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# Monitoring and Assessment of Oyster and Barnacle Larvae Settlement in an Oyster Farm in Western Taiwan

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#### **Abstract**

The Portuguese oyster (Crassostrea angulata) is an important fishery resource in Taiwan. This study investigated oyster cultures at two locations along the coast of Taisi Township from March 2012 to July 2014. The average recruitment density, measured once every two weeks, of the oyster larvae was 256.4  $\pm$  236.6 individuals/shell (N = 62) at site A and 118.5  $\pm$  140.2 individuals/shell (N = 39) at site B. The average adherence density of the barnacle larvae was 187.1  $\pm$  251.2 individuals/shell (N = 60) at site A and 60.9  $\pm$  112.5 individuals/shell (N = 37) at site B. In Taiwan, C. angulata spawned all year. The primary spawning season was from March to September. The primary of adherence spawning season of barnacles was from March to October. Rainfall was the major factor that influenced oyster C. angulata and barnacle settlement, the eigenvalue of PC1 was 1.83 and could explain 61.0%. There are two main culturing seasons that move oyster larvae from coast of Taisi to farms in other places: from March to May and from August to September. Although the oyster larvae are more abundant in spring, fishermen prefer harvesting the larvae in autumn to avoid the typhoon season (July-September).

# **Keywords**

Correlation, Influence, Rainfall, Recruitment, Spat

# 1. Introduction

## 1.1. Oyster Culture in Taiwan

Oyster aquaculture is widely distributed in the world and provides high eco-

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nomic value [1] [2]. The Pacific oyster *Crassostrea gigas* (Thunberg 1793) is a well-known commercial oyster cultivated in many countries and areas, including the United Kingdom, Japan, Korea, Australia, and Taiwan [3]-[8].

The Pacific oyster and the Portuguese oyster *Crassostrea angulata* (Lamarck 1819) were considered a single species until recently when molecular biology studies proved otherwise [9]-[15]. The Pacific oyster is native to temperate waters close to Japan and Korea, whereas the Portuguese oyster is found in the waters surrounding Taiwan [16] [17]. Studies have shown that the two oysters also differ in their filtration rates, activity levels at low temperatures, and growth rates [11] [18] [19]. The Portuguese oyster has a wide distribution in subtropical Taiwan and southern China [15] [16] [20] [21] [22] [23].

Settlement or spatfall is a vital event in the life cycle of oysters, and temperature and salinity influence oyster spatfall [24]-[29]. In addition, oyster spatfall is influenced by competition between oyster larvae and larvae of other species, such as sessile or fouling organisms [30]. Barnacles are one of these sessile fouling organisms commonly found on oyster clusters and can cause problems for oyster culturists [30] [31] [32]. Temperature and salinity affect barnacle spatfall similarly to oyster larvae [29] [33] [34] [35].

## 1.2. Oyster Predators

Barnacles feed on a wide variety of planktonic organisms ranging in size from flagellates to small crustaceans [36] [37] [38]. These and other benthic predators, such as sea anemones, barnacles, and ascidians, feed on oyster larvae or oyster veliger, thus limiting oyster larval settlement [39] [40] [41].

The coast along Taisi Township, Taiwan is a crucial oyster culture zone and source of oyster larvae. Oyster farming contributes substantially to the local economy, and the Portuguese oyster is the primary species being cultivated. Organisms that live on the shells of these oysters include barnacle *Amphibalanus* spp., rock shell *Thais clavigera*, dove shell *Mitrella bella*, mussel *Modiolus auriculatus*, and pea crab *Arcotheres sinensis*. In Taiwan, fishermen remove rock shells and barnacles manually, but they do not remove other fouling organisms.

# 1.3. Breeding Season of Oyster and Barnacles

Oysters of the genus *Crassostrea* in general had the ability to adapt well to temperature fluctuations and their larvae also generally exhibit a wide tolerance of temperature fluctuations [42] [43]. The seasonal and monthly variations of salinity, temperature, and pH have been related to variations in the settlement of meroplanktonic oyster larvae. For example, a strong correlation was found between monthly pH values and oyster spat density along the southwest coast of India [44]. Some researchers have argued that rainfall affects the water temperature, salinity and reduces the pH of the estuary, but predicting how much the winter rains would affect the summer salinity levels is difficult [44] [45] [46]. The additional environmental data may be to compare oyster settlement to local

conditions, rainfall, river flow and look for potential correlations.

Additional environmental data, such as temperature, salinity and rainfall, would allow scientists to identify potential correlations between oyster settlement and the local conditions and to determine the effects of tide and rainfall on oyster settlement [44] [45]. Chou *et al.* (2012) used principal component analysis (PCA) to identify the major sources of influence on coastal waters and reported that the coastal area was mainly affected by riverine discharge and seasonal changes (specifically transparency, turbidity and temperature) [47].

In Spring, oyster spawning becomes more regular and usually occurs on the second and sixteenth days of the Chinese lunar calendar. In addition, winds, typhoons, rough seas, and sudden temperature changes can cause oyster spawning, which lasts only 3 days according to the local fishermen. Local fishermen also claim that earthquakes and lightning can also instigate spawning; however, evidence for this claim is lacking. In autumn, oyster spat have thin, white shells and low survival rates; fishermen avoid collecting them during this period. Conversely, oyster spat have high survival rates and thick, dark shells in spring. The oyster larvae grow and develop adherence abilities quickly when the seawater temperature is high, whereas at low temperatures, these processes are prolonged. Spring is the ideal time to begin oyster farming, but oysters are often damaged by the summer typhoons, which are routine annual weather events in Taiwan. We conducted a 2-year monitoring study to verify the views of the fishermen, who—despite the oyster larvae being abundant in spring—harvest the larvae in autumn.

## 2. Materials and Methods

#### 2.1. Study Site

This study was mainly conducted at the Jiuhuwei estuary (23°42'48"N, 120°09'25"E) in Taisi Township, Yunlin County, Taiwan. Two oyster rack sites, A and B, were set up within 1 km of each other (Figure 1). Site A underwent more flooding because of its proximity to a tidal creek, and site B occupied higher ground and therefore experienced less tidal impact. From March 2, 2012, to July 26, 2014, we collaborated with a local fisherman who helped us hang ten oyster-shell strings at each site. Each string was a 5-meter long plastic rope fitted with 18 empty oyster shells, which were used as oyster spat and barnacle collectors. During high tide, the strings would submerge underwater to depths of 1 to 2 m. During low tide, they would remain above the water. Over time, the oyster larvae and barnacles adhered to the empty shells. After two weeks of accumulation, the shells were collected and replaced with new strings at each site. Furthermore, the others eight strings were keep for 6 - 12 months, then return to the laboratory for crab research [48] [49], and identification of attached oysters and barnacles [23] [50]. The strings were retrieved biweekly from each site, and ten shells were randomly chosen from each string, thus, 20 shells were sampled in

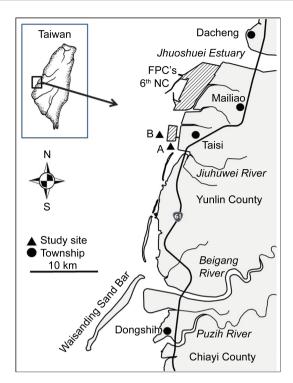


Figure 1. Adherence density of the larvae of oysters and barnacles at site A in Taisi Township once every two weeks from March 2, 2012, to July 26, 2014. Solid lines represent oyster larvae and dashed lines represent barnacle larvae.

total to count the number of oyster spats and barnacles. However, on September 13, 2013, drift sand buried the oyster rack at site B and prevented our retrieval of its strings.

### 2.2. Environmental Factors

Throughout the study period, meteorological and seismic data was collected from the Yunlin weather station, and 2-week accumulated rainfall was calculated. From March 2, 2012 to June 21, 2013, each time we retrieved the oyster shell strings at sampling site A, we recorded the seawater temperature (°C), salinity (psu), conductivity (ms/cm), pH, DO (mg/L), redox potential (mV), and chromaticity (ppm) by using portable instrument (WTW-Cond3210, -pH3210, -Oxi3210, and LaMotte-mart3). From March 2, 2012 to June 21, 2013, the others environmental factors turbidity (NTU), NH<sub>4</sub>-N (mg/L), NO<sub>3</sub>-N (mg/L), T-P (total phosphate) (mg/L), SS (suspended solids) (mg/L), BOD (mg/L), chlorophyll-a (μg/L) were measured in laboratory. NH<sub>4</sub>-N, NO<sub>3</sub>-N, T-P were determined by the Spectrophotometer Method with Spectroquant Nova 60 Photometer by Merck (Germany). Suspended solids, turbidity, chlorophyll-a, and biochemical oxygen demand in water were detected in National Institute of Environmental Analysis, NIEA W210.58A, NIEA W219.52C, NIEA E508.00B, and NIEA W510.55B, respectively.

Three L of phytoplankton was sampled from study site A. Samples were stored in the dark and low temperature. Phytoplankton samples were concentrated to 100 ml by the settling and decanting method in a refrigerator [51]. The settling time was at least 4 days. A known aliquot of phytoplankton was added to 0.2 ml of 10% syrup on a 22 × 22 mm cover slip [52], then dried in the dark and sealed onto a slide by nail polish. Algal taxa were enumerated at  $400\times$  (Olympus BX51, Tokyo, Japan). Biovolumes of algae were measured according to shape measurements of Hillebrand *et al.* (1999) [53]. At least 300 natural units were recorded [54]. For algal identification Ding and Li (1991), and Carmelo (1997) were consulted [55] [56]. The volume and cell number of planktonic algae ( $\mu$ m³/L and individual/L), were determined in a laboratory. Both the volume and cell number of planktonic algae were transformed to a natural logarithmic scale.

Moreover, each week in the study period, we collected eyed oyster larvae by using a 100-μm mesh trap filter containing approximately 5 L of seawater at a depth of 1 m. We added 70% alcohol to the specimens to maintain the laboratory count (individual/L) [57]. Finally, the data of eyed oyster larvae from these two weeks were averaged. Throughout the study period, meteorological and seismic data was collected from the Yunlin weather station (23°42'20"N, 120°31'02"E). The 2-week accumulated rainfall was calculated.

# 2.3. Data Analysis

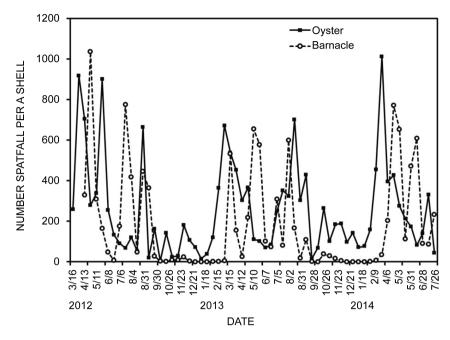
Data are presented as means ± standard deviation or ranges. The data (environmental factors, oyster larvae, and barnacle larvae) were analyzed using PCA and BEST (an approach that combines the Spearman and BIO-ENV methods) subroutines featured in the statistical analysis program PRIMER v.6 (PRIMER-E, Plymouth, UK) [58].

### 3. Results

# 3.1. Recruitment Density of Oyster Larvae and Barnacle

From March 2, 2012, to July 26, 2014, we retrieved the oyster shell strings from our two sampling sites biweekly. We collected 404,990 oyster larvae and 269,207 barnacle larvae in total. The oysters were recognized as *C. angulata*, and the barnacles were classified into two species, *Amphibalanus amphitrite* (Darwin 1854) and *A. reticulatus* (Utinomi 1967).

At site A, which was near the estuary, the recruitment densities of oyster larvae and barnacle larvae were high. The average biweekly density of the eyed oyster larvae was  $59.4 \pm 149.8$  individuals/L (N = 63). The average biweekly adherence density of the oyster larvae was  $254.2 \pm 236.6$  individuals/shell (N = 63), and the average biweekly adherence density of the barnacle larvae was  $183.0 \pm 250.7$  individuals/shell (N = 61) (**Figure 2**). In spring, the oyster larvae began settling about 2 - 3 weeks earlier than did the barnacles. However, evidence for the adult barnacles' interference and predatory nature against the oyster larvae was weak.



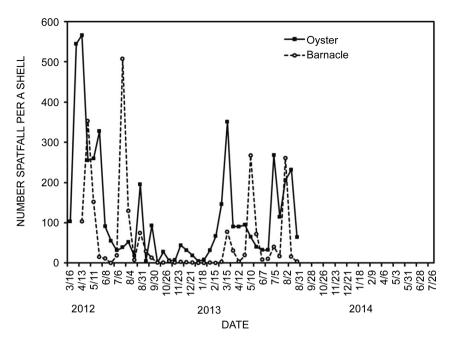
**Figure 2.** Adherence density of the oysters larvae and barnacles at Site A in Taisi Township once every two weeks from March 2, 2012, to July 26, 2014. Solid lines represent oyster larvae and dashed lines represent barnacle larvae.

On September 13, 2013, an accumulation of drift sand buried an oyster rack at site B and prevented us from collecting data. Despite this drawback, we extrapolated the following data from Site B: average biweekly adherence density of the oyster larvae and barnacle larvae was  $118.5 \pm 140.2$  individuals/shell (N = 39) and  $60.9 \pm 112.5$  individuals/shell (N = 37), respectively (**Figure 3**). Oyster larvae from sites A and B had a correlation level of 60.9%, whereas barnacle levels from both sites had a correlation level of 45.2%.

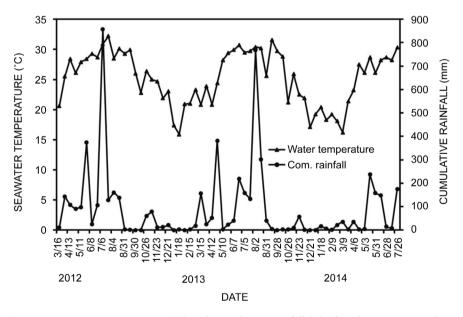
Oyster larvae levels peaked on five occasions: March 30 and August 31, 2012; March 15 and August 31, 2013; and March 23, 2014; the corresponding average biweekly adherence levels of oyster larvae were 917.5  $\pm$  232.7, 664.5  $\pm$  100.9, 671.4  $\pm$  167.5, 701.2  $\pm$  240.6, and 1012.1  $\pm$  260.7 individuals/shell (N = 20), respectively. During the same period, barnacle adherence levels peaked on six occasions: April 27 and July 19, 2012; March 29, May 25, and August 16, 2013; and April 22, 2014. The corresponding average numbers of barnacle larvae on 20 shells were 1034.8  $\pm$  346.7, 775.6  $\pm$  293.1, 531.6  $\pm$  150.8, 654.0  $\pm$  178.7, 598.9  $\pm$  145.8, and 771.4  $\pm$  466.4 individuals/shell, respectively (**Figure 2**).

#### 3.2. Environmental Factors

From March 16, 2012, to July 26, 2014, the 2-week cumulative rainfall was 90.3  $\pm$  159.9 mm (**Figure 4**); during this period, there were 14 typhoons and 3 earth-quakes. The biweekly average seawater temperature was 25.5  $\pm$  4.6°C (**Figure 4**), salinity 30.7  $\pm$  3.3 psu, conductivity 46.5  $\pm$  5.7 ms/cm, pH 8.04  $\pm$  0.10, DO 6.3  $\pm$  1.1 mg/L, redox potential 135.5  $\pm$  24.7 mV, chromaticity 283.7  $\pm$  249.14 ppm,



**Figure 3.** Adherence density of the oysters larvae and barnacles at Site B in Taisi Township once every two weeks from March 2, 2012, to September 13, 2013. Solid lines represent oyster larvae and dashed lines represent barnacle larvae.

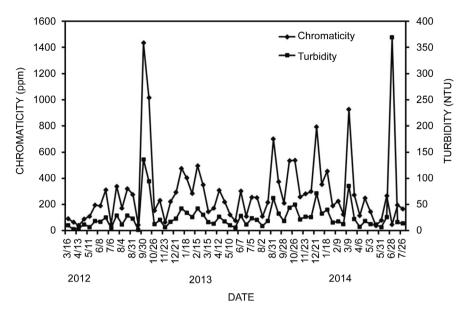


**Figure 4.** Seawater temperature (▲) and cumulative rainfall (•) of study site A in Yunlin, western Taiwan once every two weeks from March 2, 2012 to July 26, 2014.

and turbidity  $30.9 \pm 49.0 \text{ NTU } (\text{Figure 5})$ , respectively (N = 63; Table 1).

From March 16, 2012, to June 21, 2013, the biweekly average of NH4-N was 0.34  $\pm$  0.34 mg/L, NO<sub>3</sub>-N 0.61  $\pm$  0.21, PO<sub>4</sub>-P 0.10  $\pm$  0.18, SS 52.6  $\pm$  14.0 mg/L, BOD 1.23  $\pm$  0.85, and chlorophyll-a 4.40  $\pm$  2.89 µg/L, respectively (N = 34; **Table 1**).

The planktonic algae were classified as diatom (60.5%), blue-green algae



**Figure 5.** Chromaticity (♦), and Turbidity (■) of study site A in Yunlin, western Taiwan once every two weeks from March 2, 2012 to July 26, 2014.

**Table 1.** Average value and rang of each physical-chemical biology variables of the coast of Taisi Township from March 2012 to July 2014.

2012/3/16 - 2014/7/26 (N = 63)			2012/3/16 - 2013/6/21 (N = 34)		
Items	Avg ± SD	Rang	Items	Avg ± SD	Rang
cum. rainfall (mm)	93.1 ± 174.0	0.0 - 940.5	NH <sub>4</sub> - N (mg/L)	$0.34 \pm 0.34$	0.08 - 1.40
Water temperature (°C)	$25.8 \pm 4.1$	15.9 - 32.2	NO <sub>3</sub> - N (mg/L)	$0.61 \pm 0.21$	0.10 - 0.90
Salinity (psu)	$31.4 \pm 2.2$	24.4 - 37.7	PO <sub>4</sub> - P (mg/L)	$0.10 \pm 0.18$	0.01 - 1.05
Conductivity (ms/cm)	$47.0 \pm 5.6$	19.4 - 50.5	SS (mg/L)	$52.6 \pm 14.0$	28.5 - 96.0
pН	$8.04 \pm 0.09$	7.76 - 8.23	BOD (mg/L)	$1.23 \pm 0.85$	0.42 - 4.50
DO (mg/L)	$6.8 \pm 0.9$	5.0 - 8.7	Chlorophyll-a ( $\mu$ g/L)	$4.40 \pm 2.89$	0.00 - 11.85
Redox potential (mV)	131.4 ± 18.9	100.0 - 194.0	Ln(v)*	15.2 ± 3.6	2.4 - 20.2
Chromaticity (ppm)	270.3 ± 276.3	41.0 - 1435.0	Ln(indi)#	$14.4 \pm 2.8$	7.1 - 18.9
Turbidity (NTU)	$24.0 \pm 26.0$	1.4 - 136.0			

<sup>\*:</sup> ln(v), where v is the volume of planktonic algae ( $\mu m^3/L$ ); #: ln(indi), where indi is the cell number of planktonic algae (indi/L).

(31.8%), green algae (5.8%), euglena (1.3%), and alginate (0.6%), respectively. The biweekly average of planktonic algae in  $\ln(\text{volume/L})$  was 15.2  $\pm$  3.6 and in  $\ln(\text{indi/L})$  was 14.4  $\pm$  2.8 (Table 1).

We transformed our results for the 14 environmental factors, chlorophyll-a, and the 2 planktonic algae factors, eyed oyster larvae, spat oyster larvae, and spat barnacle larvae by applying the fourth root and analyzed these data using the PCA and BEST subroutines. Eigenvalues of PC1 was 1.83 could explain 61.0% of the water quality variation; among these values, rainfall and chlorophyll-a were positive, and chromaticity and turbidity were negative. Eigenvalues of PC2 was

0.813 could explain 27.1% of the water quality variation; among these values, chromaticity, turbidity and rainfall were negative, and chlorophyll-a was positive. Eigenvalues of PC3 was 0.204 could explain 6.8% of the water quality variation, and total could explain 94.9% (Table 2).

The best correlations (by BEST subroutine) environmental data with eyed oyster larvae and with oyster larvae settlement were 0.867 and 0.895, when the same following five variables were selected: rainfall, chromaticity, turbidity, chlorophyll-a, and planktonic algae ln(v). The correlation of environmental data and barnacle larvae settlement was 0.814, when the following five variables were selected: rainfall, water temperature, chromaticity, SS, and chlorophyll-a. Barnacle attachment was more susceptible to low seawater temperatures in winter than oyster attachment. In summary, rainfall, chromaticity and chlorophyll-a were the main factor governing phytoplankton biomass and production, whereas seawater temperature and suspended solids (sand, SiO<sub>2</sub>) concentration had both a direct effect on production and an indirect effect by changing community composition. We speculate that rainfall is the major sources of influence on oyster larvae *C. angulata* and barnacle larvae *Amphibalanus* spp. settlement.

#### 4. Discussion

Karuppaiyan and Raja (2007) reported that the adherence season of oyster larvae in southeast India was from September through May, except for the coldest days in December [59] (Karuppaiyan and Raja 2007). Shaw (1967) recommended that in Broad Creek, Talbot County, Maryland, the shells should be planted between July and August [60] (Shaw 1967). In this study, settlement specimen identification should wait for 6 - 12 months for the same batch of larvae to grow. The Portuguese oyster *C. angulata* spawns all year. The primary spawning season is from March to September, and the adherence numbers drop to their lowest levels in January and February. The primary spawning season for barnacles is from March to October, and barnacle adhesion ceases when the seawater temperature decreases below 21°C. We surmise that because *C. angulata*, *A. amphitrite*, and *A. reticulatus* are mainly distributed in the tropics and subtropics, their

**Table 2.** PCA of water quality data along the coast of Taisi Township in Yunlin, western Taiwan from March 16, 2012, to Jane 21, 2013 (N = 34).

Variable	PC1	PC2	PC3
Two-week cum. rainfall	0.953	-0.297	0.025
Chromaticity	-0.255	-0.805	-0.156
Turbidity	-0.153	-0.453	-0.096
Chlorophyll-a	0.029	0.197	-0.932
Eigenvalues	1.830	0.813	0.204
% variation	61.0	27.1	6.8
% % cum. variation	61.0	88.1	94.9

fertility diminishes at low temperatures.

Pan *et al.* (2016) reported that turbidity, water temperature, and SiO<sub>2</sub> were the main factor governing phytoplankton biomass and production [61] (Pan *et al.* 2016). Generally, rainfall has a direct effect on turbidity and water temperature. At site A, after heavy precipitation during the typhoon season, water temperatures at the estuary exceeded 30°C, and the pH of the seawater recovered to more than 7.9; consequently, adherence levels for oyster larvae peaked (**Figure 2** and **Figure 3**).

In Taiwan, there are two oyster spat harvest seasons exploited by oyster culturists. The first season is from March to May, when the shells of the oyster larvae are thick and dark. The larvae grow rapidly in summer and have a low death rate, but they are vulnerable to typhoons; hence, they must be cultured in lagoons. The second harvest season is from August to September, when the shells of the oyster larvae are thin and light. The larvae grow slowly in winter, when there are no typhoons. Demand for oyster larvae produced in Taisi Township is higher in the autumn. Local fishermen suggest that the occurrence of lightning, thunder, earthquakes, and other disruptions induce spawning in mature oysters; however, evidence for this claim is lacking.

In spring, nutrient levels are low and the barnacles conserve nutrition required for growth and reproduction. Oyster larvae are likely to be a source of nutrition for them during this period. In summer, typhoons wash large amounts of nutrients into the estuary from upstream, resulting in higher nutrient levels for the barnacles. They rely less on oyster larvae for nutrition during this time. We hypothesize that because barnacles feed on oyster larvae, oyster larvae levels peak first in spring, following which the barnacles thrive soon after. In summer, when the barnacles are less reliant on oyster larvae for nutrition, this dependency decreases, leading to low correlation between the population of these two species

In 2014, Kripa *et al.* had reported that "in lower pH of 6.5, the larvae did not survive beyond the first day, and in pH 7.0, only 12% larvae developed to D-larvae" from Vembanad Lagoon of Indian [44]. In this study, the pH was 8.04 (7.76 - 8.23) (**Table 1**), within a reasonable range suitable for survival and settlement of oyster larvae. It was not selected by PCA and BEST subroutines. However, in the monitoring data of Yunlin Islands Industrial Zone, there are many pH data that are very low in southwest coast of Taiwan from 1987 to 2018 [62]. Therefore, it is very important to continuously control the pollution of waste acid.

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## **Conflicts of Interest**

The authors declare no conflicts of interest regarding the publication of this paper.

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