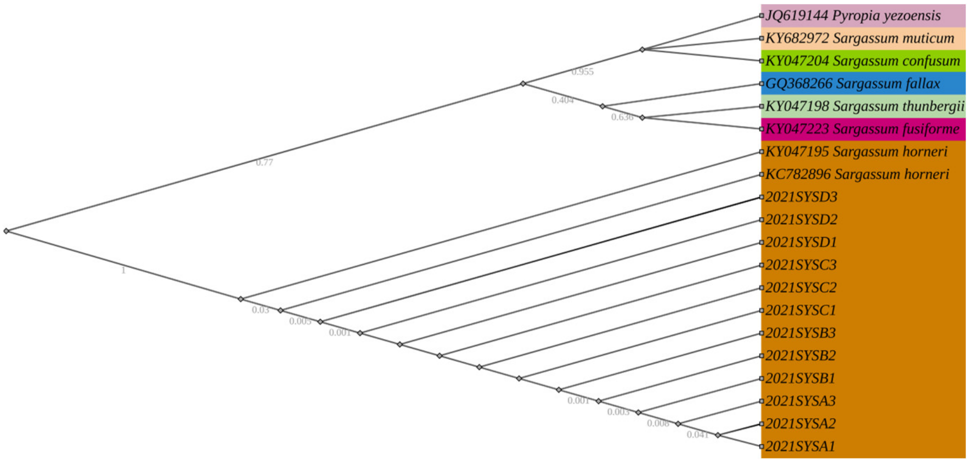

Figure 3. MCL phylogenetic tree of the golden tide macroalgae analyzed in this study; 1000 bootstrap replicates were used to verify the support rate of each node in this tree.

Based on the abovementioned detailed morphological and molecular biology identification, the golden tide macroalgae that accumulated in the SYS in the offshore sea area of

Jiangsu Province in March 2021 was *S. horneri*. The taxonomy of this species is Chromista, Ochrophyta, Phaeophyceae, Fucales, Sargassaceae, *Sargassum* [53].

3.2. Species Composition and Biological Parameters of Epizoans

Morphological and molecular identification results showed that the collected epizoans consisted of four species with the following taxonomy (Table 3, Figure 4). Taxonomic status and distribution are referred to at the Global Biodiversity Information Facility (https://www.gbif.org/, accessed on 10 February 2023). There are fewer reports about these species on floating *S. horneri* in SYS.

Table 3. Taxonomy, major morphological features, and distribution of epizoans.

Species	Taxonomic Status	Major Morphological Features	Distribution
Ampithoe lacertosa Spence Bate, 1858	Animalia; Arthropoda; Malacostraca; Amphipoda; Ampithoidae; *Ampithoe* Leach, 1814	The first and second antennae are almost equal in length. The gnathopods are sub-chelate, the second is larger than the first. The third pereiopod is shorter. The exopodite of the third uropod has hooked terminal spines and the tip of the endopodite has setae [66].	USA, Canada, Japan, South Korea, and China
Apohyale sp.	Animalia; Arthropoda; Malacostraca; Amphipoda; Hyalidae; *Apohyale* Bousfield & Hendrycks, 2002	The second antennae is slightly longer than the first, and accessory flagellum is absent. The first gnathopod is smaller than the second. The third uropod is not segmented, and the telson is cleft [68].	China [68]
Idotea metallica Bosc, 1802	Animalia; Arthropoda; Malacostraca; Isopoda; Idoteidae; *Idotea* Fabricius, 1798	The body is wide and flat. The thoracic segments are almost the same length. The number of segments of the second antennae is more. The abdomen is generally composed of 3 sections, of which each side of the base of the last section has a side suture [65].	USA, Japan, Mexico, Canada, Australia, Germany, France, U.K., Brazil, New Zealand, Spain, and Sweden
Peramphithoe tea J.L.Barnard, 1965	Animalia; Arthropoda; Malacostraca; Amphipoda; Ampithoidae; *Sunamphitoe* Spence Bate, 1857	The second gnathopod is larger than the first. Pereiopods 5~7 are similar to each other. The tip of the exopodite of the third uropod has 2 hooked spines, the endopodite has small spines and terminal setae, and the telson is complete [67].	South Korea, Canada, and the USA

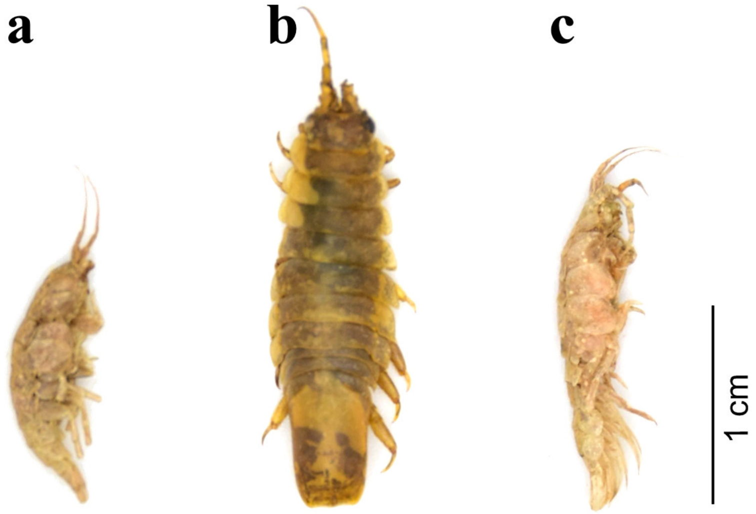

Figure 4. Morphology of the collected epizoans: (**a**) *Apohyale* sp.; (**b**) *Idotea metallica*; (**c**) *Peramphithoe tea*.

The length of COI standard gene sequences obtained from Sangon Biotechnology were 647, 606, 626, and 653 bp, respectively. The BLAST results of the 28 samples showed the similarity of the obtained sequences with the sequences of *A. lacertosa*, *Apohyale* sp., *I. metallica*, and *P. tea* from NCBI were 100%, 99.17–99.5%, 97.7%, and 99.39–99.84%. Thus, the epizoans collected from the four stations were 1 *A. lacertosa* (accounting for 3.6%), 13 *Apohyale* sp. (46.4%), 6 *I. metallica* (21.4%), and 8 *P. tea* (28.6%).

Table 2 provides details about the GenBank accession numbers for the epizoans. The MCL phylogenetic tree supported the molecular biology identification results. The sequence of *A. lacertosa* clustered with OK480914 (*A. lacertosa*) as the main clade (marked with gray), which was not the same clade as that of *A. helleri* (KX223985). The 13 sequences of *Apohyale* sp. clustered with OK180438 (*Apohyale* sp.) as the main clade (marked with orange) and were not in the same clade as *A. Pugettensis* (MG315613). The six sequences of *I. metallica* clustered with KU530515 (*I. metallica*) as the main clade (marked with green), which was not the same clade as that of *I. emarginata* (KU530492). The eight sequences of *P. tea* clustered with JN575608 (*P. tea*) as the main clade (marked with blue), and the genetic distance between different species was large (Figure 5). In summary, the richness of epizoans from the floating golden tide macroalgae in the SYS in March 2021 included *A. lacertosa*, *Apohyale* sp., *I. metallica*, and *P. tea*.

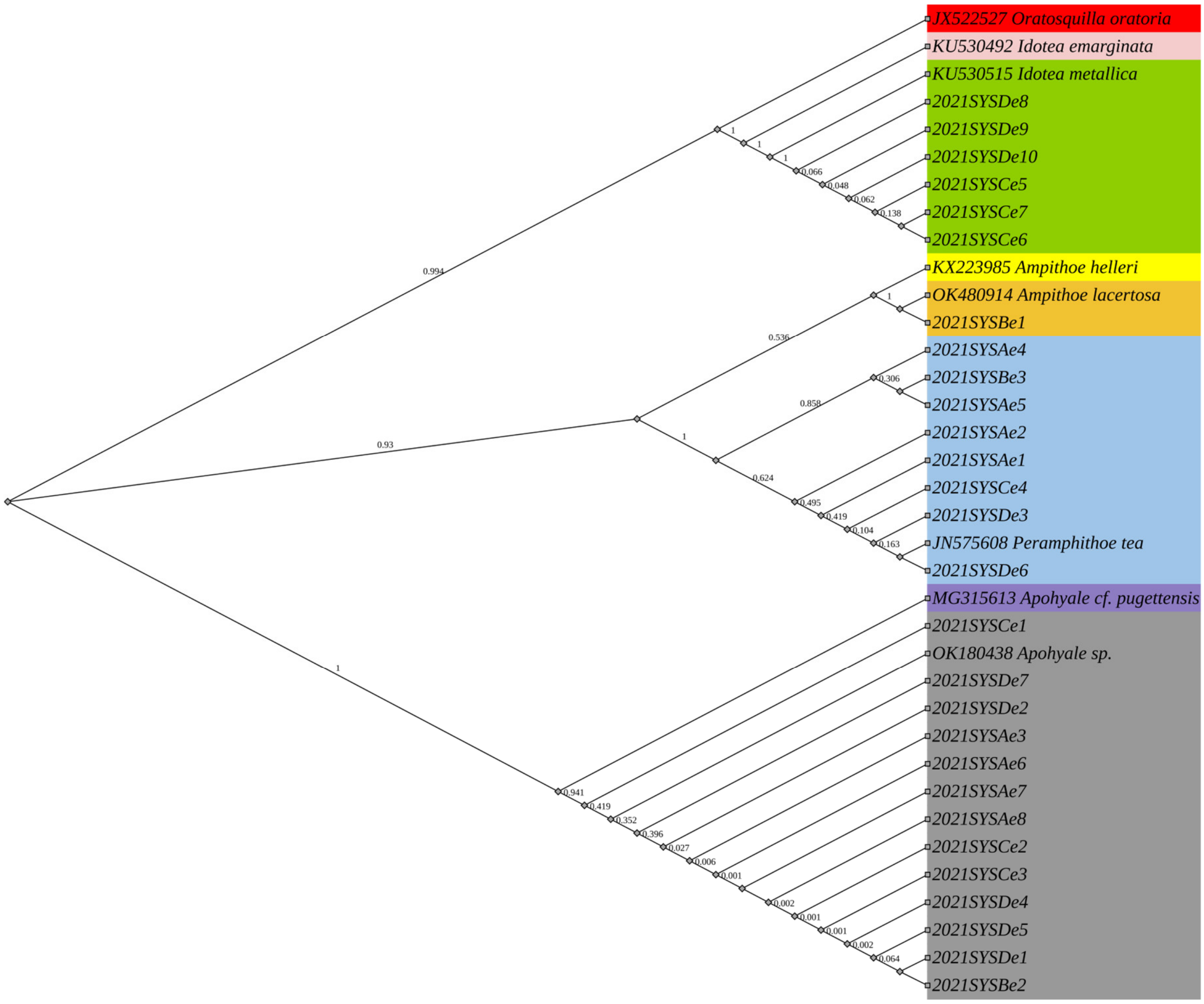

Figure 5. MCL phylogenetic tree of the epizoans analyzed in this study; 1000 bootstrap replicates were used to verify the support rate of each node in this tree.

The frequency of occurrence and fresh weight of epizoans from each station were counted and measured (Figures 6 and 7). The fresh weight of amphipods ranged from 0.0037 g to 0.0420 g each, and the weight of isopods ranged from 0.0222 g to 0.6592 g (Figure 7). The eight samples collected from the 2021SYSA station consisted of four *Apohyale* sp. (accounting for 50%, 0.0044 ~ 0.0204 g) and four *P. tea* (50%, 0.0112 ~ 0.0304 g) (Figures 6 and 7). One *A. lacertosa* (accounting for 33.3%, 0.0047 g), one *Apohyale* sp. (33.3%, 0.0420 g) and one *P. tea* (33.4 %, 0.0078 g) were collected from the 2021SYSB station (Figures 6 and 7). The seven samples collected from the 2021SYSC station included three *Apohyale* sp. (42.9%, 0.0112 ~ 0.0248 g), three *I. metallica* (42.9%, 0.1015 ~ 0.6592 g), and one *P. tea* (14.2%, 0.0143 g) (Figures 6 and 7). The samples collected from the 2021SYSD station consisted of five *Apohyale* sp. (50%, 0.0037 ~ 0.0186 g), three *I. metallica* (30%, 0.0222 ~ 0.1685 g), and two *P. tea* (20%, 0.0056 ~ 0.0278 g) (Figures 6 and 7). *Apohyale* sp. and *P. tea* were found at all stations and the frequency of occurrence is higher, while *A. lacertosa* is only found at 2021SYSB (Figure 6).

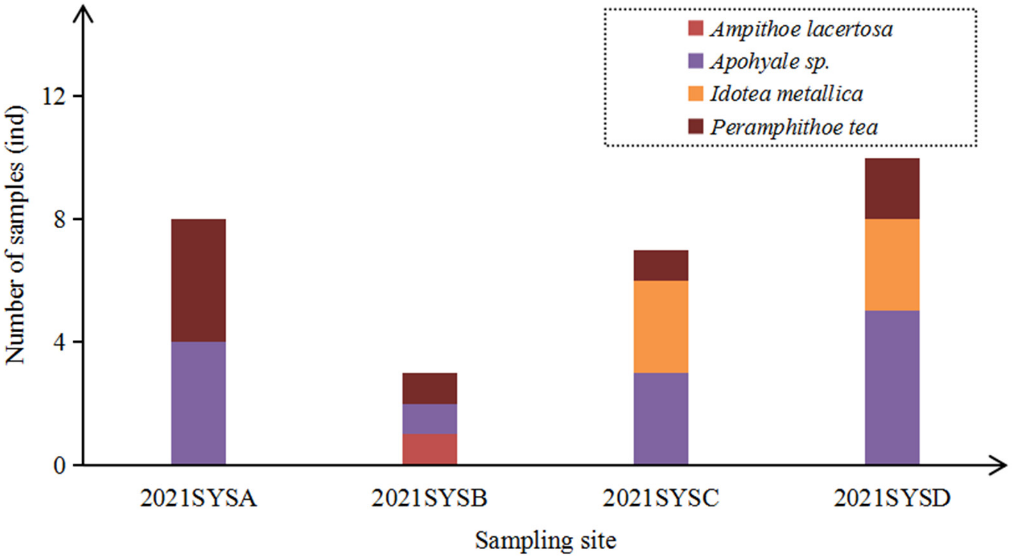

Figure 6. The occurrence frequency of epizoans from each site.

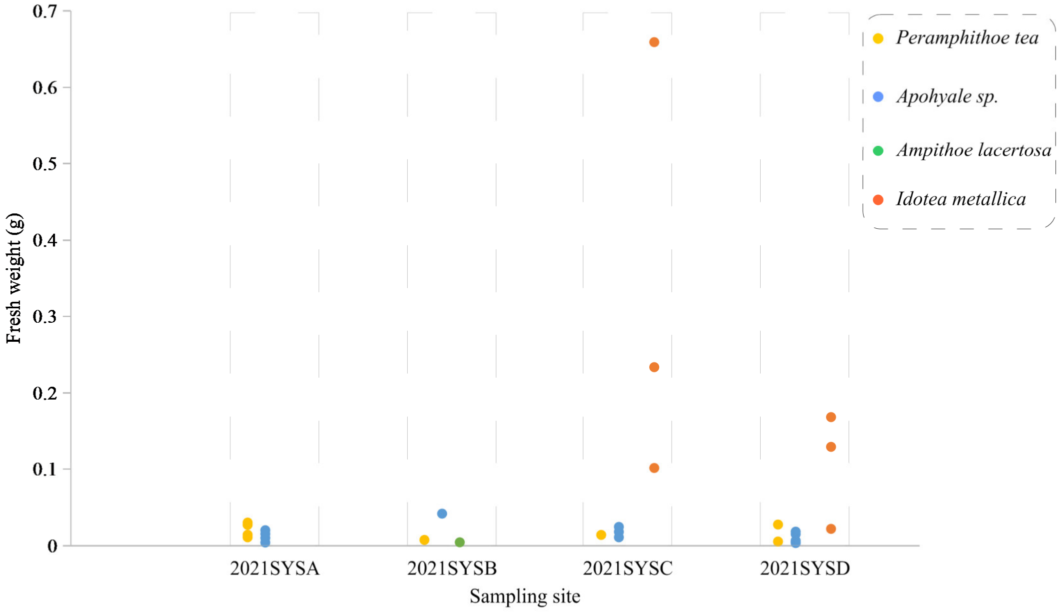

Figure 7. Scatter graph of fresh weight of epizoans from each site.

4. Discussion

4.1. Golden Tides in the SYS and the Origin of Blooms

Golden tides are caused by *Sargassum* spp. in USA, Mexico, Japan, China, South Korea, and Australia [18]. The distribution and coverage areas of golden tides in the SYS of China have been increasing in the recent decade. In 2013, 2015, 2016, 2017, and 2020, large-scale *S. horneri* blooms were observed in the Yellow Sea [17,18,30,75,76]. These results were consistent with the golden tide macroalgae reported in the SYS in previous years [39], with no changes within the dominant bloom-forming species.

The source of large-scale *S. horneri* blooms in the Yellow Sea remains unknown, although some evidence points toward two main sources originating in Zhejiang and Shandong Provinces [77–79]. During the investigation, local fishermen indicated that the spring-floating *S. horneri* may come from the coastal areas of Shandong Province and the eastern sea area. Meanwhile, some researchers speculate that benthic populations of *S. horneri* in the coastal waters of South Korea [78] could be the source of the *S. horneri* blooms observed in the Yellow Sea. Generally, suitable climatic conditions can promote large-scale proliferation and bloom of benthic *S. horneri* in seaweed beds and intertidal zones. Human activities, such as removing macroalgae from aquaculture rafts may promote the process of *S. horneri* floating into the open sea area. Over several decades, seaweed farmers in Shandong and Zhejiang Province periodically removed *S. horneri* from the aquaculture infrastructure (kelp and mussel rafts) during aquaculture activities. In addition, *S. horneri* as the dominant species were also used in marine ranching in Shandong, Zhejiang, Fujian Province, etc., which may provide the initial biomass for the golden tide. It is worth noting that more evidence is required for these perceptions, which deserve subsequent attention and in-depth study. The special gas bladder structure of macroalgae is conducive to floating to other sea areas with ocean currents to settle and gradually form local intertidal zone populations [80], which in turn causes new blooms locally. Therefore, the large scale of *S. horneri* entering the SYS and its origin deserve attention.

4.2. Potential Effects of Epizoans Entering the SYS

Rafting on artificial and natural objects is an important dispersal mechanism for coastal invertebrates [81]. Rafting on macroalgae with gas bladders, which can provide a food source and long survival time [81], is an effective marine dispersal mechanism for epizoans that may have a profound impact on coastal biodiversity [82]. *I. metallica* is an obligate rafter (as opposed to a facultative rafter) that occurs in the Atlantic, Pacific, Mediterranean, and Black Sea [83]. For this species, rafting is a specific and evolutionarily developed life strategy [84]. The low feeding rates, high assimilation efficiency, and total lipid contents of *I. metallica* allow it to adapt to long-distance rafting [84]. *I. metallica* may be used as a sensitive indicator for climate change due to its high natural capacity for dispersal and response to changing environmental conditions [85]. Isopods and amphipods, as important community members in drifting macroalgae [39,83,85], may have potential ecological value. On the one hand, epizoans can indicate environmental changes (e.g., warming) in SYS. On the other hand, epizoans can drift long distances on the macroalgae, which can be used to trace the origin of *S. horneri* in SYS by comparing the genetic relationships of different epizoan populations. However, there are few studies on the epizoans from floating *S. horneri* in the Yellow Sea, and population genetic analysis could be carried out with more epizoan sequences in the future.

4.3. Relevance of Epizoans in Marine Ecosystems

Small herbivorous marine invertebrates, also known as mesograzers, are primary consumers that are distributed in almost all marine vegetation habitats, and the top-down control by mesograzers has an important impact on the seaweed communities [37,86] and periphyton biomass [86,87]. Due to their special status in the marine food web, mesograzers have important impacts on both primary producers and higher consumers, and trophically link primary production with fish [88,89].

In this study, we reported four crustacean species belonging to the genera *Peramphithoe*, *Apohyale*, *Ampithoe*, and *Idotea* on the floating *S. horneri* in China for the first time. Sano et al. (2003) [90] also found epizoans such as *Perampithoe*, *Amphithoe*, and *Idotea* on floating *Sargassum* spp. Epizoans fed on floating macroalgae and plankton, and omnivorous epizoans (e.g., *Idotea*) also fed on crustaceans. In turn, these epizoans are preyed on by other carnivores and large predators [82,83] (Figure 8). In summary, these epizoans and floating macroalgae found in this study constitute a floating community, and herbivorous and omnivorous epizoans promote energy flows that connect primary production by macroalgae and epiphytes with carnivores [90] (Figure 8). Certainly, there may be more epizoans on the floating *S. horneri* in SYS. The above four species were found in this study, and the ecological flowchart will be improved if more species are found in the future. However, we could not quantify the biomass and density of epizoans because the density of *S. horneri* was sparse and its surface was difficult to salvage when using common nets for collection. Moreover, grappling hooks may have caused epizoans to flee the algal mat. These issues precluded quantitative analysis in our study. In this study, the sample was pulled out of the water by nets and hooks. The epizoan on the macroalgae could be startled, causing an underestimation of the epizoan biomass. To better quantify the epizoan biomass, the sample methods need be improved in future study.

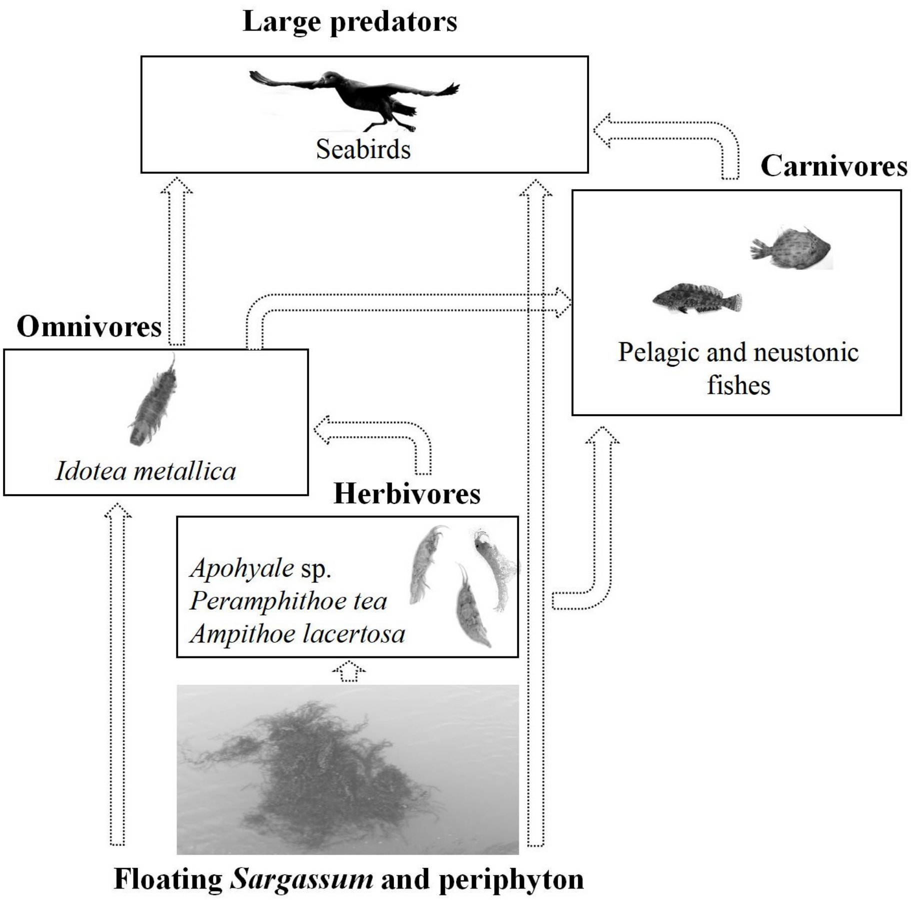

Figure 8. Flowchart of the floating *Sargassum* ecosystem.

Grazing pressure by mesograzers can influence the abundance of macroalgae in eutrophic waters [91,92]. *Apohyale* sp. found in this study is considered to be able to reduce the biomass and growth of floating macroalgae [68]. *Idotea* spp. are observed as active grazers of macroalgae [91]. This study obtained the body weights of each epizoan on floating *S. horneri* at four stations in the SYS, however, the productivity of these epizoans requires further assessment. Meanwhile, whether the grazing effect of epizoans can decrease the growth of *S. horneri* during the bloom stage when they are increasing requires further study.

Yu et al. (2021) [93] cut ~3 cm long segments from the top of the main branch or lateral branch of *S. horneri* for culture and found that the carbon and nitrogen contents of the thalli were higher during the growth phase, which might help to resist the stress of unfavorable environments. Wang et al. (2020) [94] reported that low culture density was beneficial to the growth of *S. horneri*, indicating that *S. horneri* could grow rapidly when floating on the sea surface. Epizoans may graze macroalgae into small thalli, which would quickly grow under suitable temperature and light conditions.

Furthermore, these epizoans may promote pollination in seaweed. Lavaut et al. (2022) [95] found that *Idotea balthica* can significantly increase pollination in *Gracilaria gracilis* by directly transporting spermatia of the thalli. Spermatia can adhere to the cuticles of *Idotea* that can be transmitted to female thalli by *Idotea* [95]. A similar process may also occur in the interaction between *S. horneri* and epizoans (i.e., *I. metallica* in this study). *Idotea* may also be able to transport male gametes to promote the reproduction of *S. horneri*, and contribute to the bloom development.

The SYS is rich in nutrients from different sources [96,97], and suitable hydrological conditions may promote the large-scale proliferation of *S. horneri*. The interaction between epizoans may also be an important variable. These processes may partially explain the golden tides' large coverage and distribution areas. However, these hypotheses need to be further studied and confirmed. The initial biomass of floating *S. horneri* entering the sea and environmental parameters are the primary factors determining the scale of the golden tide, but epizoans drifting into sea areas with *S. horneri* blooms likely will have a certain impact on the local ecosystem, and the related research still has a long way to go.

5. Conclusions

Combined with morphological and molecular identification, *S. horneri* was found to be the dominant species of the 2021 golden tide in the SYS. Meanwhile, molecular identification based on COI indicated that 28 epizoans on *S. horneri* were composed of four crustaceans: *A. lacertosa*, *I. metallica*, *Apohyale* sp., and *P. tea*. Among them, *Apohyale* sp. and *P. tea* were found at all stations, while *A. lacertosa* with a low frequency of occurrence. Epizoans are an important component of drifting macroalgal ecosystems, which are predicted to have a certain impact on local coastal ecosystems and have potential application value. This study provides a reference for future studies on the ecological significance of golden tide macroalgae in the SYS.

Author Contributions: Conceptualization, investigation and methodology, J.X., C.L., Y.T. and J.L. (Jinlin Liu); resource, T.W.; writing—original draft, J.X. and J.L. (Jinlin Liu); writing—review and editing, funding acquisition, J.L. (Jinlin Liu), J.Z. and J.L. (Ji Li). All authors have read and agreed to the published version of the manuscript.

Funding: This research was financially supported by Shanghai Frontiers Science Center of Polar Science (SCOPS), Technology Innovation Center for Land Spatial Eco-restoration in Metropolitan Area, M.N.R. (CXZX202006), Natural Science Foundation of Shanghai (21ZR1427400), Project of Key Laboratory of Marine Ecological Monitoring and Restoration Technologies, M.N.R. (202003), and National Natural Science Foundation of China (41576163).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

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

References

1. Fernandez, C. Boom-bust of *Sargassum muticum* in northern Spain: 30 years of invasion. *Eur. J. Phycol.* **2020**, *55*, 285–295. [CrossRef]
2. Wang, H.; Wang, G.C.; Gu, W.H. Macroalgal blooms caused by marine nutrient changes resulting from human activities. *J. Appl. Ecol.* **2020**, *57*, 766–776. [CrossRef]
3. Joniver, C.F.H.; Photiades, A.; Moore, P.J.; Winters, A.L.; Woolmer, A.; Adams, J.M.M. The global problem of nuisance macroalgal blooms and pathways to its use in the circular economy. *Algal. Res.* **2021**, *58*, 102407. [CrossRef]
4. Kang, E.J.; Han, A.R.; Kim, J.H.; Kim, I.N.; Lee, S.; Min, J.O.; Nam, B.R.; Choi, Y.J.; Edwards, M.S.; Diaz-Pulido, G.; et al. Evaluating bloom potential of the green-tide forming alga *Ulva ohnoi* under ocean acidification and warming. *Sci. Total Environ.* **2021**, *769*, 144443. [CrossRef] [PubMed]
5. Bermejo, R.; Golden, N.; Schrofner, E.; Knöller, K.; Fenton, O.; Serrão, E.; Morrison, L. Biomass and nutrient dynamics of major green tides in Ireland: Implications for biomonitoring. *Mar. Pollut. Bull.* **2022**, *175*, 113318. [CrossRef]
6. Guo, X.N.; Zhu, A.; Chen, R.S. China's algal bloom suffocates marine life. *Science* **2021**, *373*, 751. [CrossRef]
7. Liu, J.L.; Li, C.X.; Xia, J.; Sun, Y.Q.; Tong, Y.C.; Zhang, J.H.; Zhao, S.; Zhuang, M.M.; He, P.M. Epizoic *Ulva* attached to intertidal animals in the Subei intertidal zone are not the additional source of the famed Yellow Sea green tides. *J. Sea. Res.* **2021**, *174*, 102065. [CrossRef]
8. Liu, J.L.; Xia, J.; Zhuang, M.M.; Zhang, J.H.; Yu, K.F.; Zhao, S.; Sun, Y.Q.; Tong, Y.C.; Xia, L.H.; Qin, Y.T.; et al. Controlling the source of green tides in the Yellow Sea: NaClO treatment of *Ulva* attached on *Pyropia* aquaculture rafts. *Aquaculture* **2021**, *535*, 736378. [CrossRef]
9. Vazquez-Delfin, E.; Freile-Pelegrin, Y.; Salazar-Garibay, A.; Serviere-Zaragoza, E.; Mendez-Rodriguez, L.C.; Robledo, D. Species composition and chemical characterization of *Sargassum* influx at six different locations along the Mexican Caribbean coast. *Sci. Total Environ.* **2021**, *795*, 148852. [CrossRef]
10. Liu, J.L.; Tong, Y.C.; Xia, J.; Sun, Y.Q.; Zhao, X.H.; Sun, J.Y.; Zhao, S.; Zhuang, M.M.; Zhang, J.H.; He, P.M. *Ulva* macroalgae within local aquaculture ponds along the estuary of Dagu River, Jiaozhou Bay, Qingdao. *Mar. Pollut. Bull.* **2022**, *174*, 113243. [CrossRef]
11. Wang, S.Y.; Huo, Y.Z.; Zhang, J.H.; Cui, J.J.; Wang, Y.; Yang, L.L.; Zhou, Q.Y.; Lu, Y.W.; Yu, K.F.; He, P.M. Variations of dominant free-floating *Ulva* species in the source area for the world's largest macroalgal blooms, China: Differences of ecological tolerance. *Harmful Algae* **2018**, *74*, 58–66. [CrossRef]
12. Hiraoka, M. Massive *Ulva* Green Tides Caused by Inhibition of Biomass Allocation to Sporulation. *Plants* **2021**, *10*, 2482. [CrossRef]
13. Liu, D.Y.; Keesing, J.K.; Xing, Q.U.; Shi, P. World's largest macroalgal bloom caused by expansion of seaweed aquaculture in China. *Mar. Pollut. Bull.* **2009**, *58*, 888–895. [CrossRef]
14. Han, H.B.; Li, Y.; Ma, X.J.; Song, W.; Wang, Z.L.; Zhang, X.L. Factors influencing the spatial and temporal distributions of green algae micro-propagules in the coastal waters of Jinmenghaiwan, Qinhuangdao, China. *Mar. Pollut. Bull.* **2022**, *175*, 113328. [CrossRef]
15. Sun, Y.Q.; Liu, J.L.; Xia, J.; Tong, Y.C.; Li, C.X.; Zhao, S.; Zhuang, M.M.; Zhao, X.H.; Zhang, J.H.; He, P.M. Research development on resource utilization of green tide algae from the Southern Yellow Sea. *Energy Rep.* **2022**, *8*, 295–303. [CrossRef]
16. Wu, H.L.; Feng, J.C.; Li, X.S.; Zhao, C.Y.; Liu, Y.H.; Yu, J.T.; Xu, J.T. Effects of increased CO_2 and temperature on the physiological characteristics of the golden tide blooming macroalgae *Sargassum horneri* in the Yellow Sea, China. *Mar. Pollut. Bull.* **2019**, *146*, 639–644. [CrossRef]
17. Zhang, J.H.; Shi, J.T.; Gao, S.; Huo, Y.Z.; Cui, J.J.; Shen, H.; Liu, G.Y.; He, P.M. Annual patterns of macroalgal blooms in the Yellow Sea during 2007–2017. *PLoS ONE* **2019**, *14*, e0210460. [CrossRef]
18. Liu, J.L.; Xia, J.; Zhuang, M.M.; He, P.M.; Sun, Y.Q.; Tong, Y.C.; Zhao, S.; Zhang, J.H. Golden seaweed tides accumulated in *Pyropia* aquaculture areas are becoming a normal phenomenon in the Yellow Sea of China. *Sci. Total Environ.* **2021**, *774*, 145726. [CrossRef]
19. Xiao, J.; Wang, Z.L.; Liu, D.Y.; Fu, M.Z.; Yuan, C.; Yan, T. Harmful macroalgal blooms (HMBs) in China's coastal water: Green and golden tides. *Harmful Algae* **2021**, *107*, 102061. [CrossRef]
20. Gower, J.F.R.; King, S.A. Distribution of floating *Sargassum* in the Gulf of Mexico and the Atlantic Ocean mapped using MERIS. *Int. J. Remote Sens.* **2011**, *32*, 1917–1929. [CrossRef]
21. Smetacek, V.; Zingone, A. Green and golden seaweed tides on the rise. *Nature* **2013**, *504*, 84–88. [CrossRef] [PubMed]
22. Byeon, S.Y.; Oh, H.J.; Kim, S.; Yun, S.H.; Kang, J.H.; Park, S.R.; Lee, H.J. The origin and population genetic structure of the 'golden tide' seaweeds, *Sargassum horneri*, in Korean waters. *Sci. Rep.* **2019**, *9*, 7757. [CrossRef]
23. Gao, G.; Zhong, Z.; Zhou, X.; Xu, J. Changes in morphological plasticity of *Ulva prolifera* under different environmental conditions: A laboratory experiment. *Harmful Algae* **2016**, *59*, 51–58. [CrossRef] [PubMed]
24. Feng, Z.; Zhang, T.; Shi, H.; Gao, K.; Xu, J.; Wang, J.; Wang, R.; Li, X.; Gao, G. Microplastics in bloom-forming macroalgae: Distribution, characteristics and impacts. *J. Hazard. Mater.* **2020**, *397*, 122752. [CrossRef] [PubMed]
25. Feng, Z.; Zhang, T.; Wang, J.; Huang, W.; Wang, R.; Xu, J.; Fu, G.; Gao, G. Spatio-temporal features of microplastics pollution in macroalgae growing in an important mariculture area, China. *Sci. Total Environ.* **2020**, *719*, 137490. [CrossRef]
26. An, D.Y.; Yu, D.F.; Zheng, X.Y.; Zhou, Y.; Meng, L.; Xing, Q.G. Monitoring the Dissipation of the Floating Green Macroalgae Blooms in the Yellow Sea (2007–2020) on the Basis of Satellite Remote Sensing. *Remote Sens.* **2021**, *13*, 3811. [CrossRef]
27. Zhang, T.; Wang, J.X.; Liu, D.X.; Sun, Z.W.; Tang, R.K.; Ma, X.; Feng, Z.H. Loading of microplastics by two related macroalgae in a sea area where gold and green tides occur simultaneously. *Sci. Total Environ.* **2022**, *814*, 152809. [CrossRef]

28. Liu, F.; Pan, J.; Zhang, Z.S.; Moejes, F.W. Organelle genomes of *Sargassum confusum* (Fucales, Phaeophyceae): mtDNA vs cpDNA. *J. Appl. Phycol.* **2018**, *30*, 2715–2722. [CrossRef]
29. Chen, Y.L.; Wan, J.H.; Zhang, J.; Ma, Y.J.; Wang, L.; Zhao, J.H.; Wang, Z.Z. Spatial-Temporal Distribution of Golden Tide Based on High–Resolution Satellite Remote Sensing in the South Yellow Sea. *J. Coastal Res.* **2019**, *90*, 221–227. [CrossRef]
30. Zhang, J.H.; Ding, X.W.; Zhuang, M.M.; Wang, S.Y.; Chen, L.; Shen, H.; He, P.M. An increase in new *Sargassum* (Phaeophyceae) blooms along the coast of the East China Sea and Yellow Sea. *Phycologia* **2019**, *58*, 374–381. [CrossRef]
31. Zhuang, M.M.; Liu, J.L.; Ding, X.W.; He, J.Z.; Zhao, S.; Wu, L.J.; Gao, S.; Zhao, C.Y.; Liu, D.Y.; Zhang, J.H.; et al. *Sargassum* blooms in the East China Sea and Yellow Sea: Formation and management. *Mar. Pollut. Bull.* **2021**, *162*, 111845. [CrossRef]
32. Resiere, D.; Valentino, R.; Neviere, R.; Banydeen, R.; Gueye, P.; Florentin, J.; Cabie, A.; Lebrun, T.; Megarbane, B.; Guerrier, G.; et al. *Sargassum* seaweed on Caribbean islands: An international public health concern. *Lancet* **2018**, *392*, 2691. [CrossRef]
33. Liu, F.; Pang, S.J.; Li, X.; Li, J. Complete mitochondrial genome of the brown alga *Sargassum horneri* (Sargassaceae, Phaeophyceae): Genome organization and phylogenetic analyses. *J. Appl. Phycol.* **2015**, *27*, 469–478. [CrossRef]
34. Liu, F.; Liu, X.F.; Wang, Y.; Lin, Z.; Moejes, F.W.; Sun, S. Insights on the *Sargassum horneri* golden tides in the Yellow Sea inferred from morphological and molecular data. *Limnol. Oceanogr.* **2018**, *63*, 1762–1773. [CrossRef]
35. Li, J.J.; Liu, Z.Y.; Zhong, Z.H.; Zhuang, L.C.; Bi, Y.X.; Qin, S. Limited Genetic Connectivity Among *Sargassum horneri* (Phaeophyceae) Populations in the Chinese Marginal Seas Despite Their high Dispersal Capacity. *J. Phycol.* **2020**, *56*, 994–1005. [CrossRef]
36. Mei, X.Y.; Wu, C.H.; Zhao, J.; Yan, T.; Jiang, P. Community Structure of Bacteria Associated with Drifting *Sargassum horneri*, the Causative Species of Golden Tide in the Yellow Sea. *Front. Microbiol.* **2019**, *10*, 1192. [CrossRef]
37. Duffy, J.E.; Hay, M.E. Strong impacts of grazing amphipods on the organization of a benthic community. *Ecol. Monogr.* **2000**, *70*, 237–263. [CrossRef]
38. Jiang, R.J.; Zhang, S.Y.; Bi, Y.X.; Wang, Z.H. Food sources of small invertebrates in the macroalgal bed of Gouqi Island. *J. Fish. China* **2015**, *39*, 1487–1498. [CrossRef]
39. Liu, J.L.; Zhuang, M.M.; Zhao, L.J.; Liu, Y.K.; Wen, Q.L.; Fu, M.L.; Yu, K.F.; Zhang, J.H.; He, P.M. Taxonomy and Genetic Diversity of Amphipods Living on *Ulva lactuca* L. from Gouqi Coast, China. *Pac. Sci.* **2020**, *74*, 137–146. [CrossRef]
40. McDonald, P.S.; Bingham, B.L. Comparing macroalgal food and habitat choice in sympatric, tube-building amphipods, *Ampithoe lacertosa* and *Peramphithoe humeralis*. *Mar. Biol.* **2010**, *157*, 1513–1524. [CrossRef]
41. Quillien, N.; Nordstrom, M.C.; Le Bris, H.; Bonsdorff, E.; Grall, J. Green tides on inter- and subtidal sandy shores: Differential impacts on infauna and flatfish. *J. Mar. Biol. Assoc. UK* **2018**, *98*, 699–712. [CrossRef]
42. Catenazzi, A.; Donnelly, M.A. Role of supratidal invertebrates in the decomposition of beach-cast green algae *Ulva* sp. *Mar. Ecol. Prog. Ser.* **2007**, *349*, 33–42. [CrossRef]
43. Schaal, G.; Leclerc, J.C.; Droual, G.; Leroux, C.; Riera, P. Biodiversity and trophic structure of invertebrate assemblages associated with understorey red algae in a Laminaria digitata bed. *Mar. Biol. Res.* **2016**, *12*, 513–523. [CrossRef]
44. Hebert, P.D.N.; Cywinska, A.; Ball, S.L.; DeWaard, J.R. Biological identifications through DNA barcodes. *Proc. R. Soc. B Biol. Sci.* **2003**, *270*, 313–321. [CrossRef] [PubMed]
45. Ward, R.D.; Zemlak, T.S.; Innes, B.H.; Last, P.R.; Hebert, P.D.N. DNA barcoding Australia's fish species. *Proc. R. Soc. B Biol. Sci.* **2005**, *360*, 1847–1857. [CrossRef]
46. Chen, L.P.; Chang, Y.G.; Li, J.; Wang, J.M.; Liu, J.L.; Zhi, Y.C.; Li, X.J. Application of DNA Barcoding in the Classification of Grasshoppers (Orthoptera: Acridoidea)—A Case Study of grasshoppers from Hebei Province, China. *Zootaxa* **2018**, *4497*, 99–110. [CrossRef]
47. Mattio, L.; Payri, C. Assessment of five markers as potential barcodes for identifying *Sargassum* subgenus *Sargassum* species (Phaeophyceae, Fucales). *Cryptogam Algol.* **2011**, *31*, 467–485.
48. Yip, Z.T.; Quek, R.Z.B.; Huang, D.W. Historical biogeography of the widespread macroalga *Sargassum* (Fucales, Phaeophyceae). *J. Phycol.* **2019**, *56*, 300–309. [CrossRef]
49. Yip, Z.T.; Quek, R.Z.B.; Low, J.K.Y.; Wilson, B.; Bauman, A.G.; Chou, L.M.; Todd, P.A.; Huang, D.W. Diversity and phylogeny of *Sargassum* (Fucales, Phaeophyceae) in Singapore. *Phytotaxa* **2018**, *369*, 200–210. [CrossRef]
50. Donald, K.M.; Preston, J.; Williams, S.T.; Reid, D.G.; Winter, D.; Alvarez, R.; Buge, B.; Hawkins, S.J.; Templado, J.; Spencer, H.G. Phylogenetic relationships elucidate colonization patterns in the intertidal grazers *Osilinus* Philippi, 1847 and *Phorcus* Risso, 1826 (Gastropoda: Trochidae) in the northeastern Atlantic Ocean and Mediterranean Sea. *Molec. Phylogenet. Evol.* **2012**, *62*, 35–45. [CrossRef]
51. Linse, K.; Jackson, J.A.; Malyutina, M.V.; Brandt, A. Shallow-Water Northern Hemisphere *Jaera* (Crustacea, Isopoda, Janiridae) Found on Whale Bones in the Southern Ocean Deep Sea: Ecology and Description of *Jaera tyleri* sp. nov. *PLoS ONE* **2014**, *9*, e93018. [CrossRef]
52. Ng, P.K.; Chiou, Y.S.; Liu, L.C.; Sun, Z.M.; Shimabukuro, H.; Lin, S.M. Phylogeography and genetic connectivity of the marine macro-alga *Sargassum ilicifolium* (Phaeophyceae, Ochrophyta) in the northwestern Pacific. *J. Phycol.* **2019**, *55*, 7–24. [CrossRef]
53. Tseng, C.K.; Lu, B.R. *Marine Algal Flora of China Tomus III Phaeophyta Fucales*; Science Press: Beijing, China, 2000; pp. 43–44.
54. Saunders, G.W. Applying DNA barcoding to red macroalgae: A preliminary appraisal holds promise for future applications. *Phil. Trans. R. Soc. B* **2005**, *360*, 1879–1888. [CrossRef]
55. Sherwood, A.R.; Kurihara, A.; Conklin, K.Y.; Sauvage, T.; Presting, G.G. The Hawaiian Rhodophyta Biodiversity Survey (2006–2010): A summary of principal findings. *BMC Plant Biol.* **2010**, *10*, 258. [CrossRef]

56. Du, G.Y.; Wu, F.F.; Guo, H.; Xue, H.F.; Mao, Y.X. DNA barcode assessment of Ceramiales (Rhodophyta) in the intertidal zone of the northwestern Yellow Sea. *Chin. J. Oceanol. Limnol.* **2015**, *33*, 685–695. [CrossRef]
57. Hall, T.A. BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp. Ser.* **1999**, *41*, 95–98. [CrossRef]
58. Niwa, K.; Sano, F.; Sakamoto, T. Molecular evidence of allodiploidy in F1 gametophytic blades from a cross between *Neopyropia yezoensis* and a cryptic species of the *Neopyropia yezoensis* complex (Bangiales, Rhodophyta) by the use of microsatellite markers. *Aquac. Rep.* **2020**, *18*, 100489. [CrossRef]
59. Tamura, K.; Stecher, G.; Kumar, S. MEGA11: Molecular Evolutionary Genetics Analysis Version 11. *Mol. Biol. Evol.* **2021**, *38*, 3022–3027. [CrossRef]
60. Tamura, K.; Nei, M. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Mol. Biol. Evol.* **1993**, *10*, 512–526. [CrossRef]
61. Ivica, L.; Peer, B. Interactive Tree Of Life (iTOL) v5: An online tool for phylogenetic tree display and annotation. *Nucleic Acids Res.* **2021**, *49*, W293–W296. [CrossRef]
62. Zhao, X.B.; Pang, S.J.; Shan, T.F.; Liu, F. Applications of three DNA barcodes in assorting intertidal red macroalgal flora in Qingdao, China. *J. Ocean Univ. China* **2013**, *12*, 139–145. [CrossRef]
63. Silberfeld, T.; Leigh, J.W.; Verbruggen, H.; Cruaud, C.; De Reviers, B.; Rousseau, F. A multi-locus time-calibrated phylogeny of the brown algae (Heterokonta, Ochrophyta, Phaeophyceae): Investigating the evolutionary nature of the "brown algal crown radiation". *Mol. Phylogenet. Evol.* **2010**, *56*, 659–674. [CrossRef] [PubMed]
64. Rizzo, C.; Genovese, G.; Morabito, M.; Faggio, C.; Pagano, M.; Spanò, A.; Zammuto, V.; Minicante, S.A.; Manghisi, A.; Cigala, R.M.; et al. Potential Antibacterial Activity of Marine Macroalgae against Pathogens Relevant for Aquaculture and Human Health. *J. Pure Appl. Microbiol.* **2017**, *11*, 1695–1706. [CrossRef]
65. Wei, C.D.; Chen, Y.S. *Fauna of Zhejiang Crustacea*; Zhejiang Science & Technology Press: Hangzhou, China, 1991; 481p.
66. Jiang, N.C.; Lu, J.P. *Field Internship Guidance of Zhejiang Coastal Zoology*; Zhejiang Universuty Press: Zhejiang, China, 2005; 184p.
67. Ren, X.Q. *Crustacea Amphipoda Gammaridea I, Fauna Sinica Invertebrata*; Science Press: Beijing, China, 2006; 588p.
68. Miao, X.X.; Xiao, J.; Fan, S.L.; Zang, Y.; Zhang, X.L.; Wang, Z.L. Assessing Herbivorous Impacts of *Apohyale* sp. on the *Ulva prolifera* Green Tide in China. *Front. Plant Sci.* **2021**, *12*, 795560. [CrossRef]
69. Yoshino, H.; Yamaji, F.; Ohsawa, T.A. Genetic structure and dispersal patterns in *Limnoria nagatai* (Limnoriidae, Isopoda) dwelling in non-buoyant kelps, *Eisenia bicyclis* and *E. arborea*, in Japan. *PLoS ONE* **2018**, *3*, e0198451. [CrossRef]
70. Zhang, D.Z.; Ding, G.; Zhou, T.T.; Yang, W.J.; Li, J.; Peng, X.; Tang, B.P. Genetic Diversity of *Oratosquilla oratoria* Populations in Yellow Sea. *Chin. J. Zool.* **2013**, *48*, 232–240. [CrossRef]
71. Lobo, J.; Ferreira, M.S.; Antunes, I.C.; Teixeira, M.A.L.; Borges, L.M.S.; Sousa, R.; Gomes, P.A.; Costa, M.H.; Cunha, M.R.; Costa, F.O. Contrasting morphological and DNA barcode-suggested species boundaries among shallow-water amphipod fauna from the southern European Atlantic coast. *Genome* **2017**, *60*, 147–157. [CrossRef]
72. Dewaard, J.R.; Hebert, P.D.N.; Humble, L.M. A Comprehensive DNA Barcode Library for the Looper Moths (*Lepidoptera*: *Geometridae*) of British Columbia, Canada. *PLoS ONE* **2011**, *6*, e18290. [CrossRef]
73. Panova, M.; Nygren, A.; Jonsson, P.R.; Leidenberger, S. A molecular phylogeny of the north-east Atlantic species of the genus *Idotea* (Isopoda) with focus on the Baltic Sea. *Zool. Scr.* **2017**, *46*, 188–199. [CrossRef]
74. Kim, Y.H.; Hong, S.S.; Conlan, K.E.; Lee, K.S. The genus *Peramphithoe* Conlan & Bousfield, 1982 from Korean waters (*Crustacea*: *Amphipoda*: *Ampithoidae*). *Zootaxa* **2012**, *3400*, 1–19. [CrossRef]
75. Kim, K.; Shin, J.; Kim, K.Y.; Ryu, J.H. Long-Term Trend of Green and Golden Tides in the Eastern Yellow Sea. *J. Coastal Res.* **2019**, *90*, 317–323. [CrossRef]
76. Xiao, J.; Wang, Z.L.; Song, H.J.; Fan, S.L.; Yuan, C.; Fu, M.Z.; Miao, X.X.; Zhang, X.L.; Su, R.G.; Hu, C.M. An anomalous bi-macroalgal bloom caused by *Ulva* and *Sargassum* seaweeds during spring to summer of 2017 in the western Yellow Sea, China. *Harmful Algae* **2020**, *93*, 101760. [CrossRef]
77. Qi, L.; Hu, C.M.; Wang, M.Q.; Shang, S.L.; Wilson, C. Floating algae blooms in the East China Sea. *Geophys. Res. Lett.* **2017**, *44*, 11501–11509. [CrossRef]
78. Huang, B.X.; Ding, L.P.; Qin, S.; Fu, W.T.; Lu, Q.Q.; Liu, Z.Y.; Pang, Y.L.; Li, X.L.; Sun, Z.M. The taxonomical status and biogeographical distribution of *Sargassum horneri* with the origin analysis of its drifting population in the end of 2016 at the Western Yellow Sea. *Oceanol. Et Limnol. Sin.* **2018**, *49*, 214–223. [CrossRef]
79. Ding, X.W.; Zhang, J.H.; Zhuang, M.M.; Kang, X.Y.; Zhao, X.H.; He, P.M.; Liu, S.R.; Liu, J.F.; Wen, Y.; Shen, H.; et al. Growth of *Sargassum horneri* distribution properties of golden tides in the Yangtze Estuary and adjacent waters. *Mar. Fish.* **2019**, *41*, 188–196. [CrossRef]
80. Zhao, J.; Jiang, P.; Qiu, R.; Ma, Y.Y.; Wu, C.H.; Fu, H.H.; Chen, H.X.; Li, F.C. The Yellow Sea green tide: A risk of macroalgae invasion. *Harmful Algae* **2018**, *77*, 11–17. [CrossRef]
81. Thiel, M. Rafting of benthic macrofauna: Important factors determining the temporal succession of the assemblage on detached macroalgae. *Hydrobiologia* **2003**, *503*, 49–57. [CrossRef]
82. Thiel, M.; Gutow, L. The ecology of rafting in the marine environment. II. The rafting organisms and community. *Oceanogr. Mar. Biol.* **2005**, *43*, 279–418. [CrossRef]
83. Riera, R. Are marine drifting species cosmopolitan? the example of the isopod *Idotea metallica*. *Thalassas* **2014**, *30*, 75–78.

84. Gutow, L.; Strahl, J.; Wiencke, C.; Franke, H.-D.; Saborowski, R. Behavioural and metabolic adaptations of marine isopods to the rafting life style. *Mar. Biol.* **2006**, *149*, 821–828. [CrossRef]
85. Franke, H.D.; Gutow, L.; Janke, M. The recent arrival of the oceanic isopod *Idotea metallica* Bosc off Helgoland (German Eight, North Sea): An indication of a warming trend in the North Sea? *Helgol. Mar. Res.* **1998**, *52*, 347–357. [CrossRef]
86. Duffy, J.E. Amphipods on seaweeds: Partners or pests? *Oecologia* **1990**, *83*, 267–276. [CrossRef] [PubMed]
87. Liess, A.; Kahlert, M. Gastropod grazers affect periphyton nutrient stoichiometry by changing benthic algal taxonomy and through differential nutrient uptake. *J. N. Am. Benthol. Soc.* **2009**, *28*, 283–293. [CrossRef]
88. Edgar, G.J.; Shaw, C. The production and trophic ecology of shallow-water fish assemblages in southern Australia III. General relationships between sediments, seagrasses, invertebrates and fishes. *J. Exp. Mar. Biol. Ecol.* **1995**, *194*, 107–131. [CrossRef]
89. Zheng, X.Q. *A Preliminary Study on the Impacts of Amphipods' Grazing on the Macroalgal Community in Yundang Lagoon*; Xiamen University: Xiamen, China, 2008; 106p.
90. Sano, M.; Omori, M.; Taniguchi, K. Predator-prey systems of drifting seaweed communities off the Tohoku coast, northern Japan, as determined by feeding habit analysis of phytal animals. *Fish. Sci.* **2003**, *69*, 260–268. [CrossRef]
91. Geertz-Hansen, O.; Sand-Jensen, K.; Hansen, D.F.; Christiansen, A. Growth and grazing control of abundance of the marine macroalga, *Ulva lactuca* L. in a eutrophic Danish estuary. *Aquat. Bot.* **1993**, *46*, 101–109. [CrossRef]
92. Hauxwell, J.; McClelland, J.; Behr, P.J.; Valiela, I. Relative importance of grazing and nutrient controls of macroalgal biomass in three temperate shallow estuaries. *Estuaries* **1998**, *21*, 347–360. [CrossRef]
93. Yu, J.; Kuang, L.L.; Wang, Q.H.; Liu, Y.; Gong, Q.L.; Li, J.Y. Differences in photosynthesis, growth and resource accumulation bet ween drif-ting alga *Sargassum horneri* and cultured alga *Undaria pinnatifida* and their roles in interspecies competition. *Period. Ocean Univ. China* **2021**, *51*, 1–10. [CrossRef]
94. Wang, L.M.; Wang, S.Y.; Xu, W.D.; Li, H.L.; He, P. Effects of temperature, light intensity and ratio of nitrogen to phosphorus on growth of seedlings in sea weed *Sargassum horneri*. *J. Dalian Ocean Univ.* **2020**, *35*, 376–381. [CrossRef]
95. Lavaut, E.; Guillemin, M.L.; Colin, S.; Faure, A.; Coudret, J.; Destombe, C.; Valero, M. Pollinators of the sea: A discovery of animal-mediated fertilization in seaweed. *Science* **2022**, *377*, 528–530. [CrossRef]
96. Li, H.M.; Zhang, C.S.; Han, X.R.; Shi, X.Y. Changes in concentrations of oxygen, dissolved nitrogen, phosphate, and silicate in the southern Yellow Sea, 1980–2012: Sources and seaward gradients. *Estuar. Coast. Shelf Sci.* **2015**, *164*, 44–45. [CrossRef]
97. Zhao, S.B.; Xu, B.C.; Yao, Q.Z.; Burnette, W.C.; Charettef, M.A.; Su, R.G.; Lian, E.; Yu, Z. Nutrient-rich submarine groundwater discharge fuels the largest green tide in the world. *Sci. Total Environ.* **2021**, *770*, 144845. [CrossRef]