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**Moonlight cycles synchronize oyster behavior**

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Moonlight, circalunar rhythm, mollusks, bivalve, behavioral ecology, *Crassostrea gigas*

24 **Abstract**

25 Organisms possess endogenous clock mechanisms that are synchronized to external cues and  
26 orchestrate biological rhythms. Internal timing confers the advantage to anticipate  
27 environmental cycles inherent in life on Earth and to prepare accordingly. Moonlight-  
28 entrained rhythms are poorly described, being much less investigated than circadian and  
29 circannual rhythms synchronized by sunlight. Yet focus on these lunar rhythms is highly  
30 relevant to understanding temporal organization of biological processes. Here, we investigate  
31 moonlight cycle effects on valve activity behavior of the oyster *Crassostrea gigas*. Our results  
32 show that oysters modulate valve behavior according to both intensity and direction of the  
33 lunar illumination cycle. As a consequence, valve opening amplitude is significantly increased  
34 at third quarter moons (decreasing lunar illumination) compared to first quarter moons  
35 (increasing lunar illumination) despite identical lunar illumination, and this indicates that  
36 oyster modulation of valve behavior by moonlight cycles is not a direct response to lunar  
37 illumination. We propose that oysters use moonlight cycles to synchronize behavior and also  
38 other physiological and ecological aspects of this benthic mollusk bivalve.

39

40 **1. Introduction**

41 Life on earth is influenced by a multitude of cycles resulting from sun, earth and moon  
42 trajectories and interactions, including in marine ecosystems. Fitness and survival depend on  
43 the capacity to forecast oscillating ecosystem constraints and benefits. By conferring a  
44 personal time measurement, internal molecular clock(s) give organisms anticipation [1]. This  
45 gives meaning to environmental cues and leads to temporal organization, from cell to  
46 ecosystem [1,2]. Lunar rhythms, defined by a 29.5-day period length of moonlight oscillation,  
47 has received considerably less attention than circadian rhythms. In marine organisms,  
48 moonlight has mostly been observed to act on reproduction, constituting an essential cue for

49 mass synchronous external spawning events [3,4]. For a number of species, this reproductive  
50 rhythm is not a direct response to moonlight but requires the action of an endogenous clock  
51 [3,4]. Unlike reproductive processes, physiological and behavioral lunar rhythms in marine  
52 organisms are largely unknown, despite their potential significance in ecological functioning.  
53 The Pacific oyster *Crassostrea gigas* is a benthic marine invertebrate with among the largest  
54 of geographical distributions. Diploid and triploid oysters, i.e. organisms with two or three  
55 sets of chromosomes, respectively, dominate shellfish production and wild populations are  
56 increasingly becoming established, with potential impact on ecosystems [5]. Valve activity in  
57 bivalve species is closely related to physiological processes and gene expression [6–8]. In *C.*  
58 *gigas*, valve behavior follows a daily rhythm for which the corresponding circadian clock has  
59 been identified [9,10], and an annual rhythm for which endogenous circannual timing has  
60 been proposed [9,11]. This behavior also follows a strong tidal rhythm, modulated by neap-  
61 spring tidal (semilunar) and anomalistic moon cycles [12]. A recent study showed that *C.*  
62 *gigas* behavior might evolve according to a 29.5-day periodicity [11]. However, how oysters  
63 modulate their valve behavior following lunar cycles has, to our knowledge, never been  
64 studied.

65 In this study, we present the first analysis of lunar rhythm-modulated behavior in *C. gigas*.  
66 Focusing on winter, i.e. minimal reproductive efforts and spawning events, we investigated  
67 effects of both intensity and direction of lunar illumination on this species. We demonstrated  
68 that the lunar rhythm in *C. gigas* is not a direct response to nocturnal light. This finding raises  
69 the question of the underlying mechanism of the lunar-related behavior in oysters.

70

## 71 **2. Materials and methods**

### 72 **(a) Animals, study area and data collection**

73 The present study analyzed valve behavior data of 12 *C. gigas* oysters (6 diploid and 6  
74 triploid) from 22 December 2014 to 3 April 2015. Two-year old animals ( $66 \pm 1$  mm shell  
75 length) were positioned in a natural site in the proximity of wild oysters (Arcachon Bay,  
76 France, latitude  $44.66^\circ$ , longitude  $-1.16^\circ$ ) in February 2014, i.e. 9 months before this study  
77 began. They were in a permanently immersed oyster bag fixed on an oyster table, at a  
78 minimum water depth of 1 m. Valve behavior was recorded every 1.6 second using high-  
79 frequency non-invasive (HFNI) valvometer technology [13]. The astronomical data related to  
80 moon cycles was retrieved from the site <https://www.timeanddate.com/>.

81

## 82 **(b) Data treatment and statistical analyses**

83 Daily valve opening amplitude (daily VOA, 54000 averaged data, %, more details in [11])  
84 was analyzed individually and averaged based on ploidy status. Mean VOA values for the 5  
85 days around each new moon (NM), full moon (FM), first and third quarter moon (first and  
86 third QM) phases were calculated. Because of the non-normality of the data, ANOVA on  
87 ranks of the data followed by Dunn's method were applied to test moon phase effects. An  
88 illuminated moon surface value (%) was associated with each daily VOA, as a proxy of lunar  
89 illumination. A random components mixed model for repeated measures was applied to test  
90 lunar illumination, direction of lunar illumination and ploidy effects on individual daily VOA  
91 values, using XLSTAT 2018 software (Addinsoft. New York, USA). All statistical analyses  
92 were performed after checking assumptions (normality and equal variance;  $p$ -value  $< 0.05$   
93 considered significant) and using Sigma Plot software (Version 13.0; Systat Software, USA).

94

## 95 **3. Results**

96 Figure 1 presents daily valve opening amplitude (VOA, %) of diploid and triploid oysters  
97 during the entire winter season, i.e. three and a half moon cycles. In figure 2, ANOVA on

98 ranks showed significant daily VOA differences according to moon phase in both diploid and  
99 triploid oysters ( $p < 0.001$  for both). Daily VOA was maximal during NM and minimal during  
100 first QM, and increased at FM and third QM (figure 2). Finally, daily VOA was increased by  
101 16 % and 19 % at third QM compared to first QM, in diploid and triploid oysters, respectively  
102 (figure 2).

103 In table 1, the entire daily VOA dataset was used. The mixed model showed a significant  
104 random effect for oyster individuals ( $p = 0.007$ ). Moreover, this model showed significant  
105 effect of the fixed parameters: lunar illumination ( $p < 0.0001$ ) and direction of lunar  
106 illumination ( $p < 0.0001$ ) on daily VOA. No significant effect was shown for the ploidy ( $p =$   
107  $0.2916$ ). There was a significant interaction between lunar illumination and direction of lunar  
108 illumination ( $p < 0.0001$ ) independent of ploidy status.

109

#### 110 **4. Discussion**

111 Our findings clearly show behavioral modulations in *C. gigas* which follow lunar cycles, and  
112 allow innovative hypotheses. First, we indicate that oysters, independent of ploidy, are able to  
113 sense moonlight despite its extremely low intensity compared to sunlight. Secondly, we  
114 demonstrate that oysters can detect if the moonlight is increasing or decreasing. Finally, we  
115 show that oysters combine these two pieces of information to adjust their behavior.

116 The moonlight sensitivity observed in *C. gigas* is consistent with a moonlight response in  
117 another bivalve, the mussel *Pinna nobilis* [8,14]. In contrast to our results, *P. nobilis* valves  
118 were more open when the moon was more than 50 % illuminated [8]. Yet both studies imply  
119 that these mollusks possess light receptor(s) able to perceive dim nocturnal light. In other  
120 phyla, photoreceptor families such as cryptochromes or opsins, also present in oysters [10],  
121 have received significant attention, but their functional involvement in moonlight perception  
122 remains unclear [3,4].

123 Moonlight sensitivity in *C. gigas* could lead to a direct behavioral response to lunar  
124 illumination cycles. However, our results indicated significant VOA differences between first  
125 and third QM despite similar moonlight intensity. Alternatively, moonlight could influence  
126 the circadian clock mechanism, usually synchronized by sunlight, and known to regulate  
127 valve behavior in *C. gigas* [6,7,10]. Another hypothesis could be that a specific dedicated  
128 circalunar clock is behind the moonlight rhythm [3,4]. To answer to the question if the lunar-  
129 related behavior is internally or externally regulated, further laboratory experiments should be  
130 carried out in constant light and food supply, and in simulated moonlight entrainments.  
131 Internal timing offers major advantages compared to solely responding to the immediate  
132 external environment [1]. A recent study indicated that moonlight cycles drive vertical  
133 migration of pelagic zooplankton species, influencing predation success and risk [15]. Our  
134 analyses show that oyster VOA is globally higher when lunar illumination is low. This  
135 corresponds well with the nocturnal pattern of oyster circadian rhythms during winter [9].  
136 Previous experiments attest that circadian behavior in *C. gigas* is in accordance with digestive  
137 rhythms [6], and we also hypothesize that the global increase in oyster VOA in the absence of  
138 light (night and NM) could optimize their access to food, which is of low abundance in  
139 winter. Indeed, as a sessile and benthic species, oyster rhythms could be an adaptive  
140 mechanism allowing e.g., synchronization with light-dependent vertical migration of  
141 phytoplankton in the water column [16]. Previous experiments also show that *C. gigas* daily  
142 behavior is associated with circadian expression of at least 6.2 % of the genes in gills [7]. By  
143 analogy, behavioral lunar rhythms observed in this study could reflect a lunar rhythmicity of  
144 some other physiological functions, as observed in corals where gene expression was  
145 modulated over a lunar month [17]. Finally, a seasonal modulation of phase and intensity of  
146 oysters' lunar rhythm could be hypothesized, as observed concerning daily and tidal rhythms

147 in this species [9,11]. In this sense, it has been observed that moonlight response in the mussel  
148 *Pinna nobilis* depend on periods of the year [14].

149 Temporal organization of physiological processes at a species level may have significant  
150 effects on ecosystems. It has been argued that lunar rhythms of zooplankton vertical migration  
151 could have a large influence on biogeochemical cycles by facilitating monthly pulses of  
152 carbon remineralization [15]. Moreover, it has been proposed that vertical migrations at one  
153 trophic level can affect vertical migrations at the next one, leading to “cascading migrations”,  
154 from phytoplankton to fish [18]. By the oysters capacity to modulate several biogeochemical  
155 properties and processes, including phytoplankton assemblages [5], oyster lunar rhythms  
156 could have a non-negligible ecological impact both on benthic and pelagic areas.

157

158 **Ethics:** All experiments complied with the laws in effect in France and they conformed to  
159 international ethical standards.

160 **Data accessibility:** The data underlying this study are available in supplementary material  
161 (SM\_Payton&Tran\_2018).

162 **Author contributions:** L.P. and D.T. conceived the study, analyzed the data, drafted and  
163 revised the manuscript. Both authors agree to be held accountable for the content therein and  
164 approve the final version of the manuscript.

165 **Competing interests:** We have no competing interests.

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171



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231 **Legends**

232

233 **Figure 1.** *In situ* profiles of individual (thin lines) and mean (bold line) daily valve opening  
234 amplitude (VOA, %) of diploid (blue, n = 6) and triploid (green, n = 6) oysters during the  
235 winter season in Arcachon Bay (France). Moon phases are reported at the top (new moon:  
236 black circle; full moon: white circle; first quarter moon: half black and half white circle; third  
237 quarter moon: half white and half black circle).

238

239 **Figure 2.** Mean daily VOA and standard error of diploid (blue, n = 6) and triploid (green, n =  
240 6) oysters according to the moon phases. NM: new moon; First and third QM: first and third  
241 quarter of the moon; FM: full moon. Identical letters indicate no significant differences of  
242 mean daily VOA ( $p$ -value = 0.05).

243

244 **Table 1.** Random components mixed model for repeated measures applied to test the effects  
245 of lunar illumination (i.e. over or under 50 % of illuminated moon surface), direction of lunar  
246 illumination (i.e. increasing or decreasing illumination) and ploidy of oysters (diploid or  
247 triploid) on individual daily VOA. (\*\* and \*\*\*) indicate  $p$ -value < 0.01 and < 0.001,  
248 respectively.

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257 Table 1: Payton &amp; Tran

<b>Random components mixed model for repeated measures</b>			
Covariance parameters	Z		<i>p</i> -value
Random effects	2.452		0.007**
Repeated factors	16.831		< 0.0001***
Source of variation (fixed parameters)	NumDF	F	<i>p</i> -value
Lunar illumination	1	63.446	< 0.0001***
Direction of lunar illumination	1	65.204	< 0.0001***
Ploidy	1	1.240	0.2916
Interactions			
Illumination x Ploidy	1	3.332	0.0682
Direction x Ploidy	1	0.005	0.9428
Illumination x Direction	1	20.501	< 0.0001***

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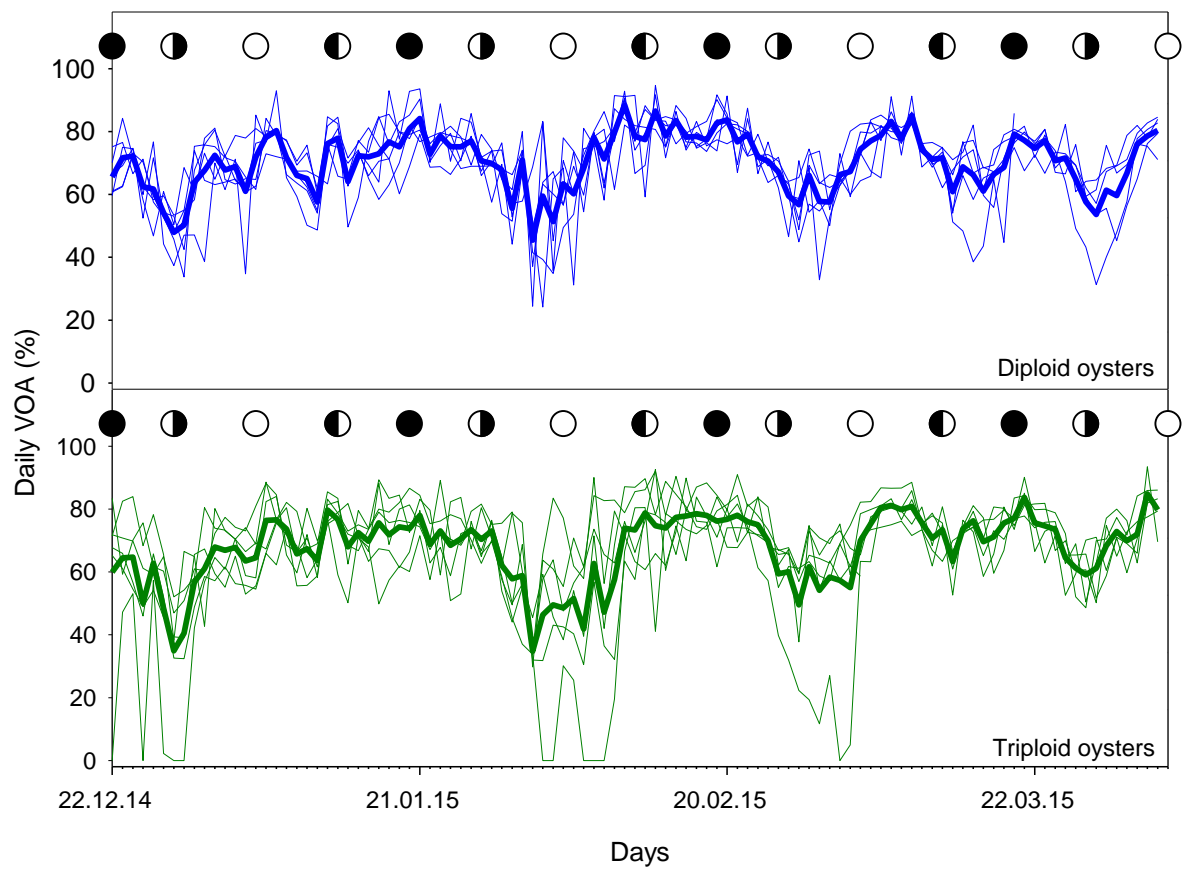


Figure 1 : Payton & Tran

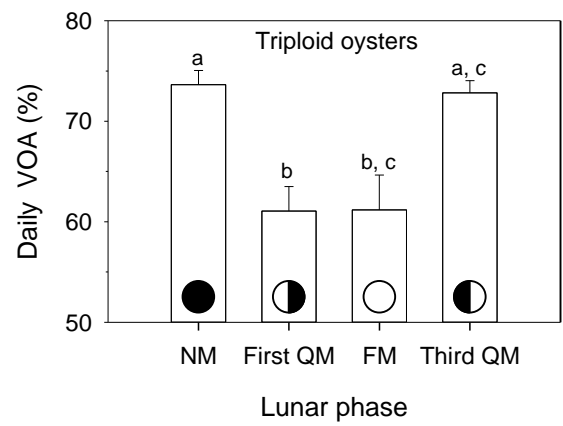
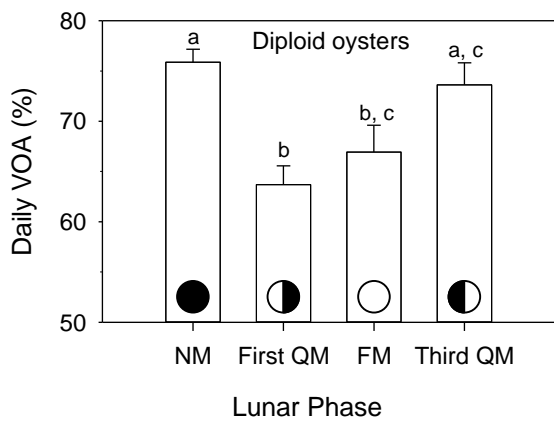


Figure 2 : Payton & Tran